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Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic–mass spectrometric analysis

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Abstract

The aroma of Greek citrus honey was investigated by means of SPME–GC/MS analysis. A total of 61 compounds could be identified, with lilac aldehydes predominating the extract. These compounds can be considered the most powerful markers for citrus honey. Additionally, the two isomeric dehydroxy linaloxides, lavender lactone, dill ether, the four isomers of 1-*p*-menthen-9-al, methyl anthranilate and nerolidol could aid the botanical discrimination. Of the compounds identified, five are reported as honey constituents for the first time, that is *trans*- and *cis*-dehydroxy linaloxides, 1,8-menthadien-4-ol, limonene-10-ol and methyl *N*-methylanthranilate.

Keywords: SPME; Aroma; Citrus honey; Linalool derivatives; Lilac aldehydes

1. Introduction

Honey has for long been an excellent nutritional option for many generations due to its health benefits. Theoretically, a unifloral honey can be produced from every honey plant. However, in practice, unifloral honeys are not so easy to produce. Thus, their price is, in most cases, higher than multifloral ones, especially for certain types of unifloral honeys. The need for finding reliable marker compounds to discriminate between unifloral honeys is obvious. Marker compounds can not only characterize a certain type of honey, but they can also show adulteration in honey.

Solid-phase microextraction (SPME), lately introduced by Arthur and Pawliszyn (1990), is highly appreciated by the food industry for the analysis of volatile compounds (Augusto, Valente, Tada, & Rivellino, 2000; Elmore, Mottram, & Hierro, 2000; Kovačevič & Kač, 2001; Sala, Mestres, Marti, Busto, & Guasch, 2000; Steffen & Pawliszyn, 1996). Even though it did not give satisfactory results in the case of unifloral Greek cotton honey (Alissandrakis, Kibaris, Tarantilis, Harizanis, & Polissiou, 2005), it has proven effective for other types of honey (Fuente de la, Martınez-Castro, & Sanz, 2005; Perez, Sánchez-Brunete, Calvo, & Tadeo, 2002; Piasenzotto, Gracco, & Conte, 2003; Soria, Martínez-Castro, & Sanz, 2003; Verzera, Campisi, Zappalà, & Bonaccorsi, 2001).

Citrus honey is one of the major harvests in Greece, comprising more than 10% of the annual production. It is light yellow to orange, with a very sweet taste and a distinct floral aroma. The scope of this work is to investigate the headspace aroma compounds of citrus honey and find characteristic compounds with respect to other unifloral honeys. Moreover, possible biochemical formations as well as connections among the volatiles identified are discussed.

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2. Materials and methods

2.1. Honey samples

Citrus honeys (33 samples) were from four different regions of Greece and from Italy. In order to ensure that honeys are as unifloral as possible, a certain procedure was followed (Alissandrakis, Daferera, Tarantilis, Polissiou, & Harizanis, 2003). This procedure was followed in all cases, except for the samples from Italy that were obtained from local beekeepers. Also, thyme (*Corydothymus capitatus*, 29 samples), cotton (*Gossypium hirsutum*, 7 samples), erica (*Erica manipuniflora*, 3 samples), chestnut (*Castanea sativa*, 3 samples), eucalyptus (*Eucalyptus* spp., 3 samples), Jerusalem sage (*Phlomis fruticosa*, 1 sample), strawberry tree (*Arbutus unedo*, 1 sample), pine (*Pinus* spp., 5 samples) and fir (*Abies* spp., 5 samples) honeys were analyzed.

2.2. Reagents

Benzaldehyde, xylene and α-terpineol were purchased from Merck (Darmstadt, Germany). Octanoic acid was purchased from Riedel-de Haën (Steinheim, Germany). Phenylacetaldehyde was purchased from Aldrich (Steinheim, Germany). The other reagents were purchased from Fluka Chemika (Buchs, Switzerland).

2.3. Isolation of volatile compounds

The isolation of the aroma compounds was performed using the SPME procedure (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2005). A DVB/carboxen/PDMS fibre was used to extract headspace volatiles from honey. The samples (honey water solution of 3 g/mL) were placed in 15 mL screw-top vials with PTFE/silicone septa. Benzophenone was used as internal standard and a portion of 20 μl (10 μg/mL in methanol) was added prior to extraction. The vials were maintained in a water bath at during equilibration and extraction and were partially submerged so that the liquid phase of the sample was in the water (Miller & Stuart, 1999).

In order to determine the optimal conditions for the extraction, the following parameters were evaluated: water bath temperature (45, 60 and 75 °C), sampling time (30, 60 and 90 min), equilibration time (30 and 60 min), sample volume (3 and 6 ml) and salt addition (NaCl and $MgSO_4$). Finally, a comparison between stirring and sonication during the whole procedure was made.

2.4. Analysis of the isolated compounds

The analysis of the extracts was performed using an Agilent 5890 II GC, equipped with an Agilent 5972 MS detector. The column used was an HP-5MS (Crosslinked 5% phenylmethylsiloxane) 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 MS-transfer line temperatures were maintained at 220 and 290 °C, respectively. Oven temperature was held at 40 °C for 3 min, raised to 160 °C at 3 °C/min and then to 200 °C at 10 °C/min.

Mass spectra were recorded in the electron ionization mode at 70 eV, scanning the 40–500 m/z range. The identification of the isolated compounds was achieved by comparing retention times and mass spectra with those of authentic samples. For tentative identification, the Nist98 and Wiley275 mass spectral libraries were employed, as well as spectral data provided by Adams (2001) are published in the literature.

Because the recovery factors vary greatly among the compounds isolated, no quantification was performed. The results are expressed as ratios of the response of each compound against the response of the internal standard (benzophenone).

3. Results and discussion

3.1. Optimization of the extraction conditions

The fiber employed was arbitrarily selected without testing different coatings, because it is suitable for extracting compounds with relatively wide range of polarities and volatilities.

The optimal conditions were selected taking into account the overall amount of the extracted volatiles, as well as the formation of artifacts. At first, the water bath temperature was tested. Even at 75 °C, no artifacts were detected, yet the overall amount of the extracted compounds was decreased. Probably, the increased temperature heated the fiber, thus decreasing its effectiveness. Keeping the fiber cool is a desired condition for the SPME procedure (Miller & Stuart, 1999). For honeys with low volatile potential (e.g., chestnut), lower temperature (45 °C) led to a very poor TIC profile. So, the water bath temperature was set at 60 °C. Sampling time was tested after a 60 min equilibration. Even though 60 min of sampling increased the overall extractives compared to 30 min, no significant differences were observed at 90 min. Moreover, for 60 min of sampling time, no substantial difference was observed for equilibration times of 30 and 60 min. Therefore, equilibration time and sampling time were set at 30 and 60 min, respectively. The sample volume of 6 ml gave better results than 3 ml. Salt addition did not improve the efficacy of the procedure; in fact it decreased the overall extractives. Finally, stirring proved more efficient than sonication.

3.2. Aroma compounds of citrus honey

A total of 33 samples of unifloral citrus honeys were analyzed by means of solid phase microextraction followed by gas chromatography and mass spectrometry. Table 1 shows the compounds isolated from the headspace of citrus

Table 1
Compounds isolated from the headspace of citrus honeys

No.	Compound	KI[f]	Min[g]	Max	Avg[h]	%[i]
1	Octane[a]	800	0.00	0.20	0.07	0.29
2	Butyl acetate[a]	820	0.00	0.33	0.10	0.42
3	Furfural[b,c]	848	0.00	0.14	0.04	0.17
4	Xylene[a]	872	0.00	0.18	0.04	0.15
5	Nonane[a]	900	0.00	0.07	0.02	0.07
6	Heptanal[a]	902	0.00	0.07	0.03	0.11
7	Benzaldehyde[a]	966	0.01	1.17	0.39	1.62
8	6-Methyl-5-hepten-2-one[b,c]	991	0.00	0.16	0.04	0.16
9	Dehydroxy-*trans*-Linaloxide[b,c] (**I**)[e]	993	0.01	0.52	0.17	0.72
10	Decane[a]	1000	0.00	0.11	0.01	0.05
11	Octanal[a]	1005	0.04	0.32	0.14	0.60
12	Dehydroxy-*cis*-Linaloxide[b,c] (**II**)	1009	0.03	0.80	0.22	0.91
13	Cymene[a]	1027	0.00	0.07	0.02	0.08
14	Limonene[a]	1030	0.23	6.52	1.62	6.81
15	Lavander lactone[b,c] (**III**)	1047	0.01	0.15	0.04	0.18
16	Phenylacetaldehyde[a]	1049	0.15	5.00	1.37	5.74
17	*trans*-Furanoid linaloxide[a] (**IV**)	1076	0.04	0.61	0.19	0.79
18	*cis*-Furanoid linaloxide[a] (**V**)	1091	0.01	0.17	0.05	0.20
19	Cymenene[b,c]	1092	0.00	0.06	0.02	0.07
20	Methyl benzoate[a]	1098	0.00	0.05	0.02	0.10
21	Undecane[a]	1100	0.00	0.14	0.03	0.12
22	Linalool[a] (**VI**)	1103	0.12	1.06	0.40	1.67
23	Nonanal[a]	1105	0.27	6.64	1.59	6.68
24	Hotrienol[b–d] (**VII**)	1109	0.00	1.07	0.27	1.14
25	Phenylethyl alcohol[a]	1124	0.00	0.10	0.04	0.15
26	Methyl octanoate[a]	1128	0.04	0.26	0.09	0.37
27	1,3,8-*p*-Menthatriene[b] (**VIII**)	1144	0.00	0.13	0.04	0.17
28	Lilacaldehyde (1st isomer)[b,d] (**IX**)	1146	1.19	6.32	3.12	13.13
29	Lilacaldehyde (2nd isomer)[b,d] (**IX**)	1154	2.17	11.07	5.20	21.86
30	Lilacaldehyde (3rd isomer)[b,d] (**IX**)	1169	0.79	4.90	2.45	10.30
31	1-*p*-menthen-9-al (1st isomer)[b] (**X**)	1179	0.01	0.43	0.08	0.34
32	1-Nonanol[a]	1180	0.01	0.22	0.05	0.19
33	1,8-Menthadien-4-ol[b] (**XI**)	1181	0.02	0.25	0.10	0.41
34	Dill ether[b,c] (**XII**)	1188	0.07	0.97	0.24	1.01
35	Octanoic acid[a]	1191	0.00	0.13	0.04	0.18
36	Unknown (*43*, 67, 109, 137, 152 M^+)	1192	0.00	0.09	0.02	0.07
37	α-Terpineol[a]	1195	0.02	0.33	0.06	0.27
38	Dodecane[a]	1200	0.00	0.24	0.01	0.05
39	Decanal[a]	1207	0.09	2.02	0.69	2.89
40	1-*p*-Menthen-9-al (2nd isomer)[b] (**X**)	1212	0.00	0.10	0.02	0.07
41	1-*p*-Menthen-9-al (3rd isomer)[b] (**X**)	1217	0.34	2.69	0.99	4.14
42	1-*p*-Menthen-9-al (4th isomer)[b] (**X**)	1221	0.32	2.64	0.96	4.04
43	Methyl nonanoate[a]	1227	0.09	0.49	0.24	0.99
44	Unknown (43, 79, *93*, 108, 121, 137, 166 M^+)	1233	0.00	0.19	0.06	0.24
45	2-Decenal[b,c]	1266	0.00	0.14	0.03	0.15
46	Nonanoic acid[a]	1291	0.00	0.38	0.10	0.41
47	Limonen-10-ol[c] (**XIII**)	1298	0.00	0.13	0.05	0.20
48	Tridecane[a]	1300	0.00	0.04	0.01	0.02
49	Undecanal[a]	1310	0.00	0.11	0.04	0.15
50	Methyl decanoate[a]	1328	0.01	0.66	0.13	0.54
51	Methyl anthranilate[a] (**XIV**)	1350	0.11	2.73	1.59	6.69
52	8-Hydroxylinalool[b,d] (**XV**)	1376	0.00	0.15	0.01	0.06
53	Decanoic acid[a]	1387	0.00	0.28	0.01	0.06
54	β-Damascenone[a]	1388	0.00	0.14	0.03	0.11
55	Tetradecane[a]	1400	0.00	0.08	0.02	0.07
56	Dodecanal[a]	1412	0.00	0.16	0.05	0.20
57	Methyl-*n*-methyl-anthranilate[b,c] (**XVI**)	1415	0.00	0.84	0.04	0.19
58	Geranyl acetone[b]	1458	0.03	0.15	0.07	0.30
59	Pentadecane[a]	1500	0.00	0.14	0.04	0.16
60	Methyl dodecanoate[a]	1527	0.02	0.15	0.07	0.29
61	Nerolidol[b,c] (**XVII**)	1569	0.00	0.29	0.05	0.23
62	Hexadecane[a]	1600	0.00	0.12	0.03	0.12
63	Internal Standard (benzophenone)	1664				
64	Heptadecane[a]	1700	0.01	0.24	0.07	0.29
		Total	6.25	65.87	23.76	99.67

Identification: a, authentic compound; b, NIST98 & Wiley275 MS libraries; c, Adams (2001); d, literature cited; e, roman numerals refer to structures in Fig. 2; f, KI values were calculated using the hydrocarbons naturally present in honey; g, min and max values refer to the internal standard calibration; h, avg refers to the average values of all the samples analyzed; i, % refers to the mean percentage of all samples analyzed against the total peak area.

honey. In Fig. 1, a representative TIC chromatogram is presented, while in Fig. 2 the structures of the most important compounds are illustrated.

Major constituents of the aroma profile of citrus honeys were the three isomeric lilac aldehydes, comprising more than 45% of the total extract. Significant proportions of limonene (6.81%), methyl anthranilate (6.69%), nonanal (6.68%) and phenylacetaldehyde (5.74%) were also obtained. Lesser amounts of the third and fourth isomer of 1-*p*-menthen-9-al (4.14% and 4.04%, respectively), decanal (2.89%), linalool (1.67%), benzaldehyde (1.62%), hotrienol (1.14%) and dill ether (1.01%) were also found. Limonene, nonanal, phenylacetaldehyde, decanal and benzaldehyde, even though they are present in relatively high amounts, they are constituents of nearly all honeys analyzed and thus they do not provide help for botanical discrimination.

Among the compounds identified, some could be used as floral markers for citrus honey as they are absent in other honeys or present in significantly lower amounts (Table 2). These are the two isomeric dehydroxy linaloxides, lavender lactone, the isomeric lilacaldehydes, dill ether, the four isomers of 1-*p*-menthen-9-al, methyl anthranilate and nerolidol. The fact that some of these compounds are present in other honeys may be due to contribution of citrus nectar to their production. In any case, the amounts found in citrus honeys are normally one to two orders of magnitude higher than other honeys. Concerning 1,8-menthadien-4-ol, limonene-10-ol and methyl *N*-methylanthranilate, they cannot be considered as potent markers as they were not detected in all of the samples. Of the aforementioned components, five are reported as honey constituents for the first time, that is the two isomeric dehydroxy linaloxides, 1,8-menthadien-4-ol, limonene-10-ol and methyl *N*-methylanthranilate. Discrimination between Greek and Italian samples could not be established as no significant differences were observed.

Lilac aldehydes have been reported as characteristic compounds of New Zealand nodding thistle honey (Wilkins, Lu, & Tan, 1993) and Greek citrus honey (Alissandrakis et al., 2003). Another linalool derivative, (*E*)-8-hydroxylinalool, was the major constituent in both cases. The isolation procedures were different than the one presented in this work; liquid–liquid extraction in the first and ultrasound-assisted extraction in the second case. Methyl anthranilate is a known constituent of citrus honey and has for long been a floral marker for this type of honey (White, 1966; White & Bryant, 1996). Its methylated derivative, methyl *N*-methylanthranilate, was detected in some of the samples, mainly those from the region of Chania. This compound is characteristic of mandarin peel essential oil (Faulhaber, Hener, & Mosandl, 1997). 1-*p*-Menthen-9-al has been reported before in haze honey (Shimoda, Wu, & Osajima, 1996) and in different types of honey from Sicily (Verzera et al., 2001). In this last work, 1,3,8-*p*-menthatriene was traced in orange honey only. Dill ether is one of the primary odorants of dill herb (Blank, Sen, & Grosch, 1992). In honey, it was detected in the extract of linden honey (Blank, Fischer, & Grosch, 1989). Finally, lavender lactone (Shimoda et al., 1996) and nerolidol (Piasenzotto et al., 2003; Overton & Manura, 1994) have been cited as honey constituents.

Some works have been conducted using the SPME procedure to isolate the volatiles of citrus honey. Lilac aldehydes (Fuente de la et al., 2005; Perez et al., 2002; Soria et al., 2003) and methyl anthranilate (Fuente de la et al., 2005; Soria et al., 2003) are mentioned as characteristic of citrus honey produced in Spain. Hotrienol is reported as characteristic of citrus honey and methyl anthranilate was found in various honeys (Verzera et al., 2001). Surprisingly, lilac aldehydes were not isolated, even though the same type of fiber with the aforementioned work of Perez et al. was used. In the same work the presence of 1,3,8-*p*-menthatriene in citrus honey only is reported. Finally, lilac

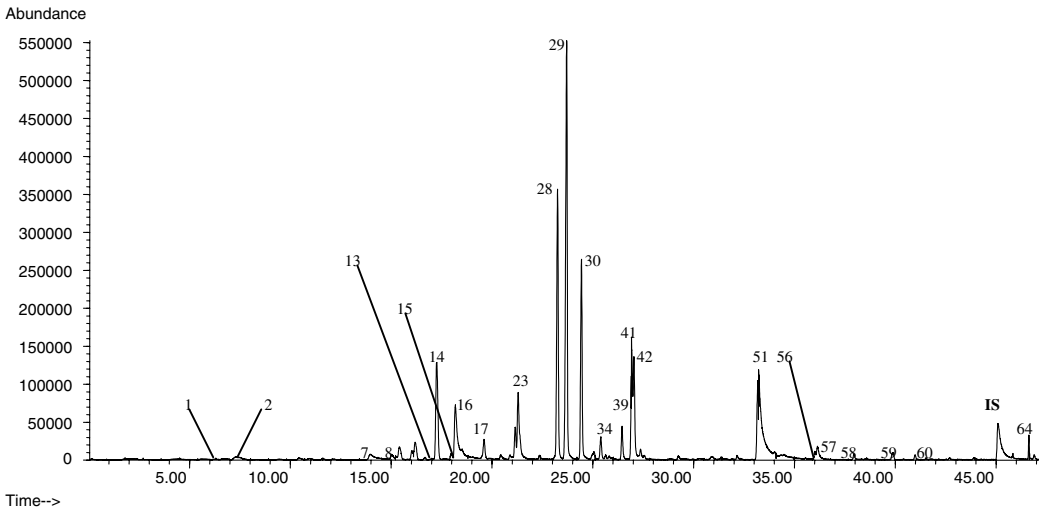

Fig. 1. Representative TIC chromatogram of citrus honey aroma extract. Numbers refer to compounds listed in Table 1.

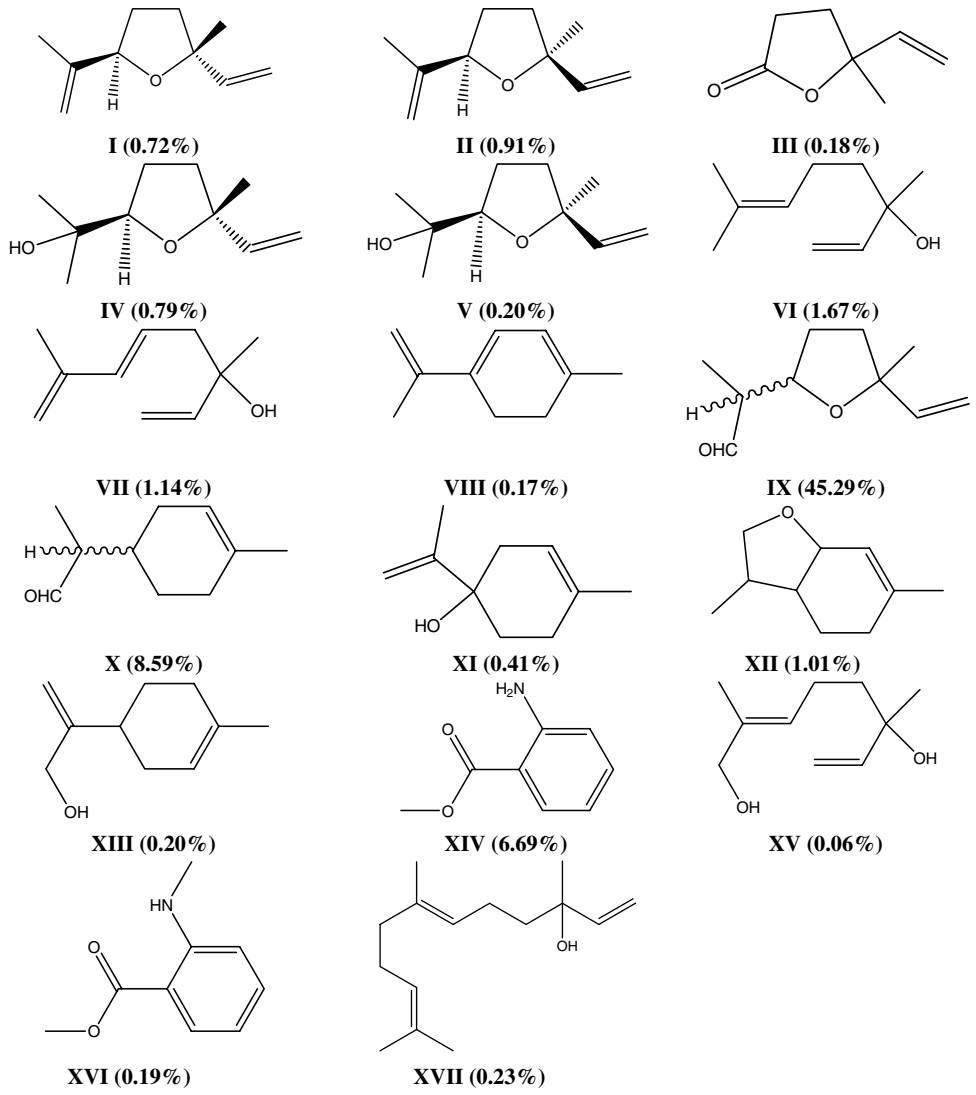

Fig. 2. Structures of the most important compounds mentioned. Numbers in parenthesis represent the percentage among the identified compounds listed in Table 1.

aldehydes, methyl anthranilate and nerolidol have been traced in citrus honey produced in Italy, using a PA fiber (Piasenzotto et al., 2003). The presence of methyl anthranilate (Verzera et al., 2001) and lilac aldehydes (Piasenzotto et al., 2003) in honeys of botanical origin other than citrus could be, to our valuation, due to contribution of citrus blossoms to their production.

3.3. Origin of the isolated compounds

Linalool has been found to be the major compound in the extract of orange, tangerine and sour tree blossoms (Alissandrakis et al., 2003). Additionally, we recently found limonene, α-terpineol, (*E*)-8-hydroxylinalool, methyl anthranilate and nerolidol in blossom extracts from different orange varieties (data not shown), indicating direct transference from nectar to honey.

Starting with linalool an array of compounds are formed, as shown in Figs. 3 and 4. These compounds are either derived by linalool degradation or liberated from glycoconjugate precursors. In literature, such precursors have been isolated for (*E*)-8-hydroxylinalool and furan linaloxides (Strauss, Gooley, Wilson, & Williams, 1997) as well as for 2,6-dimethyl-3,7-octadien-2,6-diol (Strauss et al., 1997; Wintoch, Morales, Duque, & Schreier, 1993). Within the hive, warm (about 30 °C) and acidic (pH of ripe orange honey is 3.5) conditions exist that can either lead to the oxidative degradation of linalool or the breakdown of the glycosidic bonds. Moreover, it is known that nectar contains various amounts of enzymes, as it is also enriched with others by the bees. As a conclusion, various reactions are expected to occur in honey during ripening. Oxidation reactions must be favored, as linalool was found in very low proportions, compared to the amounts present in citrus nectar.

Biogenetic studies in lilac have shown that lilac aldehydes are formed from linalool, via (*E*)-8-hydroxylinalool and (*E*)-8-oxolinalool (Kreck, Püschel, Wüst, & Mosandl,

Table 2
Characteristic compounds of citrus honey with respect to other unifloral Greek honeys

No	Compound	Or[a]	Th	Ct	Ht	Ch	Eu	Js	St	Pn	Fr
9	Dehydroxy-*trans*-linaloxide[b]	0.17[c]	–	–	–	–	–	–	–	–	–
12	Dehydroxy-*cis*-linaloxide[b]	0.22	–	–	–	–	–	–	–	–	–
15	Lavander lactone[d]	0.04	–	–	–	–	–	–	–	–	–
27	1,3,8-*p*-Menthatriene[e]	0.04	–	–	–	–	–	–	–	–	–
28	Lilacaldehyde (isomer I)[d,f,g,h,i]	3.12	0.04	0.03	–	–	–	–	–	–	0.13
29	Lilacaldehyde (isomer II)[d,f,g,h,i]	5.20	0.06	0.05	–	–	0.33	0.04	–	–	0.23
30	Lilacaldehyde (isomer III)[d,f,g,h,i]	2.45	0.04	0.02	–	–	–	–	–	–	0.15
31	1-*p*-Menthen-9-al[d,e]	0.08	–	–	–	–	–	–	–	–	–
33	1,8-Menthadien-4-ol[b]	0.10	–	–	–	–	–	–	–	–	–
34	Dill ether[j]	0.24	–	–	–	–	–	–	–	–	0.02
40	1-*p*-Menthen-9-al[d,e]	0.02	–	–	–	–	–	–	–	–	–
41	1-*p*-Menthen-9-al[d,e]	0.99	0.01	–	–	–	–	0.03	–	0.01	0.05
42	1-*p*-Menthen-9-al[d,e]	0.96	0.01	–	–	–	–	0.02	–	0.01	0.04
47	Limonen-10-ol[b]	0.05	–	–	–	–	–	–	–	–	–
51	Methyl anthranilate[e,h,i]	1.59	–	–	–	–	–	–	–	–	–
52	(*E*)-8-hydroxylinalool[f,g]	0.01	–	–	–	–	–	–	–	–	–
57	Methyl-*n*-methyl-anthranilate[b]	0.04	–	–	–	–	–	–	–	–	–
61	Nerolidol[i,k]	0.05	–	–	–	–	–	–	–	–	–

[a] Or, orange; Th, thyme; Ct, cotton; Ht, heather; Ch, chestnut; Eu, eucalyptus; Js, Jerusalem sage; St, strawberry tree; Pn, pine tree; Fr, fir.
[b] Reported as honey constituents for the first time.
[c] Values refer to internal standard calibration.
[d] See Piasenzotto et al. (2003).
[e] See Blank et al. (1992).
[f] See Kreck et al. (2003).
[g] See Faulhaber et al. (1997).
[h] See Bonnländer and Winterhalter (2000).
[i] See Elmore et al. (2000).
[j] See Rowland et al. (1995).
[k] See Sala et al. (2000).

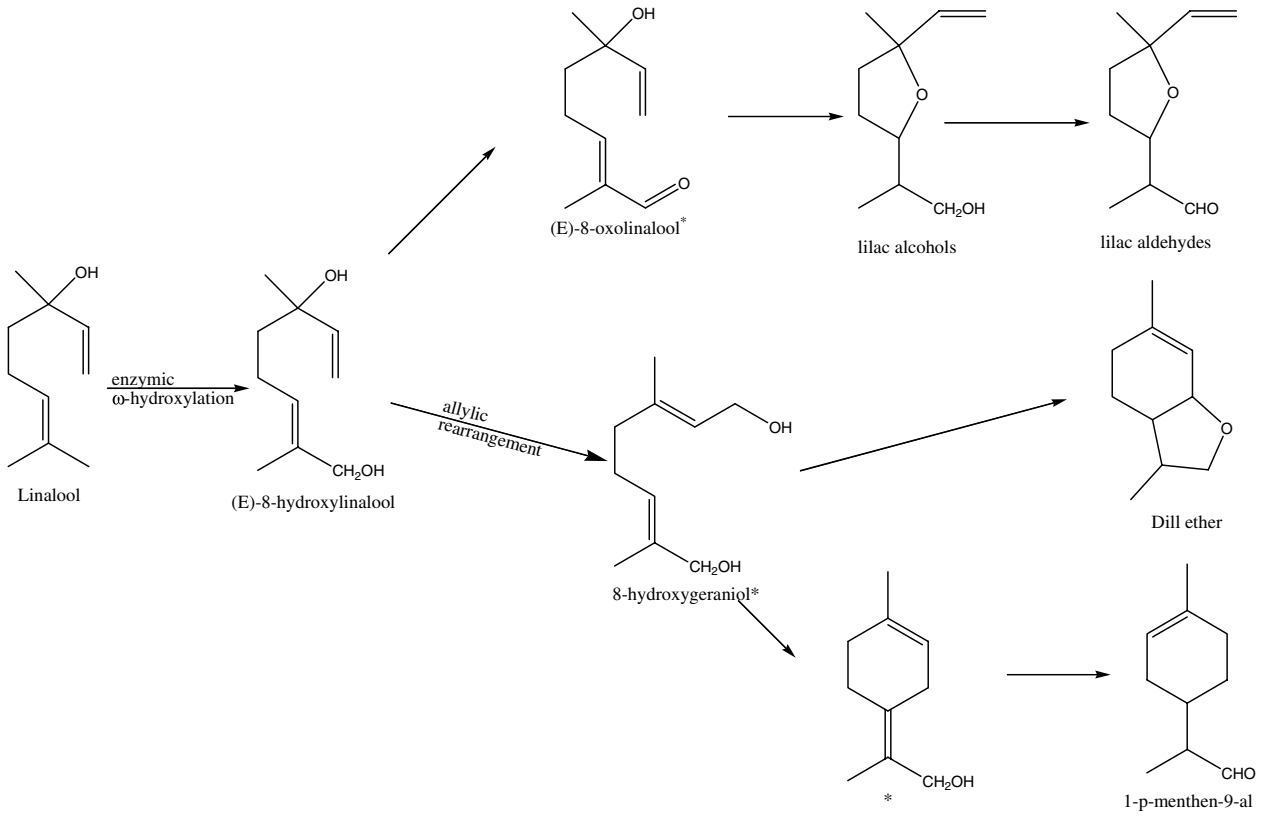

Fig. 3. Formation of lilac aldehydes, dill ether and 1-*p*-menthen-9-als. The asterisk indicates a compound not detected in this work.

2003). Also, they can be produced starting with linalool acetate (Wilkins et al., 1993). In both cases, it is proposed that lilac aldehydes are reduced to give the corresponding alcohols. However, considering the acidic nature of honey, reduction reactions are not favored. It is then plausible to assume that (*E*)-8-hydroxylinalool is isomerised to lilac alcohols that undergo oxidation to give lilac aldehydes (Fig. 3). Dill ether and 1-*p*-menthen-9-als are produced by (*E*)-8-hydroxylinalool and via the allylic rearranged 8-hydroxygeraniol (Bonnländer & Winterhalter, 2000) (Fig. 3).

Direct hydroxylation of linalool at the C_8 position forms the two isomers of 8-hydroxylinalool which can give various products. Alternatively, epoxidation of linalool gives 6,7-epoxylinalool, which undergoes further oxidation with several products (Williams et al., 1980; Winterhalter et al., 1986; Wüst and Mosandl, 1999) (Fig. 4). This epoxy derivative has been isolated from *Carica papaya* fruit (Winterhalter et al., 1986). A certain enzyme seems to be responsible for the formation of the diols 2,6-dimethyl-3,7-octadiene-2,6-diol and 3,7-dimethyl-1,7-octadiene-3,6-diol (Winterhalter et al., 1986), while acidic conditions (Williams et al., 1980a) or heating (Riberéau-Gayon, Boidron, & Terrier, 1975) issue the two furan linalool oxides.

Hotrienol is a known thermally generated product (Alissandrakis et al., 2003; Rowland, Blackman, D' Arcy, & Rintoul, 1995; Williams et al., 1980b; Wilson, Strauss, & Williams, 1984), arising after the thermal degradation of either 2,6-dimethyl-3,7-octadiene-2,6-diol (Rowland et al., 1995) or its glycoconjugate form (Wintoch et al., 1993) or the allylic rearranged 3,7-dimethyl-1,7-octadiene-3,6-diol (Wintoch et al., 1993) (Fig. 5). It is also possible to arise from (*E*)- and (Z)-8-hydroxylinalool. Nevertheless, some quantity seems to exist in non-thermally treated honey. Rowland et al. (1995) detected much lower proportions in unripe than in ripe honey and suggested that hotrienol is probably formed during honey ripening. Even though

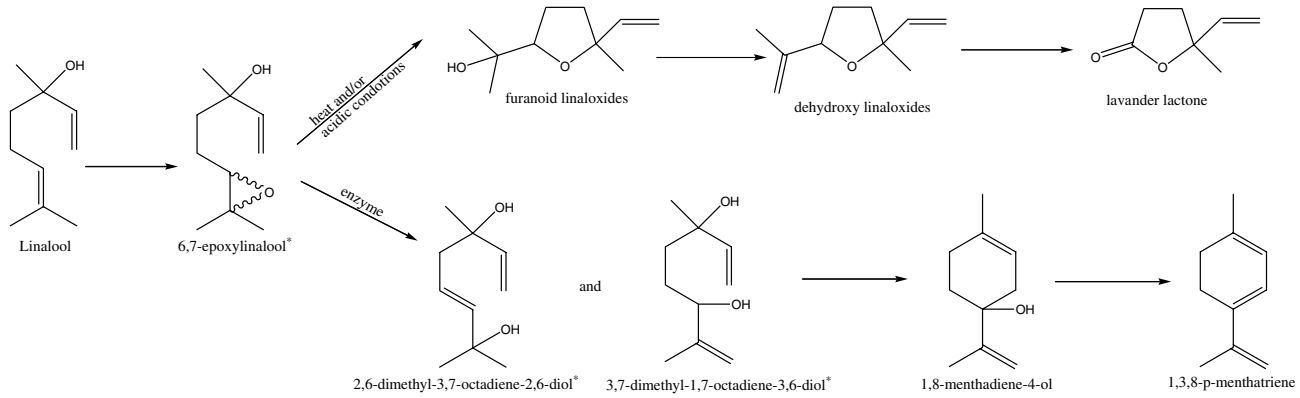

Fig. 4. Formation of furanoid linaloxides, dehydroxy linaloxides, lavender lactone, 1,8-menthadiene-4-ol and 1,3,8-*p*-menthatriene. The asterisk indicates a compound not detected in this work.

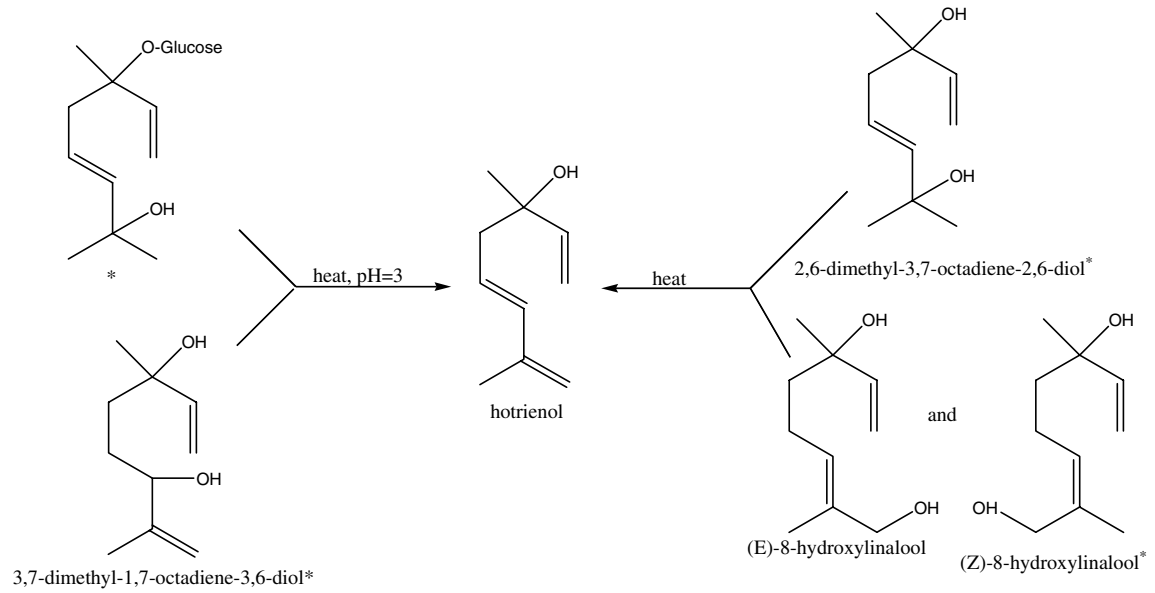

Fig. 5. Formation of hotrienol. The asterisk indicates a compound not detected in this work.

2,6-dimethyl-3,7-octadiene-2,6-diol was not isolated using SPME, it is a known constituent of Greek citrus honey (Alissandrakis et al., 2003).

4. Conclusion

The SPME/GC–MS analysis of the headspace aroma of Greek citrus honey is an effective tool to differentiate this kind of honey from others. Lilac aldehydes are the most powerful markers, followed by dill ether, methyl anthranilate and the 3rd and 4th isomers of 1-*p*-menthen-9-al. Moreover, the SPME methodology can give satisfactory results in certain cases, concerning the floral discrimination of unifloral citrus honeys. No significant differences were observed between Greek and Italian samples, and thus geographical discrimination could not be established. The procedure is easy to perform, fast and non-expensive. The fiber used is able to extract compounds with relatively wide range of polarities and volatilities. Finally, the compounds identified can be connected with each other, as well as with those of the extracts from orange blossoms. Of the compounds identified, limonene, α-terpineol, (*E*)-8-hydroxylinalool, methyl anthranilate and nerolidol have been also found in extracts from orange blossoms, indicating direct transferral from nectar to honey. Lilac aldehydes have been shown to form from linalool (the major terpene in orange blossom extract), and so have (*E*)-8-hydroxylinalool, furan linaloxides, dehydroxy linaloxides and lavender lactone. Dill ether and the 1-*p*-menthen-9-al isomers can be produced by (*E*)-8-hydroxylinalool, while the same can be assumed for hotrienol.

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