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Contribution of dynamic headspace GC–MS analysis of aroma compounds to authenticity testing of honey

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Abstract

The volatile profiles of 43 authentic honey samples of different botanical and geographical origins were obtained by means of gas chromatography–mass spectrometry. A qualitative analysis of the volatile compounds identified was performed in order to assess the marker compounds (if/when existing) for both botanical and geographical origin. The results seem to indicate the existence of certain marker compounds for the floral origins assessed (e.g. acacia, chestnut, eucalyptus, heather, lavender, lime, rape, rosemary and sunflower). Also such compounds for two geographical origins (e.g. Denmark and England) seem to exist and possible marker compounds could also be found for the honeys from The Netherlands, Spain and Portugal.

Keywords: Honey; Aroma compounds; Dynamic headspace; Floral origin

1. Introduction

The composition and the manufacture of honey are regulated by Community Directive 74/409/EEC (OJEC, 1974). In order to harmonise the common European market, the European Commission has adopted a proposal to amend this Directive. According to this amendment, the name 'honey' has now to be supplemented by information referring to the product's floral and geographical origin.

Traditionally, the determination of the floral and geographical origin of honey is achieved by analysis of the pollen (mellisopalynology) present in honey (Maurizio, 1975; Sawyer, 1975). This method is based on the identification of pollen by microscopic examination. It requires a very experienced analyst, it is very time consuming and dependent on the expert's ability and judgement (Howells, 1969). Other methods that could be more widely used for characterising honeys have been sought for many years.

The aroma profile is one of the most typical features of a food product, for both organoleptic quality and authenticity (Careri, Mangia, Barbieri, Bolzoni, Virgili & Parolari, 1993; Virgili et al., 1994). Volatile substances are the main factors responsible for aroma which, together with other factors such as taste and physical factors, contribute to the flavour. This is particularly true for a flavour-rich product such as honey. Owing to the high number of volatile components, the aroma profile represents a "fingerprint" of the product, which could be used to determine its origin. It has already been pointed out that a careful analysis of the volatiles in honey could be a useful tool for characterisation of its botanical origin (Overton & Manura, 1994). While reporting the role of volatile compounds in assessing honey's floral origin, some authors actually suggest certain specific compounds as being characteristic for the honeys from a specific floral source (Bonaga, Giumanini & Gliozzi, 1986; Serra Bonvehi,

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1988). Most of them report generally about groups of compounds being indicative while not actually specifying the volatile itself (Bonaga & Giumanini, 1986; Bouseta, Collin & Dufour, 1992; Tan, Holland, Wilkins & Molan, 1988, Tan, Wilkins, Holland & McGhie, 1989, 1990). Although the existence of such compounds in assessing the origin of honeys is extremely advantageous, the present knowledge on this subject is rather limited. Moreover, the determination of the geographical origin, on the basis of volatile compounds, did not receive any attention in the past. For this reason, honey samples covering a wider range of different botanical and geographical origins were analysed. Furthermore, a simple qualitative evaluation of obtained data was performed in order to assess the marker compound (if/when existing) for either floral or geographical origin.

2. Materials and methods

2.1. Honey samples

Authentic honey samples (43) were obtained from various hive sites in different Member States. Standard pollen analysis (Louveaux, Maurizio & Vorwohl, 1978) was performed on all honey samples in order to confirm their floral authenticity. The variety of unifloral samples was limited by the collection scheme. Honey samples were of nine different botanical origins (seven acacia, nine chestnut, three eucalyptus, eight heather, two lavender, four lime, four rape, two rosemary and four sunflower) and from eight different countries (one from Denmark, 10 from Germany, 13 from Italy, eight from France, four from The Netherlands, two from Spain, two from Portugal and three from England).

2.2. Honey flavour extraction

Portions of each honey sample (25 g) containing 5 μg of 1-dodecene as an internal standard (added in order to check extraction recoveries and instrumental performances), were placed in a 200-ml Erlenmeyer flask at 45°C. Purified helium (60 ml min^{-1}) was passed through the system for 30 min and the entrained volatiles adsorbed on a Tenax TA trap. The adsorbent was then back-flushed with the purified gas for 5 min to remove trapped moisture. The volatile compounds were subsequently thermally desorbed and transferred to the GC by using a TCT thermal desorption cold trap (TD800, Fisons Instruments, Milan, Italy). Desorption was performed at 280°C for 10 min under a helium flow (10 ml min^{-1}) and the substances were cryofocused in a glass lined tube at −120°C with liquid nitrogen. The volatile components were injected into the GC capillary column by heating the cold trap to 240°C.

The trap used for adsorption (Chrompack, Middelburg, The Netherlands) consisted of glass tubes (16×0.4 cm i.d.) filled with Tenax TA (90 mg, 20–35 mesh) and pre-conditioned at 300°C for 8 h.

2.3. Gas chromatography–mass spectrometry

GC–MS analysis of the honey headspace was carried out using a Fisons system consisting of a GC8000 gas chromatograph, a MD800 quadrupole mass spectrometer and a Fox computer. The interface and the source temperature were 230°C and 190°C, respectively. Electron impact mass spectra were recorded in the 35–250 amu range at 70 eV ionization energy.

Separation was performed on a fused-silica bonded-phase capillary column DB-WAX (J. & W. Scientific, Folsom, CA, USA) (30 m×0.25 mm, d.f. = 0.25 μm). The temperature program was isothermal at 30°C for 8 min, then raised to 60°C at 4°C min^{-1}, to 160°C at 6°C min^{-1} and to 200°C at 20°C min^{-1}; this temperature was held for 1 min. Blank analyses were carried out with the same trapping material and following the same procedure, starting from distilled water as the sample.

2.4. Data analysis and data evaluation

The identification of the isolated volatile compounds was achieved through retention times, (Kovàts retention indices; KI) and mass spectrometry by comparing mass spectra of unknown peaks with those stored in the National Institute of Standards and Technology, (NIST), US Government library and, where possible, were confirmed by comparison with authentic substances used as references. Kovàts retention indices were determined by injection of a solution containing the homologous series of normal alkanes (C_8–C_{28}) in a temperature-programmed run, as described above, and these values were compared with those reported in the literature (Careri, Mazzoleni, Musci & Molteni, 1999 and references therein).

Volatile compounds identified in the volatile fraction from each honey sample analysed (Table 1) were then used for the qualitative data evaluation. The evaluation was performed according to the following pattern. For each sample, a value of 1 was attributed if a compound was detected and 0 if absent. The probability of the presence of each compound in each sample analysed was then calculated, first versus honey groups of identical botanical origin and second versus honey groups of identical geographical origin. Marker compounds for both botanical and for geographical origin were then highlighted where appropriate (Tables 2 and 3). A compound was considered as a marker only in cases where its presence/absence was confirmed in all samples from the same floral/geographical origin analysed.

Table 1
Volatile compounds identified in 43 unifloral honeys analysed

No.	Compound	RT	KI calc.	ID[a]	Occurrence[b]								
					Ch	He	Eu	Lim	Rs	Sun	Lav	Ros	Ac
1	acetone	2.97	808	MS,RT	9	8	3	4	4	4	2	2	7
2	1-octene	3.32	833	MS,RT			3						
3	ethyl acetate	4.09	892	MS,RT, KI	8	7				2	1		5
4	2-methylfuran	4.17	898	MS				4					
5	2-butanone	4.32	904	MS,RT	9	8	3	4	4	4	2	2	7
6	2-methylbutanal	4.59	909	MS,KI	9	8	3	4	3	4	2	2	7
7	3-methylbutanal	4.64	915	MS,RT, KI	9	8	3	4	3	4	2	2	7
8	3-methyl-2-butanone	5.01	918	MS				4					
9	2-propanol	5.26	935	MS,RT	9	8	3	4	4	4	2	2	7
10	ethanol	5.54	944	MS	9	8	3	4	4	4	2	2	7
11	2-ethylfuran	5.67	949	MS,RT		8							
12	pentanal	6.77	964	MS,RT, KI	9	8		4	4		2		7
13	2-pentanone	6.94	970	MS,RT,KI	9	8	3	4	4	4	2	2	7
14	N.I.[c] (m/z 55, 67, 85, 97)	7.48	994	MS							1		
15	2-methylpropanenitrile	7.67	1009	MS					1				
16	α-pinene	8.61	1029	MS,RT,KI						4			
17	chloroform	9.07	1038	MS, RT	9	8	3	4	4	4	2	2	7
18	short chain carbonilic compound	10.1	1067	MS			1						
19	a methylbutenol	10.6	1069	MS	8	7	3	2	2	4	2	2	7
20	2,3-pentanedione	11.2	1083	MS			3						
21	dimethyl disulphide	11.4	1086	MS, KI	9	8	3	1	4				
22	hexanal	12	1099	MS,RT, KI	9	8	3	4	4	4	2	2	7
23	methyl-2-butenale	12.4	1107	MS	8	1	3	1	1	4	2	1	7
24	2-methyl-1-propanol	13.2	1124	MS,RT, KI	9	8	3	4		4	2	2	6
25	N.I. (m/z 91, 119)	13.6	1132	MS				4					
26	N.I. (55, 83, 98)	14.4	1147	MS							1		
27	3-methyl-2-butanol	14.7	1153	MS						4			
28	2-pentanol	14.7	1153	MS		1					1		
29	1-butanol	15.7	1173	MS,RT, KI	9	8	3	4	4	4	2	2	7
30	α-terpinene	16.4	1187	MS,RT, KI				4					
31	1-penten-3-ol	16.4	1187	MS		3							
32	2-heptanone	16.9	1198	MS,RT, KI			2						
33	heptanal	17	1200	MS,RT, KI	9		3	4	4		2	2	7
34	2,3-dihydro-4-methylfurane	17.4	1212	MS	9	8	3	4	4	4	2	2	7
35	a terpene (m/z 91,119,134)	18	1229	MS				4					
36	α-pinene oxide	18.2	1236	MS				4					
37	3-methyl-1-butanol	18.3	1237	MS,RT, KI	9	8	3		4	4	2	2	7
38	a cyclic ether	18.8	1251	MS			3						
39	a terpene (m/z 93,121,136)	19.2	1263	MS				4					
40	bicyclo 3,2,1-octane-2,3-bis(methylene)	19.2	1265	MS				4					
41	3-methyl-3-buten-1-ol	19.6	1277	MS,RT, KI	9	8	3	4	4	4	2	2	7
42	1-pentanol	19.8	1280	MS,RT, KI		8					1		
43	2-methyldihydrofuranone	19.9	1284	MS	9								
44	methyl isopropyl benzene	20	1287	MS				4					
45	3-hydroxy-2-butanone (acetoin)	20.6	1304	MS,RT, KI	9	8	3	4	4	4	2	2	7
46	octanal	20.8	1312	MS,RT, KI	9	8		4	4	4	2	2	7
47	an aromatic hydrocarbon	20.9	1315	MS				4					
48	hydroxyacetone (acetol)	21	1321	MS,RT, KI	9	8	3	4	4	4	2		7
49	2,2,6-trimethylcyclohexanone	21.5	1335	MS							1		
50	1-pentanol-4-methyl	21.8	1347	MS							1		
51	N.I. (m/z 91, 105)	21.8	1348	MS				2					
52	methyl-2-butenol	21.9	1353	MS	9	8	2	3	4	4	2	2	7
53	5-hepten-2-one-6-methyl	22.3	1365	MS,RT, KI	4	3			1		1	2	2
54	3-hydroxy-2-pentanone	22.4	1368	MS			1					1	
55	ethyl-2-hydroxypropanoate	22.5	1372	MS							1		
56	1-hexanol	22.9	1386	MS,RT, KI		2		1			1		

(*continued on next page*)

Table 1 (*continued*)

No.	Compound	RT	KI calc.	ID[a]	Occurrence[b]								
					Ch	He	Eu	Lim	Rs	Sun	Lav	Ros	Ac
57	2-hydroxy-3-pentanone	22.9	1386	MS			3					1	
58	1-octen-3-ol	23.3	1399	MS			1						
59	3-hexenylformate	23.7	1416	MS							1		
60	3-hexen-1-ol	23.7	1417	MS,RT	9	5							
61	nonanal	23.9	1423	MS,RT, KI	9	8	3	4	4	4	2	2	7
62	2-butoxyethanol	24.1	1435	MS							1		
63	1,3,8-menthatriene	24.6	1453	MS				1					
64	N.I (m/z 67, 85, 110)	24.8	1460	MS				2					
65	N.I. (m/z 43, 71, 114)	24.8	1460	MS			3						
66	2-cyclohexene-1-one	24.8	1462	MS				1					
67	dimethylstyrene	24.9	1465	MS	9			4					
68	5-methyl-1-hexanol	24.9	1466	MS							1		
69	N.I. (m/z 43, 55, 73, 114)	24.9	1469	MS			3						
70	*cis*-linalool oxide	25.2	1477	MS	9	8			1	4	2		7
71	N.I. (m/z 43, 57, 85)	25.3	1482	MS			3						
72	furfural	25.6	1493	MS	9	8	3	4	4	4	2	2	7
73	N.I. (m/z 57, 69, 85)	25.9	1508	MS			3						
74	decanal	26.5	1533	MS,RT,KI	9	8	3	4	4	4	2	2	6
75	2-acetylfuran	26.6	1538	MS	9	8	3	4	4	4	2		7
76	benzaldehyde	27	1555	MS,RT, KI	9		3	4	4	4	2		7
77	3,7-dimethyl-1,6-octadien-3-ol (linalool)	27.6	1583	MS	2		1	4			1		4
78	byciclo 2,2,2-octan-1-ol-4-methyl	27.8	1590	MS		7							
79	3-cyclohexen-1-ol-5-methylene-6-isopropylene	28	1597	MS				4					
80	5-methylfurfural	28.2	1608	MS	9	3	3			4			
81	cyclopentendione	28.4	1620	MS	4	4					2		
82	2-methylpropanoic acid	28.5	1625	MS	1								
83	2-cyclohexen-1-one-3,5,5-trimethyl	28.7	1632	MS		8	1				1		
84	3-methyl-1-undecene	28.8	1638	MS	1			2					
85	1,5,7-octatrien-3ol-3,7-dimethyl	29	1648	MS	3	1	3	4	4	4	2		5
86	2(3H)-furanonedihydro-5-methyl	29	1648	MS	2	1							
87	γ-butirrolactone	29.4	1666	MS	2	8	1			4			
88	2-allyl-4-methylphenol	29.4	1668	MS				2					
89	4-acetyl-1-methylcyclohexene	29.5	1672	MS				1					
90	phenylacethaldehyde	29.7	1680	MS		8	3				2		
91	acetophenone	29.9	1689	MS	9	8		4	4		1	2	5
92	furfuryl alcohol	30.1	1698	MS	8	6	3	4		4	1		
93	N.I. (m/z 70, 154)	30.2	1704	MS		8							
94	Ethylbenzoate	30.2	1706	MS		5							
95	N.I. (m/z 56, 85, 125)	30.3	1709	MS		4							
96	2- + 3-methylbutanoic acid	30.7	1730	MS	9	8			3				
97	4-oxoisophorone	30.8	1733	MS	9	8	3	4	4			2	5
98	N.I. (m/z 60, 68, 96, 152)	30.8	1734	MS							1		
99	N.I. (m/z 95, 145)	31	1747	MS	2			4					
100	2,4-cycloheptadien-1-one-2,6,6-trimethyl	31.6	1775	MS		8	3					1	
101	N.I. (m/z 67, 91, 110)	31.6	1776	MS		8							
102	4-methyl acetophenone	31.7	1778	MS				4					
103	2-methyl acetophenone	32.4		MS				1					
104	1,4-cyclohexanedione-2,6,6-trimethyl	32.5		MS	6								
105	α-methylpropyl phenyl acetate	32.6		MS		1		4	1				
106	α-methylbenzyl alcohol (sec-phenethyl alcohol)	33.1		MS	9								
107	4-ethylphenylacetate	33.6		MS		7							
108	benzyl alcohol	33.9		MS	9	7	3	4	4	4	2	2	5
109	3-aminoacetophenone	34.3		MS		8	3						
110	phenylethyl alcohol	34.4		MS	5	8	1	2	4	4	2		4

[a] Method of identification: MS, identification by comparison with mass spectrum stored in NIST library. RT, identification by comparison with retention time of authentic reference compounds. KI, identification by comparison with literature data.

[b] Botanical origin: Ch, Chestnut (nine samples). He, Heather (eight samples). Li, Lime (four samples). Sun, Sunflower (four samples). Eu, Eucalyptus (three samples). Rs, Rape (four samples). Lav, Lavender (two samples). Ros, Rosemary (two samples). Ac, Acacia (seven samples).

[c] N.I., not identified.

Table 2
Marker compounds typical for certain floral and geographical origins

Botanical/ geographical origin	Marker compound	
	Presence of	Absence of
Acacia	Both *cis*-linalool oxide and heptanal	Both phenylacetaldehyde and dimethyl disulphide
Chestnut	2-methyldihydrofuranone or α-methylbenzyl alcohol or both 3-hexen-1-ol and dimethylstyrene	–
Eucalyptus	1-octene or 2, 3-pentanedione or a-cyclic ether or N.I.[a] (*m*/*z* 43, 55, 73, 114) or N.I.[a] (*m*/*z* 43, 71, 114) or N.I.[a] (*m*/*z* 43, 57, 85) or N.I.[a] (*m*/*z* 57, 69, 85)	–
Heather	N.I.[a] (*m*/*z* 70, 154) or N.I.[a] (*m*/*z* 67, 91, 110) or byciclo-2,2,2-octan-1-ol-4-methyl or 4-ethylphenyl acetate	–
Lime	2 methylfuran or N.I.[a] (*m*/*z* 91, 119) or α-terpinene or α-pinene oxide or α-terpene (*m*/*z* 91, 119, 134) or terpene (m/z 93,121,136) or bicyclo-3,2,1-octane-2,3 bis (methylene) or methyl isopropyl benzene or an aromatic hydrocarbon or 3-cyclohexen-1-ol-5-methylene-6-isopropylene or 4-methylacetophenone	3-methyl-1-butanol
Lavender	Heptanal	4-oxoisophorone
Rape	Dimethyl disulphide	2-methyl-1-propanol
Rosemary	–	2-acetylfuran
Sunflower	α-pinene or 3-methyl-2-butanol	Both heptanal and 4-oxoisophorone
Denmark	–	3-methylbutanal
England	1-penten-3-ol	–

[a] N.I., not identified (characteristic fragment ions in parenthesis).

Table 3
Possible marker compounds for certain geographical origins

Geographical origin	Possible marker compound	
	Presence of	Absence of
The Netherlands	N.I.[a] (*m*/*z* 91, 105) or N.I. (m/z 67, 85, 110)	–
Portugal	N.I.[a] (*m*/*z* 55, 67, 85, 97) or 2,2,6-trimethylcyclohexanone or ethyl-2-hydroxypropanoate or 3-hexenylformate or N.I. (*m*/*z* 60, 68, 96, 152)	–
Spain	1-octen-3-ol or 2,4-cycloheptadien-1-one-2,6,6-trimethyl	Pentanal or *cis* linalool oxide

[a] N.I., not identified (characteristic fragment ions in parenthesis)

3. Results and discussion

3.1. Characteristic aroma profiles and compounds of honeys

In this section, characteristic aroma profiles of honeys analysed will be described and the marker compounds resulting from qualitative data evaluation will be given. In cases where the figures are superfluous to the described data, they are not given. The marker compounds and the possible marker compounds identified are summarised in Tables 1 and 2 respectively.

In general, linear and branched aldehydes, ketones, short-chain alcohols were found in most or all of the honeys analysed. Seven acacia honey samples (France 1, Germany 2 and Italy 4) were analysed by means of GC–MS. Acetone, furfural and benzaldehyde were the principal aroma components detected by headspace analysis. 6-

Methyl-5-hepten-2-one was detected in two Italian samples; 3,7-dimethyl-1,6-octadien-3-ol (linalool) occurred in one honey sample from Germany, in two Italian samples and in one French sample. It is to be noticed that dimethyl disulphide, which was detected in most samples of other floral sources, was not found in any of the seven acacia honeys analysed.

According to the qualitative data analysis performed, acacia honeys could be identified (Fig. 1) if the presence of *cis*-linalooloxide and heptanal and the absence of dimethyl disulphide and phenylacetaldehyde in a honey sample can be confirmed. As can be seen in this figure, the presence of *cis*-linalooloxide excludes the presence of eucalyptus, lime, and rosemary honeys while the presence of heptanal rules out sunflower and heather floral origins. Further, the absence of dimethyl disulphide eliminates chestnut, and rape honeys and the absence of phenylacetaldehyde excludes lavender honeys.

Nine unifloral chestnut honeys from three European countries (two from France; two from Germany and five from Italy) were analysed. Fifty volatile substances were isolated and identified in the samples analysed. A variety of furan-related compounds were detected, i.e. furfural, 5-methylfurfural, 2-acetylfuran, 5-methyl-2(3H) dihydrofuranone, (γ-valerolactone) (pentanoic acid, 4-hydroxy-,lactone), furfuryl alcohol and the dihydroxyfuranone γ-butyrolactone. 5-Methyl-2(3H)dihydrofuranone (γ-valerolactone) (pentanoic acid, 4-hydroxy-, lactone), and γ-butyrolactone were detected only in two chestnut honeys (coming from France and Italy). In addition, 2-methylpropanoic acid was identified only in one Italian sample. Among carbonyl compounds, 6-methyl-5-hepten-2-one, which is derived from the oxidation of carotenoids (Belitz & Grosch, 1987), was found in three Italian samples and in one German sample. 3-Aminoacetophenone, which is reported as possibly specific to chestnut honey (Bonaga & Giumanini, 1986a), was detected in six out of nine chestnut honeys analysed and in most cases it was present in trace amounts only.

Qualitative data evaluation suggested (Fig. 2) that the presence of either 2-methyldihydrofuranone or α-methylbenzyl alcohol or both 3-hexen-1-ol and dimethylstyrene seem to be characteristic for chestnut honeys. While the presence of the first two compounds could confirm the authenticity of this floral origin independently, the presence of both 3-hexen-1-ol and dimethylstyrene seems to be needed in order to exclude the presence of heather and lime honey, respectively.

Three (two from Italy and one from Spain) unifloral eucalyptus honey samples were analysed. The GC–MS analyses revealed that the three samples had a common profile characterised by the presence of fifty volatile compounds. 3-Hydroxy-2-butanone, known as acetoin, 2-pentanone and two not identified compounds with the characteristic fragment ions m/z (43, 57, 85) and *m*/*z* (57, 69, 85), respectively, were the major volatile components of these samples. High concentrations of acetoin and other hydroxy ketones have already been reported to be characteristic of eucalyptus honey (Graddon, Morrison & Smith, 1979). Acetoin was, however, found in all honey samples analysed.

Qualitative data analysis showed that the presence of any of the following volatile organic compounds was characteristic for eucalyptus honeys: 1-octene or 2,3-pentanedione or a cyclic ether. In addition, the presence of either of the following not identified volatile compounds with the characteristic fragment ions listed in parentheses is typical for eucalyptus honeys: *m*/*z* (43-55, 73, 114) or m/z (43, 71, 114) or m/z (43, 57, 85) or *m*/*z* (57, 69, 85).

Eight (three from England, one from France, two from Germany and two from The Netherlands) unifloral heather honeys were analysed and exhibited more

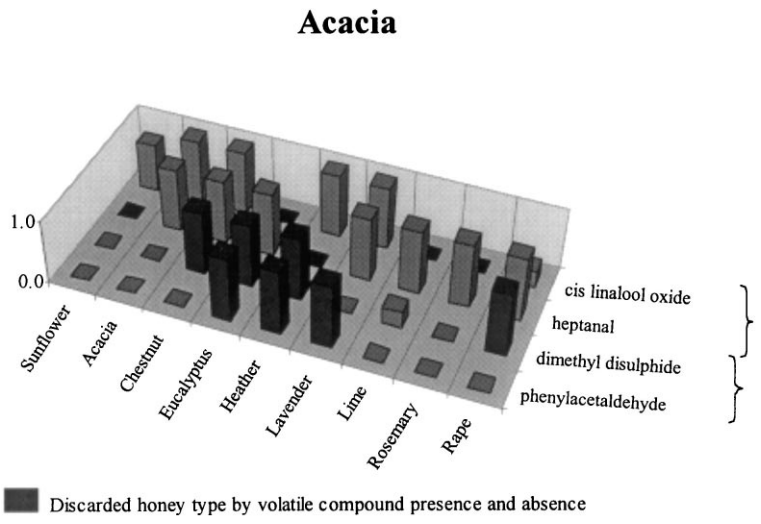

Fig. 1. Marker compounds for acacia honey.

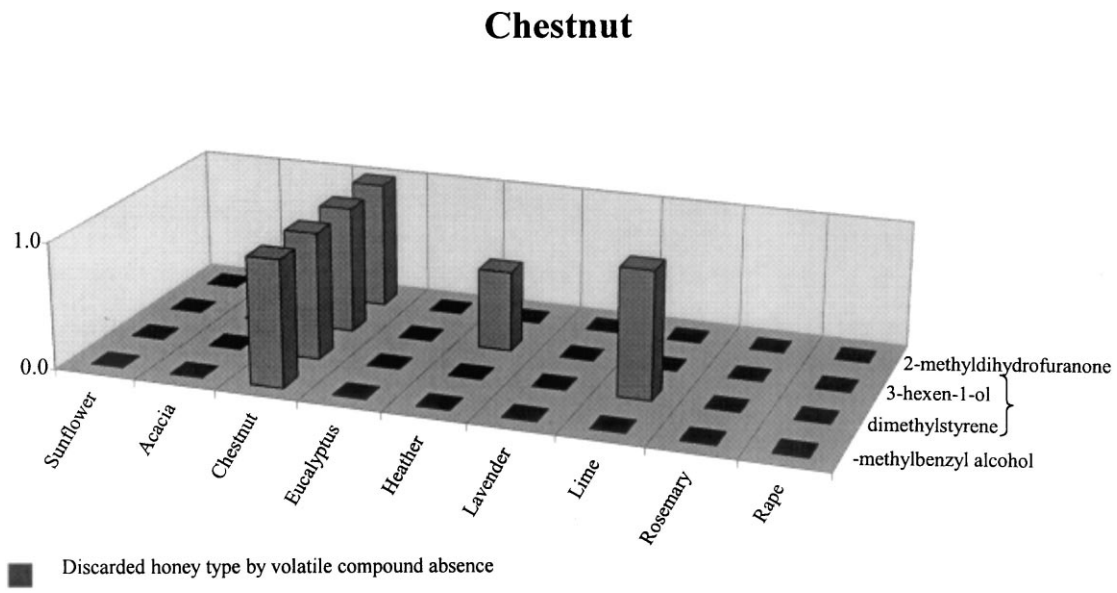

Fig. 2. Marker compounds for chestnut honey.

than 50 peaks in each of their chromatograms, many of these corresponding to alcohols, carbonyl compounds and esters. Among other alcohols, 1-penten-3-ol and bicyclo-2,2,2-octan-1-ol,4-methyl were found only in honeys of this floral source. However, 1-penten-3-ol was found in only three heather samples (from England) analysed. Among carboxylic acids, 2-methylpropanoic acid, which was also found in chestnut honeys, was detected in the German samples, in one Dutch sample and in one sample from England. Although a high concentration of phenyl lactic acid (above 200 mg/kg) (Steeg & Montag, 1987) was reported already as being characteristic of this floral origin, it was not identified in any of the samples analysed. Another characteristic compound in heather samples analysed was phenylacetaldehyde, isolated also from eucalyptus honey samples. Even though Tan et al., (1989) suggested the presence of degraded carotenoids (3,5,5-trimethyl-cyclohex-2-ene derivatives) as being characteristic of heather honey, no such compounds were identified in the samples analysed. The explanation for this could be the different geographical origin of samples analysed; e.g. our study was limited to European honeys only, while the heather honeys analysed by Tan et al. originated from New Zealand.

According to the qualitative analyses performed, the presence of one of two unidentified volatile compounds with the fragment ions: m/z (70, 154) and m/z (67, 91, 110), respectively, seems to be characteristic of heather honeys. In addition, the qualitative data analysis showed that the presence of bicyclo-2,2,2-octan-1-ol-4-methyl and 4-ethylphenyl acetate could be characteristic for this floral origin, as the probability of appearance of these two compounds was rather high (it was identified in seven of eight heather samples analysed).

Several alcohols were identified in the two (one from France and one from Portugal) unifloral lavender honey samples analysed; in particular, ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-methyl-3-buten-1-ol, 3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol) and furfuryl alcohol were the largest GC peaks in the chromatogram of the honey sample from Portugal while hexanal, heptanal, 1-hexanol, furfural, phenylacetaldehyde and benzaldehyde were the principal aroma components detected by headspace analysis in the French lavender sample. Bouseta et al., (1992) pointed out that hexanal and heptanal appeared to be characteristic of this floral source, which we could confirm for the French sample but not for the Portuguese. This could be attributed to the geographical origin, especially since Bouseta et al., (1992) only analysed lavender honeys of French origin.

Qualitative data evaluation showed that the presence of heptanal and the absence of 4-oxoisophorone is characteristic of lavender honeys. As exhibited in Fig. 3, the presence of heptanal rules out sunflower and heather honeys while the absence of 4-oxoisophorone excludes the rest of the floral origins analysed, e.g. acacia, chestnut, eucalyptus, lime, rosemary and rape.

A more complex pattern (more than 60 compounds were detected) was obtained in the case of four (two from Germany and two from The Netherlands) unifloral lime honey samples analysed. 2-Pentanone was one of the major compounds of one German sample; also, other carbonyl compounds, such as acetoin and furfural, contributed to the flavour of this sample. 4-Methylacetophenone was found in all of the four samples analysed; it was identified only in honeys of this floral source. Aromatic hydrocarbons, among which methyl isopropylbenzene and dimethylstyrene, together

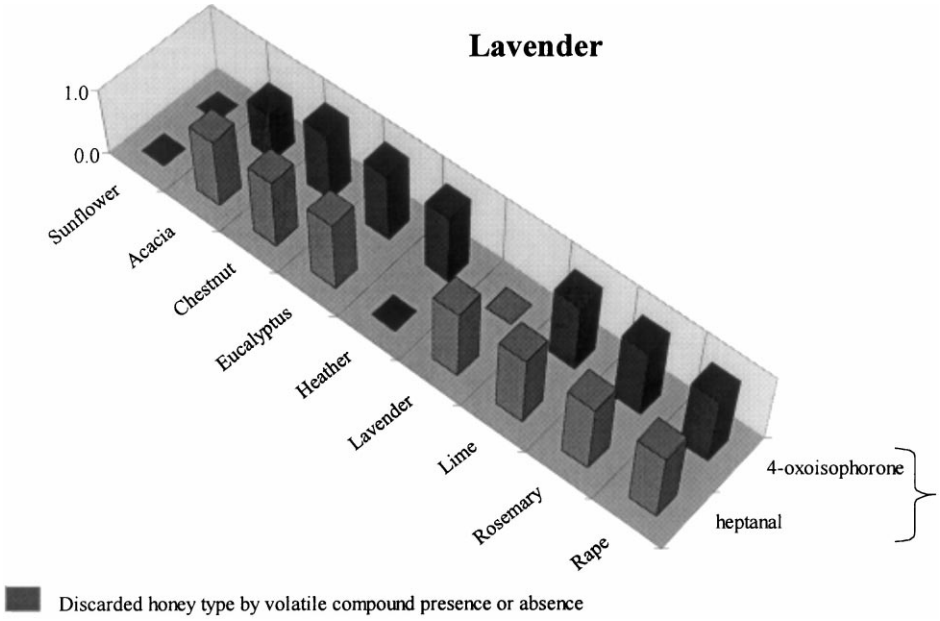

Fig. 3. Marker compounds for lavender honey.

with different terpenes, characterise the volatile fraction of lime honey samples.

According to the qualitative data evaluation, the authenticity of lime honeys could be confirmed if the analysis of the volatile fraction confirms the existence of any of the following compounds: 2 methylfuran or α-terpinene or α-pinene oxide or bicyclo-3,2,1-octane-2,3 bis (methylene) or methyl isopropyl benzene or aromatic hydrocarbon or 3-cyclohexen-1-ol-5 methylene-6-isopropylene or 4-methylacetophenone or α-terpene with the fragment ions m/z 91, 119 and 134 or terpene with the following fragment ions m/z 93, 121 and 136. The presence of an unidentified volatile compound with the fragment ions m/z 91 and 119 is also typical for lime honeys. In addition, the absence of 3-methyl-1-butanol seems to confirm the authenticity of this floral type as well.

Acetone, ethanol, nonanal, benzaldehyde and benzyl alcohol were the largest gas chromatographic peaks in the volatile fraction of the four (one from Denmark, one from France and two from Germany) rape honeys analysed. It is interesting to note that, differing from rape honeys of other geographical origin (e.g. Germany and France), in the Danish sample branched aldehydes (2- and 3-methylbutanal) and branched acids (2- and 3-methylbutanoic acid) were not detected. Methyl-2-butenol and the sesquiterpenoid-related compound, 5-hepten-2-one-6-methyl, were detected only in the French sample, whereas only the Danish sample contains *cis*-linalooloxide.

According to the qualitative data analysis performed, the authenticity of rape honeys could be confirmed by the absence of 2-methyl-1-propanol (Fig. 4). However, as this compound was found to be absent in one of seven acacia samples analysed, the simultaneous presence of dimethyl disulfide is necessary in order to confirm the authenticity of rape honey.

The volatile fraction of the two (one from Portugal and one from Spain) unifloral rosemary honey samples analysed exhibited different profiles. While methyl-2-butenal and 2,4-cycloheptadien-1-one,2,6,6-trimethyl (eucarvone) were found in the Spanish sample, acetoin and furfural were present only in the rosemary sample from Portugal. In general, carbonyl compounds, such as acetone, 2-pentanone, benzaldehyde and 4-oxoisophorone were the most abundant compounds. Analogously to acacia and sunflower honeys, no sulphur compound was found in rosemary honeys analysed.

Qualitative data evaluations performed showed that the absence of 2-acetylfuran is characteristic of rosemary honeys.

Headspace of four (two from France and two from Italy) unifloral sunflower honeys showed them to be mainly characterised by alcohols (e.g. 1-butanol-3-methyl, 3-methyl-3-buten-1-ol, methyl-2-buten-1-ol, benzyl alcohol) and aromatic aldehydes (e.g. benzaldehyde and furfural). Acetoin was one of the principal components of one Italian sample.

According to the qualitative data analysis, α-pinene, 3-methyl-2-butanol, heptanal, and 4-oxoisophorone are marker compounds for sunflower honeys. As shown in Fig. 5, the presence of either α-pinene or 3-methyl-2-butanol alone could confirm the sunflower floral origin. On the other hand, the absence of both heptanal, and 4-oxoisophorone is needed in order to confirm this floral origin.

Qualitative analysis, following the same pattern described in the previous section and applied so far, was performed to characterise the geographical origin of the

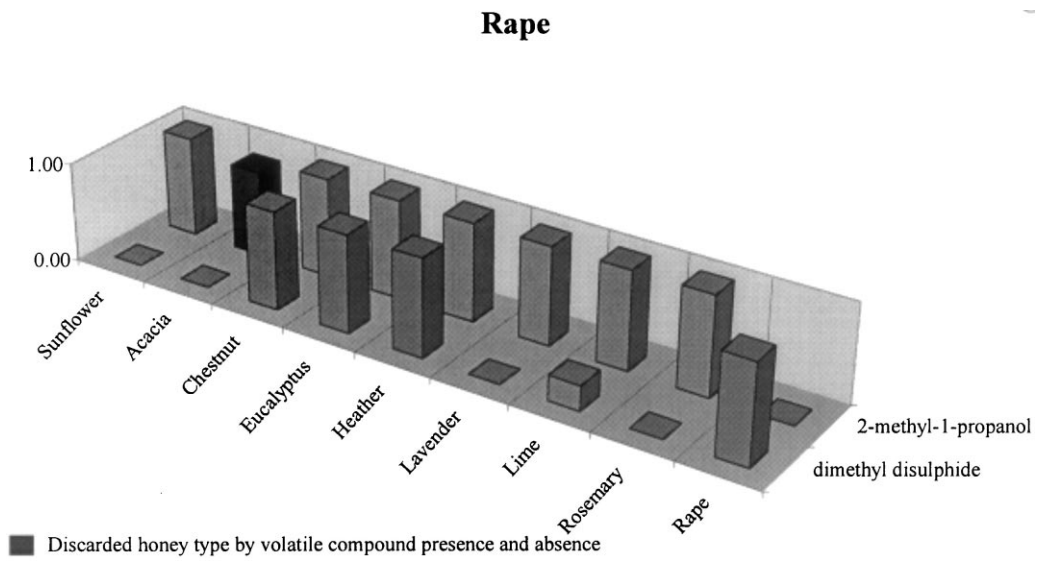

Fig. 4. Marker compounds for rape honey.

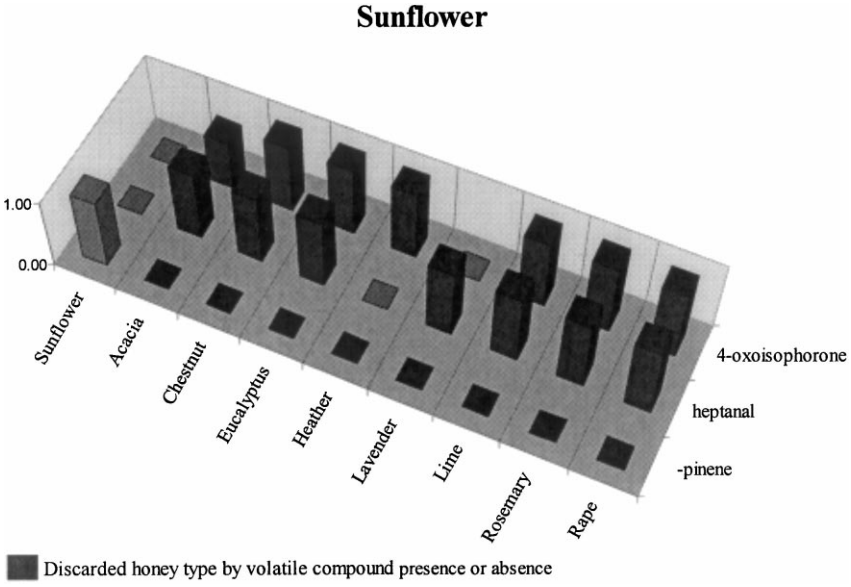

Fig. 5. Marker compounds for sunflower honey.

honeys analysed. According to this analysis, English honeys could be identified if the presence of 1-penten-3-ol in honey samples can be confirmed. Furthermore, it seems that the absence of 3-methylbutanal confirms the authenticity of Danish honeys. However, one should bear in mind that only one Danish honey was analysed.

Table 2 shows that aroma profiles are more suitable for the determination of floral origin than geographical origin. Marker compounds for only two geographical origins could be found. For honeys coming from The Netherlands, Portugal and Spain, marker compounds are possible only as their presence/absence was confirmed, not in all but in at least half of samples analysed. We feel that these possible marker compounds could be useful as a starting point for some future study and they are therefore listed in Table 3.

4. Conclusions

This study suggests that volatile compounds play an important role in assessing the origin of honey. Furthermore, the results seem to indicate the existence of certain marker compounds for the floral origins assessed (e.g. acacia, chestnut, eucalyptus, heather, lavender, lime, rape, rosemary and sunflower). Also such compounds seem to exist for two geographical origins (e.g. Denmark and England) and possible marker compounds

could also be found for the honeys from The Netherlands, Spain and Portugal.

Although the results obtained should be confirmed with a broader set of samples, we believe that they represent a valuable contribution for the determination of nine different floral origins of honey and a possible determination of the geographical origin of two.

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