Food Chemistry 112 (2009) 1022–1030

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Differentiation of monofloral citrus, rosemary, eucalyptus, lavender, thyme and heather honeys based on volatile composition and sensory descriptive analysis

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ARTICLE INFO

Article history:
Received 4 March 2008
Received in revised form 16 May 2008
Accepted 18 June 2008

Keywords:
Honey
Volatile compounds
Gas chromatography
Sensory analysis

ABSTRACT

The volatile profiles of 49 Spanish honey samples of different botanical origins were obtained by means of gas chromatography–mass spectrometry and sensory analysis. Citrus honeys were characterised by higher amounts of linalool derivatives, limonyl alcohol, sinensal isomers, and α-4-dimethyl-3-cyclohexene-1-acetaldehyde, together with fresh fruit and citric aromas; eucalyptus honeys had hydroxyketones (acetoin, 5-hydroxy-2,7-dimethyl-4-octanone), *p*-cymene derivatives, 3-caren-2-ol and spathulenol, cheese and hay aromas; lavender honeys had hexanal, nerolidol oxide, coumarin, important concentrations of hexanol and hotrienol and sensorial attributes, including balsamic and aromatic herb aromas; finally, heather honeys were characterised by high contents of benzene and phenolic compounds and ripe fruit and spicy aromas. Some of these compounds and sensory attributes were only found in honeys from a specific floral source and could thus be of interest for use as markers of their botanical origin.

1. Introduction

Nowadays a current tendency is to define the distinctive character of unifloral honeys in order to obtain a standard of quality and authenticity for these products that will allow them to be competitive on the market. Many of the volatile compounds of honey come from the nectar of the flowers. For this reason, monofloral honeys have a distinctive pattern of volatiles composition that can be used to discriminate them from honeys of different botanical origins. Over 300 volatile compounds have been identified as honey aroma components, including acids, alcohols, ketones, aldehydes, terpenes and esters (Alissandrakis, Daferera, Tarantilis, Polissiou, & Harizanis, 2003; Alissandrakis, Tarantilis, Harizanis, Daferera, & Polissiou, 2005; Bouseta, Scheirman, & Collin, 1996; Castro-Vázquez, Díaz-Maroto, Guchu, & Pérez-Coello, 2006a; D'Arcy, Rintoul, Rowland, & Blackman, 1997; Santford & Manura, 1994; Shimoda, Wu, & Osajima, 1996; Soria, Gonzalez, De Lorenzo, Martinez-Castro, & Sanz, 2005; Wilkins, Lu, & Tan, 1993).

Although honey volatile compounds may arise from various sources, only compounds deriving from plants, or their metabolites (Blank, Fischer, & Grosch, 1989; Rowland, Blackman, D'Arcy, & Rintoul, 1995), might be useful for differentiating between floral origins.

Some ramified aldehydes and alcohols may be formed by microbial metabolism, whilst pyran and furan derivatives arise from Maillard reactions or dehydration of sugars in an acid medium; these reactions may be accelerated if honey is subjected to high temperatures during processing or storage (Bouseta, Collins, & Dufour, 1992).

Several authors have identified specific volatile compounds as being characteristic of a particular floral origin and thus useful as "floral markers" (Guyot, Bouseta, Scheirman, & Collin, 1998; Guyot-Declerck, Renson, Bouseta, & Collin, 2002; Häusler & Montag, 1989; Radovic et al., 2001; Serra Bonvehí & Ventura Coll, 2003). Although the identification of such compounds would be highly advantageous, there is not always agreement on the compounds proposed as markers, since there may be differences, even within a single type of monofloral honey, due to the plant variety, the geographical origin or local beekeeping practices.

Descriptive sensory analysis has proved an effective means of distinguishing various types of food on the basis of origin or source (Dairou & Siefferman, 2002; Setser, 1994). Its application to honeys may – taken in conjunction with data obtained from the analysis of volatile compounds – help to differentiate between floral origins. Mannas and Altug (2007) have recently used volatile composition, together with sensory profile, for estimation of authenticity of thyme honey.

The aim of this work was to identify the volatile compounds and the sensory descriptors more representative of honeys from different botanical sources, allowing differentiation among them, and establishing a relationship between chemical and sensory data for each kind of honey.

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doi:10.1016/j.foodchem.2008.06.036

Table 1
Mean concentrations (μg/kg), assuming a response factor equal to 1, and mean relative standard deviations (%) of volatile compounds in each group of the monofloral honey extracts

Compounds	RI	Citrus		Rosemary		Lavender		Thyme		Eucalyptus		Heather	
		(*n* = 10)		(*n* = 10)		(*n* = 7)		(*n* = 7)		(*n* = 10)		(*n* = 5)	
		Mean	RSD	Mean	RSD	Mean	RSD	mean	RSD	Mean	RSD	Mean	RSD
Hexanal	1044	n.d.	–	n.d.	–	26.5	69	n.d.	–	14.9	33	n.d.	–
Dimethyl disulphide	1061	14.0	44	45.7	72	50.7	55	34.3	63	31.6	77	55.9	71
Heptanal	1133	n.d.	–	n.d.	–	11.2	38	n.d.	–	16.8	27	n.d.	–
3-Methyl-1-butanol	1142	26.0	48	42.4	54	70.4	29	36.2	50	151	56	119	53
3-Methyl-3-buten-1-ol	1179	34.2	40	51.0	33	74.4	16	72.7	49	36.5	43	114	51
2 Methyl-3-(2H)dihydrofuranone[a]	1200	12.2	34	9.0	27	24.5	21	21.5	47	18.3	33	37.1	51
p-Cymene	1209	n.d.	–	n.d.	–	n.d.	–	n.d.	–	31.3	28	n.d.	–
3-Hydroxy-2-butanone	1214	72.0	42	42.8	41	155	35	75.4	43	1042	19	402	71
1-Hydroxy-2-propanone[a]	1228	27.8	49	23.6	46	34.7	22	35.6	51	22.4	45	46.2	37
2-Methyl-2-buten-1-ol	1243	43.0	39	62.6	52	118	47	65.5	53	33.2	36	112	61
3-Hydroxy-2-pentanone[a]	1261	tr	–	tr	–	tr	–	17.4	34	128	53	38.1	66
Hexanol	1271	n.d.	–	9.7	53	281	54	6.9	49	21.3	53	n.d.	–
(73/55)	1285	n.d.	–	n.d.	–	n.d.	–	n.d.	–	87.5	83	n.d.	–
1-Hydroxy-2-butanone	1292	9.4	44	10.0	39	30.7	27	26.4	54	18.2	45	49.1	56
Dimethyl trisulphide	1298	23.8	41	9.7	44	10.3	36	20.3	46	14.2	50	2.1	11
(*Z*)-3-hexen-1-ol	1300	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	9.1	59
Nonanal	1316	80.5	55	50.5	28	44.1	32	93.5	52	258	53	103	136
5-Methyl-2-(3H)-furanone[a]	1347	9.9	49	11.2	40	17.5	43	12.6	27	21.9	47	16.0	35
(*Z*)-linalool oxide	1353	384	37	118	53	150	34	92.4	55	22.6	46	386	81
Acetic acid	1355	13.4	71	8.9	42	29.2	32	14.7	39	0.0		16.8	30
2-Hydroxy-5-methyl-3-hexanone[a]	1355	n.d.	–	n.d.	–	n.d.	–	n.d.	–	218	66	n.d.	–
3-Methyl thiopropanal[a]	1367	n.d.	–	n.d.	–	n.d.	–	n.d.	–	18.8	34	n.d.	–
Furfural	1378	1270	36	859	33	1160	44	1558	37	934	16	1508	15
3-Hydroxy-5-methyl-2-hexanone[a]	1379	n.d.	–	n.d.	–	n.d.	–	n.d.	–	550	40	n.d.	–
(*E*)-linalool oxide	1379	177	23	11.3	23	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Nerolidol oxide[a]	1381	n.d.	–	n.d.	–	39.9	34	n.d.	–	n.d.	–	n.d.	–
2-Acetyl furan[a]	1411	87.6	42	56.8	46	111	17	95.8	36	112	31	135	15
Benzaldehyde	1428	154	39	45.3	49	46.1	22	77.7	59	86.2	37	184	29
Lilac aldehyde (isomer I)[a]	1445	331	46	17.4	79	n.d.	–	12.5	53	n.d.	–	tr	–
Linalool	1448	112	43	14.9	57	84.8	42	101	35	29.8	30	tr	–
(67/109/136)[a]	1448	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	90.0	58
Lilac aldehyde (isomer II)[a]	1457	451	46	32.0	47	n.d.	–	18.1	56	n.d.	–	n.d.	–
2-Methyl propanoic acid	1459	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	84.7	9
Pentadecane	1459	tr	–	tr	–	tr	–	tr	–	139	44	tr	–
Lilac aldehyde (isomer III)[a]	1464	284	48	21.2	48	n.d.	–	12.3	56	n.d.	–	n.d.	–
5-Methyl furfural	1475	50.5	42	57.9	48	82.4	47	69.2	32	120	48	87.1	60
(71/43/67/55)	1478	163	57	11.5	50	tr	–	5.0	24	n.d.	–	n.d.	–
2-Cyclopentene-1,4-dione[a]	1484	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	22.8	71
Lilac aldehyde (isomer IV)[a]	1486	368	51	25.7	46	n.d.	–	16.3	46	n.d.	–	n.d.	–
Isophorone	1488	49.1	43	48.5	119	tr	–	29.5	48	44.0	50	76.0	48
6-Methyl-3,5-heptadien-2-one	1491	20.1	48	36.5	58	tr	–	11.0	51	11.5	51	n.d.	–
Hotrienol[a]	1503	153.3	34	86.5	36	710	48	130	52	97.5	67	275	46
γ-Valerolactone	1504	tr	–	1.3	39	n.d.	–	n.d.	–	n.d.	–	26.6	50
α-4-Dimethyl-3-cyclohexene-1-acetaldehyde[a]	1513	825	38	19.3	37	11.9	54	16.8	60	31.5	78	n.d.	
Butanoic acid	1516	n.d.	–	19.7	59	17.8	54	13.7	29	n.d.	–	74.7	145
γ-Butyrolactone	1524	6.9	24	6.8	34	16.6	49	16.8	42	10.7	32	35.2	50
Phenylacetaldehyde	1538	1040	31	1143	49	4481	44	3196	57	1519	48	4909	46
Nonanol	1553	7.7	44	9.8	46	19.3	32	14.4	55	27.2	50	28.2	47
2-Methyl butanoic acid	1553	9.4	27	8.0	43	8.7	49	32.4	48	97.5	50	38.0	92
Furfuryl alcohol	1553	11.0	45	16.2	36	56.1	46	19.8	53	28.3	48	89.6	72
5-Ethenyl-5-methyl-2(3H)-furanone[a]	1558	60.9	32	15.6	44	24.2	21	30.1	24	24.8	33	tr	–
Ketoisophorone (isomer I)	1583	139	51	89.2	100	34.5	54	57.0	51	131	40	97.2	52
α-Terpineol	1585	42.3	47	11.0	47	37.9	47	20.0	34	24.3	39	33.9	37
3-Formyl-pyridine[a]	1593	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	50.1	56
2-Hydroxycineol (isomer I)[a]	1603	n.d.	–	n.d.	–	n.d.	–	n.d.	–	21.8	17	n.d.	–
3-Caren-2-ol[a]	1603	n.d.	–	n.d.	–	n.d.	–	n.d.	–	19.7	8	n.d.	–
2-Furancarboxilic acid[a]	1603	n.d.	–	10.6	42	61.3	32	22.4	44	18.0	16	50.1	46
Lilac alcohol (isomer I)[a]	1605	132	59	19.2	46	14.0	44	26.5	26	tr	–	tr	–
2-*p*-Menthene-1,8-diol[a]	1611	n.d.	–	n.d.	–	19.9	29	8.1	103	27.2	36	49.4	66
Car-2-en-4-one[a]	1613	93.1	69	70.5	99	n.d.	–	24.5	136	n.d.	–	n.d.	–
Pentanoic acid	1615	6.1	23	4.6	26	7.2	48	14.8	53	8.3	42	10.8	44
Epoxylinalool (isomer I)[a]	1618	29.1	29	7.4	46	52.3	51	19.2	31	19.7	27	37.8	11
Lilac alcohol (isomer II)[a]	1623	177	41	15.1	49	tr	–	12.3	47	27.2	54	19.9	20
Epoxylinalool (isomer II)[a]	1644	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	63.4	32
Heptadecane	1655	122	36	79.2	14	186	43	242	79	325	56	160	68
Ketoisophorone (isomer II)	1658	28.9	47	21.3	62	22.9	50	29.7	16	178	50	71.6	57
Lilac alcohol (isomer III)[a]	1663	189	45	14.0	42	17.4	44	15.2	36	27.3	28	tr	–
1-Phenyl ethanol + 1-phenyl-1,2-propanedione[a]	1689	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	103	152
β-Damascenone	1698	5.3	33	5.5	25	6.6	42	11.5	39	7.4	31	10.5	45
Lilac alcohol (isomer IV)[a]	1702	54.6	34	10.7	50	47.9	18	16.3	41	21.5	46	tr	–
Hexanoic acid	1715	92.1	63	31.5	47	34.4	34	143	88	92.3	29	113	82
p-Cymen-8-ol (isomer I)[a]	1723	36.7	56	8.4	39	18.5	53	24.3	52	109	31	36.0	43

Table 1 (*continued*)

Compounds	RI	Citrus		Rosemary		Lavender		Thyme		Eucalyptus		Heather	
		(*n* = 10)		(*n* = 10)		(*n* = 7)		(*n* = 7)		(*n* = 10)		(*n* = 5)	
		Mean	RSD	Mean	RSD	Mean	RSD	mean	RSD	Mean	RSD	Mean	RSD
2-Hydroxycineol (isomer II)[a]	1729	n.d.	–	n.d.	–	n.d.	–	n.d.	–	34.8	31	43.6	56
Guaiacol	1734	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	19.2	34
Benzyl alcohol	1749	92.8	35	129	47	225	36	150	51	198	46	490	33
2-Phenylethanol	1780	484	46	165	44	652	45	427	60	287	39	1156	102
Sinensal (isomer I)	1788	137	47	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Limonyl alcohol[a]	1803	26.2	44	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
1-*p*-Menthen-9-ol	1805	24.5	40	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Heptanoic acid	1815	26.2	46	15.0	44	50.0	41	60.1	62	31.1	48	49.9	20
Nonadecane	1849	104	16	90.8	31	212	38	269	65	444	45	170	59
p-Mentha-1(7),8(10)-dien-9-ol [a]	1860	119	37	10.6	48	n.d.	–	28.0	47	8.5	30	n.d.	–
2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1,4-dione[a]	1875	n.d.	–	n.d.	–	20.3	65	12.0	171	n.d.	–	n.d.	–
p-Anisaldehyde	1888	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	231.2	175
Sinensal (isomer II)	1894	228	41	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Nerolidol	1904	54.7	48	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
Octanoic acid	1914	95.6	50	45.0	35	170	41	318	78	331	49	160	37
Propylanisole [a]	1927	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2802	216
p-Cresol	1939	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	78.7	134
p-Cymen-8-ol (isomer II)[a]	1955	n.d.	–	n.d.	–	n.d.	–	n.d.	–	67.9	43	n.d.	–
Spathulenol[a]	1976	n.d.	–	n.d.	–	n.d.	–	n.d.	–	19.9	43	n.d.	–
2-Hydroxyacetophenone	1984	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	208	91
Nonanoic acid	2012	104	56	46.9	52	50.4	43	202	48	523	53	130	119
Eugenol	2014	n.d.	–	n.d.	–	50.9	31	n.d.	–	n.d.	–	55.7	63
Thymol	2020	7.2	32	3.1	22	10.0	60	tr	–	18.1	42	tr	–
Vinylguaiacol	2045	39.4	51	36.1	51	46.2	28	19.5	50	32.0	29	132	50
Carvacrol	2060	10.7	47	10.2	15	7.0	53	tr	–	17.0	43	tr	–
2-Aminoacetophenone	2064	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	18.1	83
Methyl anthranilate	2083	1280	52	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
(97/68)	2079	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	20.7	43
Decanoic acid	2110	93.6	71	20.9	43	37.8	52	155	47	83.4	49	73.1	61
3-Phenyl-2 propen-1-ol	2125	n.d.	–	n.d.	–	79.9	36	14.4	128	n.d.	–	94.4	39
3,4,5-Trimethylphenol	2203	257	51	179	76	148	86	276	34	380	50	165	61
Coumarin	2275	n.d.	–	n.d.	–	63.6	56	n.d.	–	n.d.	–	n.d.	–
Dodecanoic acid	2306	85.6	92	28.3	37	81.5	44	268	45	134	79	54.1	98
Tetradecanoic acid	2514	96.1	96	33.3	46	56.7	93	299	34	98.2	53	53.4	138

RI were calculated on a BP-21 column (50 m × 0.32 mm × 0.25 μm).
n.d., not detected; tr, traces.
[a] Compounds identified using Wiley library.

2. Materials and methods

2.1. Honey samples

The study was carried out on 49 commercial Spanish honey samples from different floral origins. The monofloral honeys were selected from citrus (10), rosemary (10), eucalyptus (10), lavender (7), thyme (7) and heather (5).

2.2. Isolation and analysis of volatile compounds

A micro scale simultaneous distillation-extraction apparatus (Chrompack, Middelburg, The Netherlands) was used as previously described (Castro-Vázquez, Pérez-Coello, & Cabezudo, 2003; Godefroot, Sandra, & Verzele, 1981). Fifteen grammes of honey dissolved in 40 ml of deionized water, with 15 μl of 2-pentanol (1 g/l) as internal standard, were extracted using 2 ml of dichloromethane as solvent over 2 h. The extracts obtained were concentrated to 200 μl under nitrogen flow. Sample extractions and analysis were carried out in duplicate.

A Hewlett–Packard G 1800 B GCD System (Hewlett–Packard, Palo Alto, CA), equipped with a gas chromatograph and a quadrupole mass detector in electron impact mode at 70 eV, was used to carry out the GC–MS analysis of the extracts. An amount of 2 μl of the extract was injected in splitless mode (0.6 min) on a polyethylene glycol capillary column BP-21 (50 m × 0.32 mm × 0.25 μm of film thickness). Oven temperature programme was: 60° C (3 min)–2 °C/min–200 °C (30 min). Carrier gas was helium (0.8 ml/min). Injector and transfer line temperatures were 250 °C and 280 °C, respectively. Mass detector conditions were as follows: source temperature, 178 °C; scanning rate, 1 scan/s; mass acquisition range, *m*/*z* 40–450. Peak identifications were based on comparison with spectral data and retention indices from pure standard compounds when they were available; otherwise the Wiley G 1035 spectrum library was used. Semiquantitative analyses were carried out, assuming a response factor equal to 1 for all the compounds.

2.3. Descriptive sensory analysis

Honeys cited in Section 2.1 were presented at room temperature in 40 ml glass vials sealed with a twist-off to generate an adequate headspace. Three different coded samples were presented to each assessor. Honeys were evaluated in duplicate by every assessor. The assessment took place in a standard sensory analysis chamber equipped with separate booths (ISO 8589, 1988).

The panel consisted of a group of ten assessors, ranging between 25 and 40 years old with previous experience in sensory analysis.

At initial sessions, assessors underwent training in descriptive sensory analysis. Then they generated descriptors individually over the course of several sessions. Thirteen odour attributes were selected in order to describe the differences among the honeys. After that, they spent additional sessions evaluating the intensity of each attribute according to unstructured 10 cm scales, delimited at the ends by the terms "weak" and "strong".

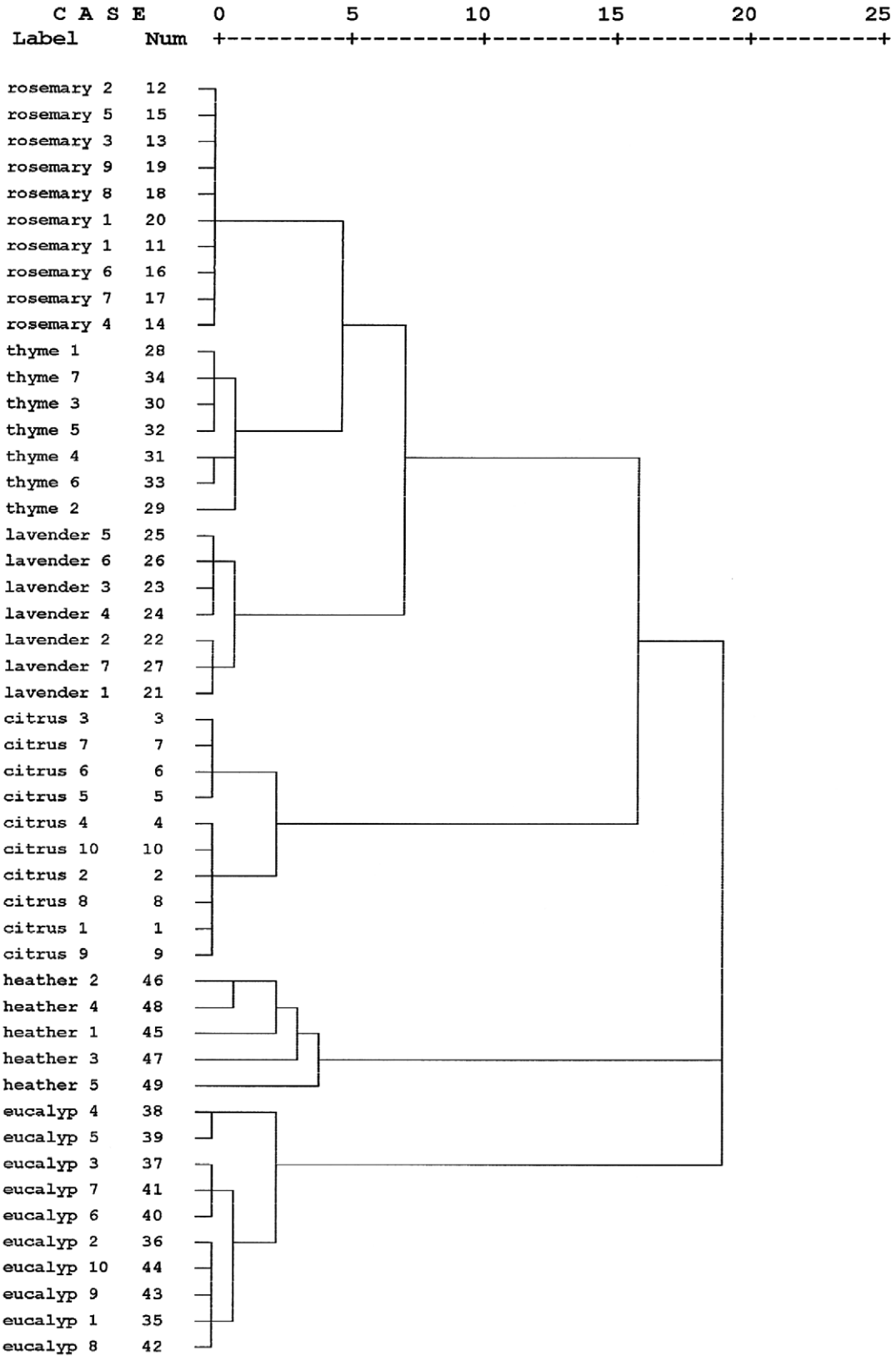

Fig. 1. Cluster analysis of volatile compounds of monofloral honeys. Dendrogram of honey samples using the Euclidean distance matrix.

2.4. Multivariate data processing

Processing of data was carried out by using the SPSS for Windows programme package. The unsupervised techniques used were principal components analysis and cluster analysis. Correlation between the volatile compounds and sensory intensity attributes was determined by calculating the Spearmańs rank order correlation coefficient (Siegal, 1956).

3. Results and discussion

3.1. Volatile composition of monofloral honeys

In total, 106 volatile compounds were identified in the 49 honey samples analysed. Mean concentrations (μg/kg) and relative standard deviations (%) for each of them in the different monofloral honeys, are shown in Table 1.

The data matrix was first subjected to hierarchical cluster analysis, which seeks to place cases in homogeneous groups or clusters not previously known, but suggested, on the basis of information drawn from the table of variables, so that the most similar samples are assigned to the same cluster.

The dendrogram obtained (Fig. 1) shows that honeys from the same floral origin were assigned to the same cluster, thus highlighting their similarity. Successive higher-level clusters indicated similarities between different types of honey: rosemary honeys were fairly similar to thyme honeys, but rather less similar to lavender and citrus honeys. Heather and eucalyptus were placed further away from this group, and there were even some subgroups, indicating small "intragroup" differences.

To obtain more detailed information on the volatile compounds involved in differentiating the monofloral honeys studied, factorial principal component analysis was applied to the whole data matrix. The first four principal components accounted for 71% of total variance. Projection of samples in the space formed by the principal components, PC-1, PC-2 and PC-4, are shown in Fig. 2. Eucalyptus honeys were grouped in the positive area of PC-1, whilst PC-2 separated citrus honeys from the rest. Lavender honeys were grouped in the positive area of PC-4, and the remaining samples in the negative area. PC-3 separated heather honeys from the rest (not shown).

The compounds most strongly correlated with the first four principal components are listed in Table 2, which also gives mean concentrations for those compounds in the honeys separated by each axis. PC-1, which separated eucalyptus honeys from the rest, displayed a strong correlation with terpene compounds, including 3-caren-2-ol, *p*-cymene and its derivate alcohols (the two isomers of *p*-cymen-8-ol) and 2-hydroxycineol. *p*-Cymen-8-ol is one of the major volatile compounds in eucalyptus essential oil extracts (Fadel, Marx, El-Sawy, & El-Ghorab, 1999) and it has been measured in Australian eucalyptus honeys (D'Arcy, Rintoul, Rowland, & Blackman, 1997). However, isomers of *p*-cymen-8-ol and 2-hydroxycineol have also been reported in Italian and Japanese monofloral honeys of various origins (Shimoda et al., 1996; Verzera, Campisi, Zappala, & Bonaccorsi, 2001). The sesquiterpene, spathulenol, is fairly common in plants, but this is the first time that it is identified as a component of honeys.

The norisoprenoid, 2,2,6-trimethyl-2-cyclohexen-1,4-dione (isomer II) (ketoisophorone), was identified at high concentrations in eucalyptus honeys. Others norisoprenoids, such as 8,9-dehydroteaspirone and 3-oxo-α-ionone, have been proposed as markers for Australian eucalyptus honeys (D'Arcy et al., 1997).

High concentrations of diketones and hydroxyketones, are widely reported in the literature as markers for eucalyptus honeys (Bianchi, Cereri, & Musci, 2005; Bouseta et al., 1992, 1996; Graddon, Morrison, & Smith, 1979; Pérez, Sánchez-Brunete, Calvo, & Tadeo, 2002; Radovic et al., 2001; Serra Bonvehí, & Ventura Coll, 2003). 3-Hydroxy-2-butanone (acetoin) was the most abundant ketone in the eucalyptus honeys analysed, with a mean concentration of 1.04 ppm. 3-Hydroxy-5-methyl-2-hexanone and 2-hydroxy-5-methyl-3-hexanone have also been identified exclusively in eucalyptus honeys and have recently been proposed as markers

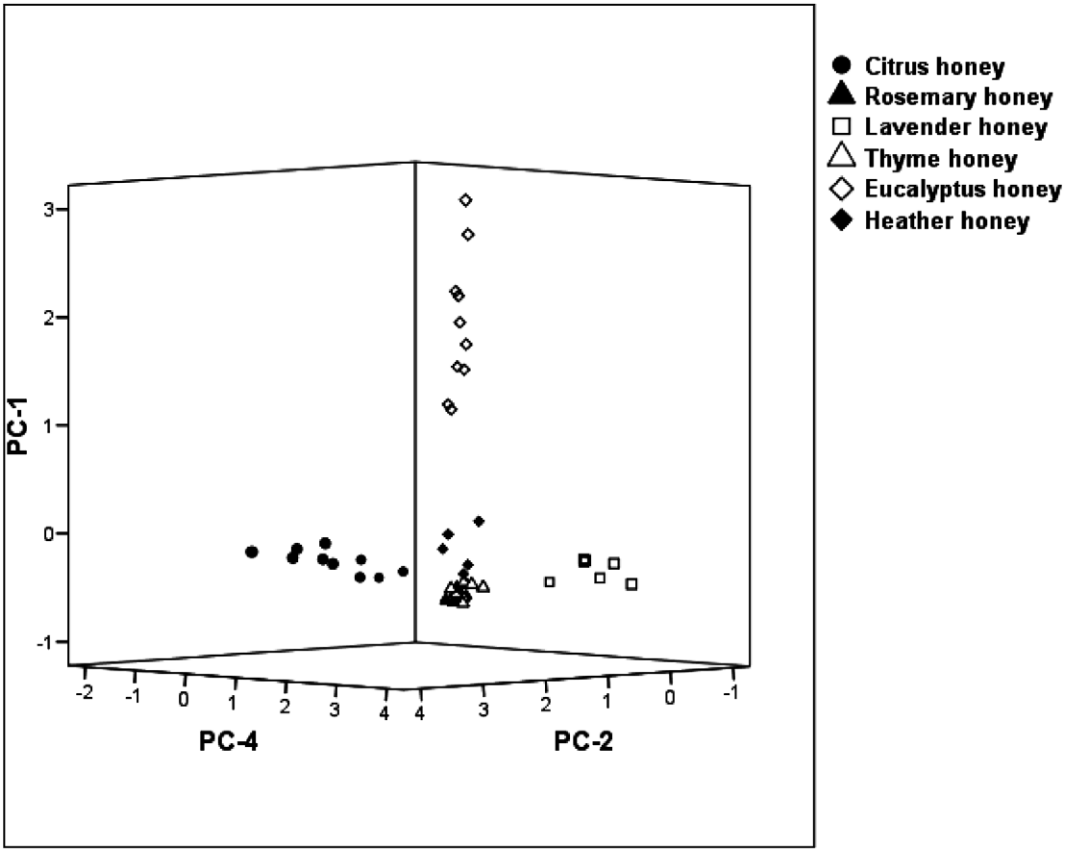

Fig. 2. Principal component analysis of the volatile composition of monofloral honeys. Projection of the honey samples in the space formed by the PC-1, PC-2 and PC-4.

Table 2
Results of the application of the principal component analysis to the volatile composition of monofloral honeys

	Compounds	Mean concentration in eucalyptus honeys (μg/kg)
PC-1	*p*-Cymen-8-ol (isomers I and II[a])	67.9–109
	3-Hydroxy-2-butanone	1042
	3-Hydroxy-2-pentanone	128
	Ketoisophorone (isomer II)	178
	3-Hydroxy-5-methyl-2-hexanone[a]	550
	p-Cymene[a]	31.3
	Spathulenol[a]	19.9
	3-Caren-2-ol[a]	19.7
	2-Hydroxycineol (isomer I)	21.8
	2-Hydroxy-5-methyl-3-hexanone[a]	218
	3-Methylthiopropanal[a]	18.8
	Compounds	Mean concentration in citrus honeys (mg/kg)
PC-2	α-4-Dimethyl-3-cyclohexene-1-acetaldehyde	825
	Lilac aldehyde (isomers I–IV)	284–451
	p-Mentha-1(7),8(10)-dien-9-ol	119
	Lilac alcohol (isomers I–III)	132–189
	(*E*)-linalool oxide	177
	Sinensal (isomers I and II)[a]	137–228
	Limonyl alcohol[a]	26.2
	Methyl anthranilate[a]	1175
	Nerolidol[a]	54.7
	1-*p*-Menthen-9-ol[a]	24.5
	Compounds	Mean concentration in heather honeys (mg/kg)
PC-3	2-Cyclopenten-1,4-dione[a]	22.8
	2-Aminoacetophenone[a]	18.1
	2-Hydroxyacetophenone[a]	208
	Guaiacol[a]	19.2
	Propyl anisol[a]	2802
	p-Anisaldehyde[a]	231
	p-Cresol[a]	78.7
	Compounds	Mean concentration in lavender honeys (mg/kg)
PC-4	Nerolidol oxide[a]	39.9
	Coumarin[a]	63.6
	Hotrienol	710
	Hexanol	281
	Hexanal	26.5

Volatile compounds most correlated with the first four principal components and mean concentrations (μg/kg) in the separated honeys.
[a] Compounds quantified exclusively in that botanical origin.

for this botanical origin (De la Fuente, Valencia-Barrera, Martinez-Castro, & Sanz, 2007).

Another compound identified in eucalyptus honeys was 3-methyl-thiopropanal (methional), derived from sulfur-containing aminoacids. Since its olfactory detection threshold is very low (0.02 ppb) (Piasenzotto, Gracco, & Conte, 2003), it may have a considerable sensory impact in this type of honey. Its aroma has been described as "baked potato", and it is among the compounds with the greatest sensorial impact in linden honeys analysed using GC-olfactometry (Blank et al., 1989).

PC-2 separated citrus honeys from the other monofloral honeys. The compounds correlating most strongly with this axis were the linalool derivates, namely (*E*)-linalool oxide, lilac aldehydes and lilac alcohols, whose concentrations were much greater in citrus honeys than in other honeys (Alissandrakis et al., 2005; Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2007; De la Fuente, Martinez-Castro, & Sanz, 2005; Soria, Martinez-Castro, & Sanz, 2003). Lilac alcohol and aldehyde isomers were first identified in lilac oils (Wakayama & Namba, 1974) and later reported as components of gardenia flowers (Serra Bonvehí, 1988). They have been suggested as floral markers in New Zealand thistle honeys (Wilkins et al., 1993) and in citrus honeys (Alissandrakis et al., 2003; Alissandrakis et al., 2005; Pérez et al., 2002).

α-4-Dimethyl-3-cyclohexene-1-acetaldehyde was one of the most abundant volatile compounds in the citrus honeys tested. It has recently been reported in Greek citrus honeys (Alissandrakis et al., 2005).

Methyl anthranilate, at concentrations of over 0.50 ppm, has also been suggested as a floral marker for citrus honeys (Serra Bonvehí, 1988). However, use of an additional marker is recommended wherever samples display concentrations of less than 0.50 ppm, or the volatile extraction method used does not fully recover this compound (Pérez et al., 2002). In our samples, methyl anthranilate concentrations ranged from 0.53 to 2.3 ppm. In this way, the two isomers of sinensal, volatile components of orange essence oil and recently identified in Spanish citrus honeys (Castro-Vázquez et al., 2007), could be additional floral markers of citrus honeys.

The fourth principal component separated lavender honeys. Among the compounds correlating most strongly with this axis were nerolidol oxide and coumarin which were only present in the lavender honeys; the latter have been described as characteristic of this type of honey by other authors (Bouseta et al., 1992, 1996; Radovic et al., 2001; Shimoda et al., 1996), although Guyot-Declerck et al. (2002) have shown changes in its concentration as a function of honey storage time.

Hotrienol and alcohols and aldehydes with six atoms of carbon may be found in honeys of various origins, but their concentrations in lavender honeys analysed were greater than in other types, and they may thus contribute to differentiation of this type of honey.

Heather honeys were grouped in the positive area of PC-3, on the basis of their major content of phenolic compounds, compared with other monofloral honeys, such as guaiacol, *p*-anisaldehyde, propylanisole and *p*-cresol, although most of them have also been found in honeydew honeys (Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2006b).

2-Aminoacetophenone, a compound derived from aminoacids, and 2-hydroxyacetophenone were detected at important concentrations in all heather honey samples. The former has also been identified in Chestnut honey (Guyot et al., 1998).

Some norisoprenoids, such as 3,5,5-trimethyl-2-cyclohexene-1-one (isophorone) and dehydrovomifoliol, have been suggested by many authors as markers for heather honeys (D'Arcy et al., 1997; Guyot, Scheirmann, & Collin, 1999; Häusler & Montag, 1989; Häusler & Montag, 1991; Piasenzotto et al., 2003; Radovic et al., 2001; Steeg & Montag, 1988; Tan, Holland, Wilkins, & McGhie, 1989), although they are common in various types of honey.

Principal component analysis could not differentiate thyme and rosemary honeys. Carotenoid derivatives and 3,4,5-trimethoxybenzaldehyde have been reported as characteristic of thyme honeys (Mannas, & Altug, 2007; Piasenzotto et al., 2003; Tan, Holland, Wilkins, & McGhie, 1990). In our case, the most representative compound of thyme honey is linalool, with a concentration similar to that in citrus honey, and the presence of fatty acids in greater concentration than in the rest of the honeys analysed.

Lilac aldehyde isomers were present in rosemary honeys and could contribute, with floral notes, to rosemary honey aroma. High concentrations of aromatic acids, some terpenes, and the presence of carbonyl compounds, such as benzaldehyde, as well as sulfur-containing compounds, have been reported as characteristic of Spanish and Portuguese rosemary honeys (Bouseta et al., 1992; Castro-Vázquez et al., 2003; Radovic et al., 2001; Serra Bonvehí, & Ventura Coll, 2003), but in general it is difficult to identify marker compounds for this type of honey (Pérez et al., 2002).

The difficulty of finding volatile compounds exclusively in honeys from a specific botanical origin justifies the use of sensory analysis to make this differentiation possible.

Table 3
Mean scores and standard deviations of the attributes selected by descriptive sensory analysis of honeys

Attribute	Citrus		Rosemary		Lavender		Thyme		Eucalyptus		Heather	
	(n = 10)		(n = 10)		(n = 7)		(n = 7)		(n = 10)		(n = 5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Floral	7.12^{a}	(0.38)	4.65^{b}	(0.45)	3.96^{d}	(0.93)	0.95^{e}	(1.19)	0.00^{c}	(0.00)	0.39$^{c\ e}$	(0.33)
Aromatic herbs	0.00^{a}	(0.00)	2.99^{b}	(0.47)	5.80^{c}	(0.95)	5.32^{c}	(0.92)	2.10^{b}	(0.24)	4.20^{d}	(1.70)
Citric	6.00^{a}	(0.49)	4.06^{b}	(0.61)	4.35^{b}	(0.94)	3.34^{d}	(0.54)	0.00^{c}	(0.00)	0.17^{c}	(0.29)
Fresh fruit	5.40^{a}	(0.74)	4.59^{b}	(0.96)	3.95^{d}	(1.12)	0.04^{c}	(0.13)	0.00^{c}	(0.00)	0.09^{c}	(0.28)
Ripe fruit	0.00^{a}	(0.00)	0.00^{a}	(0.00)	0.00^{a}	(0.00)	1.40^{c}	(1.29)	3.51^{b}	(0.90)	5.92^{d}	(0.84)
Caramel	6.68^{a}	(0.33)	4.09^{b}	(0.97)	1.82^{c}	(1.05)	2.37^{c}	(1.80)	1.56^{c}	(1.04)	4.00^{b}	(1.30)
Wood	0.00^{a}	(0.00)	0.00^{a}	(0.00)	0.25^{a}	(0.38)	0.20^{a}	(0.21)	1.63^{b}	(0.42)	2.45^{c}	(0.29)
Hay	0.00^{a}	(0.00)	0.13$^{a.b}$	(0.48)	0.62$^{a.b}$	(0.54)	0.77^{b}	(0.69)	5.26^{c}	(0.58)	1.44^{d}	(0.70)
Spices	0.00^{a}	(0.00)	0.00^{a}	(0.00)	0.82^{b}	(0.68)	1.25^{b}	(0.99)	1.51^{b}	(0.51)	4.14^{c}	(1.11)
Resin	0.00^{a}	(0.00)	0.00^{a}	(0.00)	0.37^{a}	(0.28)	0.06^{a}	(0.18)	1.11^{b}	(0.51)	2.40^{c}	(1.32)
Balsamic	0.00^{a}	(0.00)	1.86^{b}	(1.10)	4.43^{d}	(0.83)	1.80$^{b\ c}$	(1.15)	1.47$^{b.c}$	(0.97)	1.50$^{b\ c}$	(0.68)
Cheese	0.00^{a}	(0.00)	0.00^{a}	(0.00)	0.00^{a}	(0.00)	0.00^{a}	(0.00)	5.75^{b}	(0.40)	0.00^{a}	(0.00)

$^{a–e}$ Different superscripts in the same row indicate statistical differences at the $\alpha = 0.05$ level according to the Student–Newman–Keuls test.

3.2. Sensory analysis of monofloral honeys and correlation with volatile compounds

Mean scores and standard deviations for the sensory attributes detected by the assessors in the monofloral honeys studied are shown in Table 3. Principal component analysis was applied to the sensorial data, resulting in 90% of total variance being explained by the three first principal components. Fig. 3 shows the projection of the samples in the plane defined by the three first components that grouped the honeys from the same botanical origin according to the sensorial characteristics. Citrus honeys were grouped in the positive area of the PC 2 axis due to their high scores for the floral and fresh attributes, whereas thyme and lavender honeys, grouped in the negative area, presented high scores for the "aromatic herbs" and "balsamic" attributes, respectively, that were negatively correlated with the PC 2 axis. "Aromatic notes" have also been described in some monofloral Italian honeys (Esti, Panfili, Marconi, & Trivisonno, 1997).

Rosemary honeys displayed sensory profiles intermediate between citrus and thyme honeys, presenting floral and fresh attributes, although they were less intense than in citrus honey, and caramel notes. Higher scores for flowery and fruity notes have been

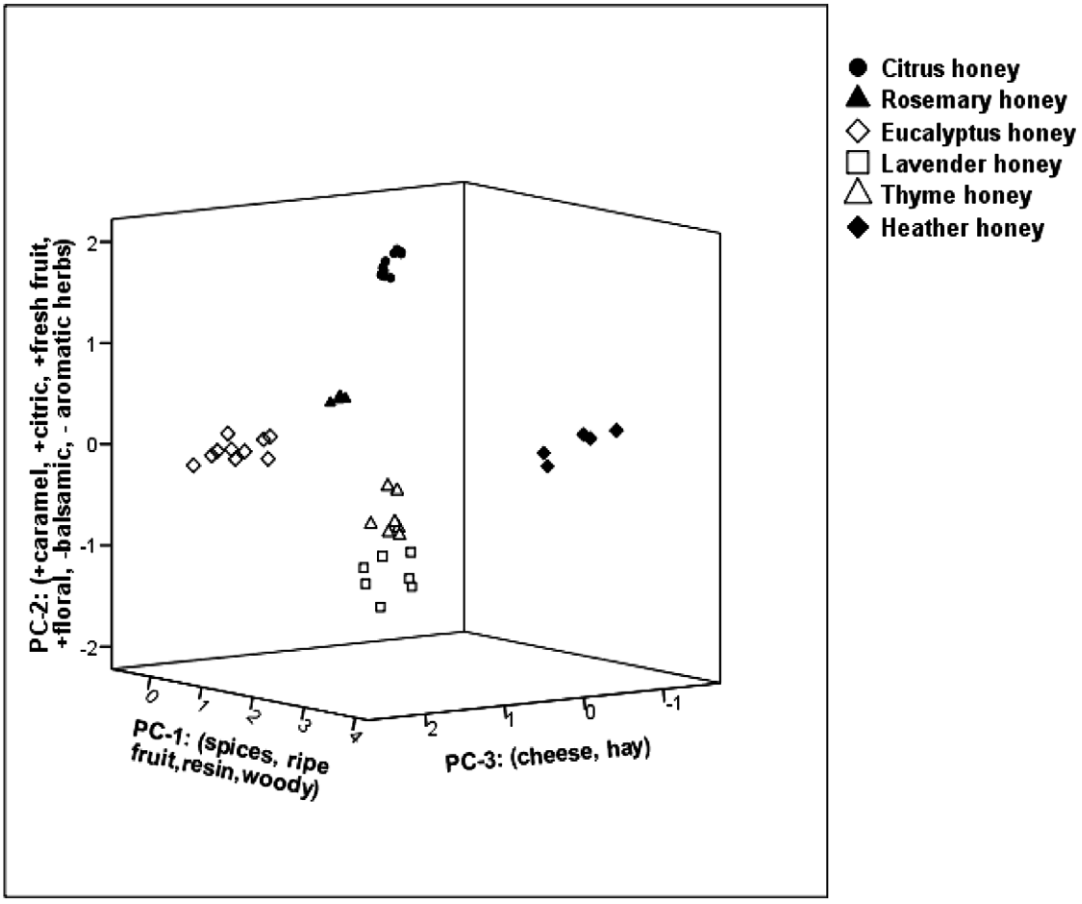

Fig. 3. Principal component analysis of the sensory scores of monofloral honeys. Projection of the honey samples in the space formed by PC-1, PC-2 and PC-3.

proposed as indicative of superior honey quality (Anupama, Bhat, & Sapna, 2003). Despite this, there are other pleasant sensory attributes present in honey from different sources.

Eucalyptus honeys were grouped in the positive area of the PC 3 axis, resulting in highest scores for the attributes "hay" and "cheese", both positively correlated with this axis. Tasters described the aroma of eucalyptus honey as an unpleasant lactic-like aroma, only identified in this type of honey. Finally, heather honeys were grouped in the positive area of the PC 3 axis, according with their high scores for the attributes "ripe fruit", "spicy", "woody" and "resin" correlated with this axis. Some of these terms coincided with the attributes described in a previous work (Galan-Soldevilla, Ruiz-Pérez-Cacho, Serrano Jimenez, Jodral Villarejo, & Bentabol Manzanares, 2005).

Sigh and Kaur-Bath (1997) reported that organoleptic evaluation showed a significant variation in Indian honeys from different floral sources. Sensory analysis showed that trained tasters were able to differentiate among honeys from different floral sources, using the attributes previously selected. These attributes, characteristic of each type of honey, can be related to the volatile composition of the honey, establishing a global aroma profile that can be useful for differentiating unifloral honeys.

Table 4 shows the correlations between the sensory attributes and the concentration of the volatile compounds selected by principal components analysis in the 49 honey samples analyzed. The presence of aromatic notes is influenced by the presence of a few components. Sinensal isomers, recently identified in orange essence oils (Högnadóttir & Rouseff, 2003), were the compounds most closely correlated with the "citric", "floral", "fresh" and "fresh fruit" aromas of citrus honey. Other volatile compounds characteristic of citrus honeys, such as methyl anthranilate, nerolidol, 1-*p*-menthen-9-ol, lilac aldehydes and limonyl alcohol, some present in lower concentrations in rosemary honeys, were also correlated with these attributes.

Many eucalyptus characteristic volatile compounds, such as *p*-cymen-8-ol, 3-hydroxy-5-methyl-2-hexanone, *p*-cymene, spathulenol, 3-caren-2-ol and 2-hydroxycineol, presented the highest Spearman's coefficients for the attributes "cheese" and "hay", which were also selected by the tasters for this kind of honey. The presence of spathulenol in eucalyptus honeys might be related to the woody notes (Jirovetz, Buchbauer, Abraham, & Shafi, 2006), whereas 3-hydroxy-2-butanone (acetoin) could be an important key odorant with "cheese" and "cream" aromas (http://www.flavornet.org) since its concentrations were higher than its odour threshold (800 ppb) (Buttery, Teranishi, Ling, & Turnbaugh, 1990).

Some phenolic compounds, characteristic of heather honeys, such as *p*-anisaldehyde, *p*-cresol and guaiacol have been correlated with "spicy" and "wood" notes ($p < 0.01$). 2-Aminoacetophenone and 2-hydroxyacetophenone also presented a significant correlation with the "ripe fruit" and "resin" attributes. All of these attributes were selected by principal component analysis as relevant in the aroma of heather honeys.

Coumarin and nerolidol oxide are the compounds that presented highest Spearmańs coefficients correlating with the attributes "aromatic herbs" and "balsamic". These volatile compounds and sensorial attributes were characteristic of lavender honeys. However, other compounds, such as hexanal and hotrienol, with odour thresholds of 4.5 ppb and 110 ppb, respectively, could contribute to the aroma of lavender honeys (Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 2000).

The joint study of chemical and sensory variables in the characterisation of monofloral honeys may improve the differentiations

Table 4
Spearman's rank-order correlation matrix applied to volatile compounds and sensory descriptors for the 49 monofloral honeys

Compounds	Floral	Aromatic herbs	Fresh	Citric	Fresh fruit	Ripe fruit	Caramel	Wood	Hay	Spices	Resin	Balsamic	Cheese
p-Cymen-8-ol (isomer I)	−0.464**	−0.333*	−0.467**	0.109	−0.469**	0.626**	−0.347*	0.637**	0.620**	0.473**	0.583**	−0.196	0.687**
3-Hydroxy-2-butanone	−0.656**	0.004	−0.647**	−0.260	−0.600**	0.662**	−0.583**	0.729**	0.756**	0.709**	0.758**	0.141	0.679**
Nonanoic acid	−0.550**	−0.202	−0.430**	−0.040	−0.532**	0.547**	−0.379**	0.440**	0.586**	0.433**	0.406**	−0.199	0.670**
3-Hydroxy-2-pentanone	−0.871**	0.058	−0.843**	−0.428**	−0.854**	0.931**	−0.551**	0.771**	0.815**	0.747**	0.702**	0.056	0.738**
Ketoisophorone (isomer II)	−0.036	−0.556**	−0.081	0.302*	0.038	0.204	0.173	0.167	0.109	−0.039	0.108	0−.356*	0.341*
p-Cymen-8-ol (isomer II)	−0.629**	−0.318*	−0.645**	−0.252	−0.533**	0.564**	−0.534**	0.587**	0.720**	0.379**	0.553**	0.002	0.988**
3-Hydroxy-5-methyl-2-Hexanone	−0.629**	−0.318*	−0.645**	−0.252	−0.533**	0.557**	−0.537**	0.581**	0.716**	0.377**	0.546**	−0.006	0.992**
p-Cymene	−0.629**	−0.312	−0.645**	−0.252	−0.533**	0.558**	−0.533**	0.583**	0.712**	0.384**	0.547**	−0.004	0.986**
Spathulenol	−0.629**	−0.315*	−0.645**	−0.252	−0.533**	0.558**	−0.532**	0.596**	0.719**	0.381**	0.545**	0.012	0.990**
3-Caren-2-ol	−0.629**	−0.324*	−0.645**	−0.252	−0.533**	0.553**	−0.531**	0.587**	0.717**	0.376**	0.546**	0.005	0.986**
2-Hydroxycineol (isomer I)	−0.629**	−0.318*	−0.645**	−0.252	−0.533**	0.557**	−0.537**	0.581**	0.716**	0.377**	0.546**	−0.006	0.992**
3-Methylthiopropanal	0.465**	−0.701**	0.472**	0.691**	0.474**	−0.394**	0.366**	−0.359**	−0.355*	−0.612**	−0.388**	−0.514**	0.106
Lilac aldehyde (isomer II)	0.758**	−0.501**	0.748**	0.727**	0.681**	−0.625**	0.769**	−0.713**	−0.802**	−0.801**	−0.774**	−0.590**	−0.503**
p-Mentha-1(7),8(10)-dien-9-ol	0.745**	−0.254	0.827**	0.691**	0.672**	−0.696**	0.617**	−0.789**	−0.749**	−0.674**	−0.798**	−0.372**	−0.612**
(*E*)-linalool oxide	0.668**	−0.147	0.557**	0.597**	0.548**	−0.394**	0.652**	−0.363*	−0.606**	−0.320*	−0.301*	−0.230	−.689**
Sinensal (isomer I)	0.702**	−0.695**	0.675**	0.987**	0.617**	−0.428**	0.692**	−0.399**	−0.549**	−0.533**	−0.428**	−0.678**	−0.252
Sinensal (isomer II)	0.701**	−0.695**	0.673**	0.984**	0.614**	−0.428**	0.689**	−0.399**	−0.549**	−0.533**	−0.428**	−0.678**	−0.252
Limonyl alcohol	0.696**	−0.695**	0.667**	0.974**	0.626**	−0.428**	0.690**	−0.399**	−0.549**	−0.533**	−0.428**	−0.678**	−0.252
Methyl anthranilate	0.695**	−0.695**	0.673**	0.980**	0.624**	−0.428**	0.693**	−0.399**	−0.549**	−0.533**	−0.428**	−0.678**	−0.252
Nerolidol	0.697**	−0.695**	0.667**	0.975**	0.618**	−0.428**	0.689**	−0.399**	−0.549**	−0.533**	−0.428**	−0.678**	−0.252
1-*p*-Menthen-9-ol	0.698**	−0.695**	0.664**	0.976**	0.624**	−0.428**	0.688**	−0.399**	−0.549**	−0.533**	−0.428**	−0.678**	−0.252
2-Cyclopenten-1,4-dione	−0.193	0.207	−0.363*	−0.169	−0.303*	0.575**	0.101	0.569**	0.237	0.540**	0.565**	−0.002	−0.169
2-Aminoacetophenone	−0.160	0.191	−0.339*	−0.150	−0.255	0.513**	0.052	0.503**	0.206	0.474**	0.508**	−0.016	−0.150
2-Hydroxyacetophenone	−0.194	0.206	−0.359*	−0.169	−0.301*	0.572**	0.116	0.565**	0.241	0.543**	0.561**	0.005	−0.169
Guaiacol	−0.192	0.205	−0.360*	−0.169	−0.303*	0.575**	0.101	0.569**	0.237	0.541**	0.565**	−0.007	−0.169
Propyl anisole	−0.194	0.204	−0.356*	−0.169	−0.305*	0.574**	0.110	0.568**	0.238	0.542**	0.563**	−0.004	−0.169
p-Anisaldehyde	−0.194	0.205	−0.358*	−0.169	−0.303*	0.573**	0.113	0.566**	0.240	0.542**	0.562**	0.002	−0.169
p-Cresol	−0.192	0.206	0.360*	−0.169	−0.296*	0.572**	0.119	0.563**	0.241	0.544**	0.561**	0.004	−0.169
Hexanal	−0.415**	0.121	−0.384**	−0.355*	−0.323*	0.151	−0.707**	0.362*	0.575**	0.399**	0.497**	0.442**	0.602**
1-Hexanol	−0.404**	0.405**	−0.254	−0.612**	−0.261	−0.047	−0.745**	0.075	0.396**	0.214	0.206	0.612**	0.368**
Hotrienol	0.170	0.300*	0.177	0.063	0.112	−0.095	−0.048	−0.003	−0.014	0.200	0.129	0.327*	−0.322*
Coumarin	0.095	0.509**	0.188	−0.204	0.142	−0.347*	−0.374**	−0.098	0.044	0.109	0.084	0.584**	−0.204
Nerolidol oxide	0.095	0.510**	0.181	−0.204	0.139	−0.347*	−0.379**	−0.088	0.041	0.103	0.094	0.580**	−0.204

*,** Significant levels at 0.05 and 0.01, respectively.

of honey types, and may also provide some insights of the consumer preferences based on the honey aromas.

Acknowledgements

The authors are grateful to the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) for financial support under the project API 03-004.

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