

Aroma composition and new chemical markers of Spanish citrus honeys

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Abstract

Sixty six volatile compounds were detected in Spanish citrus honeys by GC–MS. Certain compounds appeared to be characteristic of this floral source, particularly high concentrations of terpenes and derivatives, such as linalool, (*Z*) (*E*)-linalool oxide, α -terpineol, terpineol and isomers of lilac aldehyde and lilac alcohol. Sinensal isomers are also proposed as new chemical markers for citrus honeys, since these compounds are present only in this floral source. OAV values suggested that these compounds contributed strongly to the characteristic aroma of citrus honeys.

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1. Introduction

Honey is a complex natural product, mostly made up of carbohydrates and water; flavours and aromas vary considerably. A major concern of producers is to be able to demonstrate that a given honey possesses certain features characteristic of its botanical source, governed both by the surrounding flora and by geographical origin.

The botanical origin of honey has traditionally been determined by melissopalynological analysis, based on the identification of pollen by microscopic examination (Louveaux, Maurizio, & Worwohl, 1978). However, this analysis does not always enable reliable identification, especially in the case of honeydews or honeys containing only small amounts of pollen. Today, it is widely accepted that this technique alone is not sufficient to authenticate the floral origin of honey.

The chief factor in honey characterization is its aroma. Unifloral honeys possess highly characteristic aromas,

due to the presence of specific volatile organic components deriving from the original nectar sources. A number of authors have reported the same volatile compounds or their metabolites in honey as in nectar and in the flowers from which it is obtained (Alissandrakis, Daferera, Tarrantis, Polissiou, & Harizanis, 2003; Blank, Fischer, & Grosch, 1989; Moreira & De María, 2005; Rowland, Blackman, Darcy, & Rintoul, 1995; Soria, Gonzalez, De Lorenzo, Martinez-Castro, & Sanz, 2005; Steeg & Montag, 1988).

Some of these organic compounds have been identified and used as marker compounds for certain commercial honeys. For example, 3,9-epoxy-1-*p*-menthadiene, *t*-8-*p*-menthen-1,2-diol and *cis*-rose oxide have been proposed as markers for lime tree honey (Blank et al., 1989; Tsuneya, Shibai, Yoshioka, & Shiga, 1974); diketones, alkanes and sulfur compounds are characteristic of eucalyptus honeys (Bouseta, Collins, & Dufour, 1992, 1996; Pérez, Sánchez-Brunete, Calvo, & Tadeo, 2002; Radovic et al., 2001), while hexanal and heptanal are the major flavour compounds in lavender honeys (Bouseta et al., 1992). Asian longan and Australian leatherwood honeys contain some linalool derivatives (Ichimura, 1994; Rowland et al., 1995). The

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norisoprenoid, dehydrovomifoliol, has been reported as an important marker for European heather honeys (Häusler & Montag, 1991).

High contents of certain shikimate-pathway derivatives, such as phenylacetic acid, benzoic acid and 4-methoxybenzaldehyde, also appear to be of value for authenticating heather honeys (Guyot, Scheirmann, & Collin, 1999; Häusler & Montag, 1990). Chestnut honeys are distinguishable from those of other origins by high concentrations of acetophenone and 3-aminoacetophenone (Bonaga & Giumanini, 1986; Guyot, Bouseta, Scheirman, & Collin, 1998; Serra Bonvehí & Ventura Coll, 2003).

The most widely-used method for determining the volatile composition of honeys is based on gas chromatography–mass spectrometry (GC–MS), although prior fractionation is required to separate volatile compounds from the sugar matrix. SDE has been used to characterize floral origins and establish markers for European chestnut, lime and heather honeys (Guyot et al., 1998, 1999), to differentiate between French and Portuguese lavender honeys (Guyot-Declerck, Renson, Bouseta, & Collin, 2002) and, more recently, to characterize Greek citrus honeys (Alisandrakis, Tarantilis, Harizanis, Daferera, & Polissiou, 2005).

Methyl anthranilate has been reported as a marker of citrus honeys. In analyses of Spanish and Mediterranean citrus honeys, Serra Bonvehí (1988) and Ferreres et al. (1994) found methyl anthranilate in concentrations ranging from 0.50 to 3.60 ppm, i.e. the range required to characterise a honey as a *Citrus sp.* However, this compound may also be present in honeys of other origins (Verzera, Campisi, Zappala, & Bonaccorsi, 2001).

Other compounds, including hotrienol and the isomers of lilac aldehyde and α -4-dimethyl-3-cyclohexen-1-acetaldehyde, have been proposed for differentiating citrus honeys (Alisandrakis et al., 2005; De la Fuente, Martínez-Castro, & Sanz, 2005; Pérez et al., 2002; Verzera et al., 2001).

Not all volatile compounds have a significant impact on honey aroma. The impact of a given compound depends on the extent to which concentrations exceed its odour threshold. Thus, even compounds present in low concentrations may contribute strongly to honey aroma.

The aim of this research was to investigate volatile compounds which might enable differentiation of citrus honeys, either through their presence solely in these honeys or in terms of their sensory impact.

2. Material and methods

2.1. Simultaneous extraction and distillation (SDE)

A micro-scale simultaneous distillation–extraction apparatus (Chrompack, Middelburg, The Netherlands) was used with 10 samples of Spanish citrus honeys, as previously described (Godefroot, Sandra, & Verzele, 1981). 15 g of honey, dissolved in 40 ml of deionised water,

with 15 μ l of 2-pentanol (1 g/l) as internal standard, were extracted, using dichloromethane as solvent during 2 h (Castro-Vázquez, Pérez-Coello, & Cabezado, 2003). The extracts obtained were concentrated to 200 μ l under nitrogen flow.

2.2. Chromatographic conditions

All extracts were analysed using a Hewlett Packard G 1800 B GCD System with a mass detector (Hewlett-Packard, Palo Alto, CA, USA). 2 μ l of extracts were injected in splitless mode (0.6 min) on a BP-21 capillary column (60 m \times 0.32 mm \times 0.32 μ m of film thickness). Oven temperature programme was: 60° (3 min)–2 °C/min–200 °C (30 min). Injector and transfer line temperatures were 250 and 280 °C, respectively. Mass detector conditions were: electronic impact (EI) mode at 70 eV; mass acquisition range: 40–450 amu. Peak identifications were based on comparison with spectral data 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 one.

3. Results and discussion

GC–MS analysis of SDE extracts of 10 unifloral Spanish citrus honeys enabled the quantification of 66 volatile compounds. Maximum and minimum concentrations of the volatile compounds identified are shown in Table 1. A typical GC citrus–honey profile is shown in Fig. 1.

Some benzene derivatives identified, such as benzaldehyde, benzyl alcohol and 2-phenylethanol (peaks 16, 48 and 49, respectively) have also been reported in most European and Australian honeys from a wide range of floral sources (Bonaga & Giumanini, 1986; D’Arcy, Rintoul, Rowland, & Blackman, 1997; Soria, Martínez-Castro, & Sanz, 2003). Phenylacetaldehyde (peak 30) is considered one of the compounds that contributes to the “honey-like” aroma (Blank et al., 1989).

Other natural honey volatiles include norisoprenoids, such as isophorone, ketoisophorone and β -damascenone. High levels of isophorone are characteristic of heather honeys (Häusler & Montag, 1991; Soria et al., 2003; Tan, Holland, Wilkins, & McGhie, 1989). β -Damascenone, due to its low odour threshold, is among the compounds contributing most strongly to the characteristic “honey-like” aroma.

Furan derivatives, such as furfural, 5-methylfurfural and furfuryl alcohol (peaks 13, 21 and 33, respectively) are usually indicators of heat treatment and storage conditions, and cannot therefore be considered good floral markers (D’Arcy et al., 1997; Sancho, Muniategui, Huidrobro, & Simal Lozano, 1992; Visser, Allen, & Shaw, 1988; Wootton, Edwards, & Faraji-Haremi, 1978). These compounds indicate a possible loss of freshness due to prolonged storage or exposure to high temperatures.

Table 1
Volatile compounds ($\mu\text{g}/\text{kg}$) characterized by GC–MS in 10 samples of Spanish citrus honeys by simultaneous extraction-distillation (SDE)

Peak	Compounds	Min	Max	Mean concentration	RSD (%)
(1)	3-Methyl-1-butanol	9.1	44.5	26.0	47.7
(2)	3-Methyl-3-buten-1-ol	17.8	66	34.2	39.6
(3)	2 Methyl-3[2H]-dihydrofuranone	6.7	19.3	12.2	33.8
(4)	3-Hydroxy-2-butanone	32.5	99.1	72.0	42.1
(5)	1-Hydroxy-2-propanone	15.6	55.8	27.8	48.9
(6)	2-Methyl-2-buten-1-ol	24.7	78.5	43.0	39.4
(7)	1-Hydroxy-2-butanone	6.0	16.2	9.4	44.4
(8)	Dimethyl trisulfide	12.6	39	23.8	40.7
(9)	Nonanal	34.2	134	80.5	55.0
(10)	5-Methyl-2[3H]-furanone	5.2	13	9.9	48.6
(11)	(Z)-Linalool oxide (furan type)	190.9	559.7	383.7	36.6
(12)	Acetic acid	3.3	26.4	13.4	71.4
(13)	Furfural	826	2426	1270	35.6
(14)	(E)-Linalool oxide (furan type)	124.3	247	177	22.7
(15)	1-(2-furanyl) ethanone	45.2	143	87.6	42.1
(16)	Benzaldehyde	66.8	265	154	38.8
(17)	Lilac aldehyde (<i>isomer I</i>)	85.0	468	331	46.2
(18)	Linalool	58.7	181	112	42.7
(19)	Lilac aldehyde (<i>isomer II</i>)	258	858	451	45.7
(20)	Lilac aldehyde (<i>isomer III</i>)	73.8	537	284	48.0
(21)	5-Methyl furfural	28.8	98.8	50.5	41.8
(22)	(71/43/67/55)	92.7	406	163	57.4
(23)	Lilac aldehyde (<i>isomer IV</i>)	109	732	368	50.6
(24)	Isophorone	14.9	96.4	49.1	43.4
(25)	6-Methyl-3,5 heptadien-2-one	8.0	48.0	20.1	48.0
(26)	Hotrienol	75.3	192	153	33.6
(27)	γ -Valerolactone	Tr	Tr		
(28)	Terpineol	429	1391	825	38.1
(29)	γ -Butyrolactone	4.9	9.9	6.9	24.2
(30)	Phenylacetaldehyde	605	1356	1040	30.5
(31)	Nonanol	5.5	16.3	7.7	43.5
(32)	2-Methyl butanoic acid	5.1	13.1	9.4	27.2
(33)	Furfuryl alcohol	5.5	23.5	11.0	45.0
(34)	5-Ethenyl-5-methyl-2[3H]-furanone	35.7	80.5	60.9	31.8
(35)	2H-Pyran-2-one	34.3	24.2	139	51.2
(36)	α -Terpineol	20.4	73.5	42.3	47.0
(37)	Lilac alcohol(<i>isomer I</i>)	36.8	247	132	59.1
(38)	Car-2-en-4-one	45.9	206	93.1	69.3
(39)	Pentanoic acid	4.3	8.6	6.1	23.2
(40)	Epoxylinool	16.8	41.2	29.1	28.6
(41)	Lilac alcohol (isomer II)	89.6	302	177	40.8
(42)	Ketoisophorone	14.3	49.0	28.9	46.6
(43)	Lilac alcohol (<i>isomer III</i>)	99.6	308	189	45.3
(44)	β -Damascenone	2.3	7.2	5.3	33.0
(45)	Lilac alcohol (<i>isomer IV</i>)	25.6	82.3	54.6	34.2
(46)	Hexanoic acid	23.1	165	92.1	62.9
(47)	<i>p</i> -Cymen-8-ol	19.8	59.9	36.7	56.3
(48)	Benzyl alcohol	61.9	138	92.8	34.6
(49)	2-Phenylethanol	184	809	484	45.8
(50)	Sinensal (<i>isomer I</i>)	67.3	252	137	46.7
(51)	Limonil alcohol	11.2	44.3	26.2	44.3
(52)	1- <i>p</i> -Menthen-9-ol	11.8	41.7	24.5	40.0
(53)	Heptanoic acid	10.0	45.4	26.2	46.3
(54)	<i>p</i> -Mentha-(7),8(10)-dien-9-ol	53.6	180	119	37.4
(55)	Sinensal (<i>isomer II</i>)	118	391	228	40.9
(56)	Nerolidol	22.1	93.4	54.7	47.5
(57)	Octanoic acid	40.1	175	95.6	49.7
(58)	Nonanoic acid	38.5	199	104	56.4
(59)	Thymol	4.3	10.3	7.2	32.4
(60)	Vinilguaiacol	12.3	75.2	39.4	50.8
(61)	Carvacrol	5.4	20.3	10.7	47.1
(62)	Methyl antranilate	537	2315	1175	52.4
(63)	Decanoic acid	12.7	184	93.6	71.0
(64)	3,4,5-trimethylphenol	78.2	443	257	50.5
(65)	Dodecanoic acid	16.8	250	85.6	92.2
(66)	Tetradecanoic acid	14.3	289	96.1	96.3

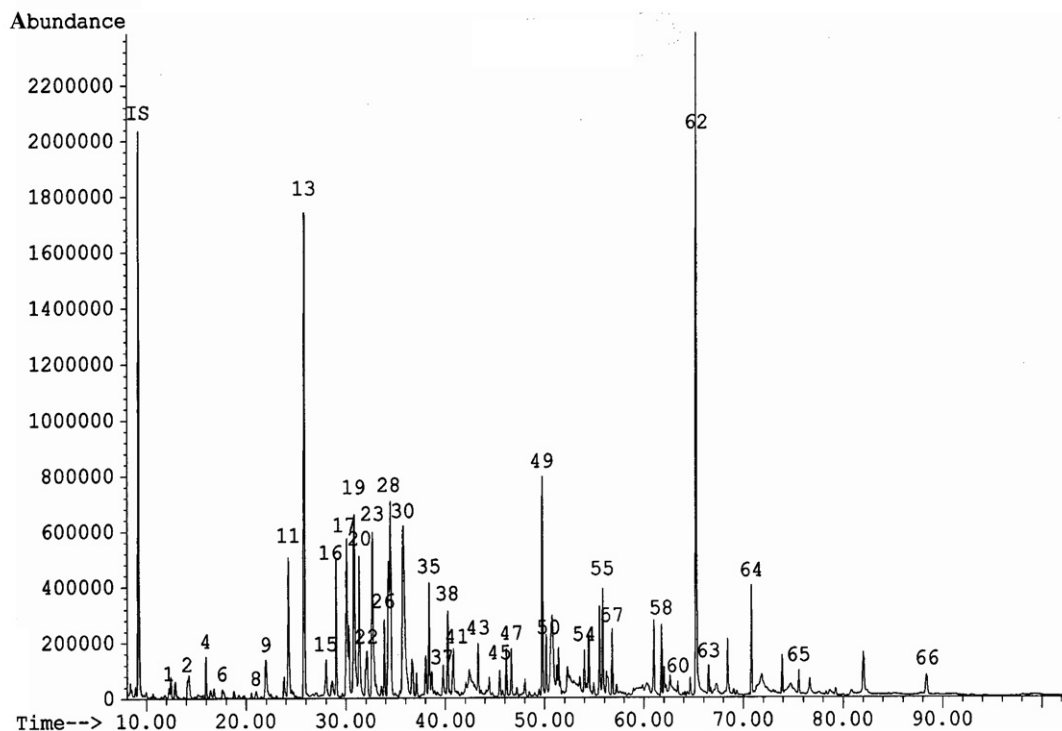


Fig. 1. GC-EM profile of SDE extract from Spanish citrus honey.

A number of 5-carbon-atom methyl alcohols detected, including 3-methyl-1-butanol, 3-methyl-3-buten-1-ol and 2-methyl-2-buten-1-ol, whilst contributing fresh notes, do not appear to be exclusive to citrus honeys, since they are not linked to floral origin (Bouseta et al., 1992; Bouseta, Scheirman, & Collin, 1996).

Spanish citrus honey extracts are rich in terpenes and derivatives. Present in the samples were linalool (peak 18), and its derivatives: (*Z*) and (*E*)-linalool oxides, lilac aldehydes (peaks 17, 19, 20 and 23) and lilac alcohol isomers (peaks 37, 41, 43 and 45), reported to be the most significant floral markers for citrus honeys (Alissandrakis et al., 2005; De la Fuente et al., 2005; Piasenzotto, Gracco, & Conte, 2003; Soria et al., 2003).

Hotrienol (peak 26), found in citrus honeys, has also been detected in honeys of various floral origins, and especially in lavender honeys (Pérez et al., 2002). The mean concentration recorded here (around 153 ppb) was lower than that reported for Australian leatherwood honey, New Zealand nodding thistle honey and Asian longan honey, whose concentrations range between 200 and 6800 ppb (Overton & Manura, 1994; Wilkins, Lu, & Tan, 1993).

Terpineol (peak 28) was one of the major volatile compounds in all Spanish citrus honeys tested, with a mean concentration of 825 ppb. It is associated with a “lilac aroma” (<http://schulen.eduhi.at/chemie/sci5a.htm>), and may be among the compounds with the greatest sensory impact. It has recently been reported as a component of Greek citrus honeys (Alissandrakis et al., 2005).

Other terpenic compounds characteristic of citrus honeys were α -terpineol, *p*-mentha,1(7),8(10)-dien-9-ol, *p*-cymen-8-ol, limonyl alcohol, 1-*p*-menthen-9-ol, and nerolidol (peaks 36, 54, 47, 51, 52 and 56), present at mean concentrations of no more than 180 ppb.

Peaks 50 and 55 corresponded to two sinensal isomers (Fig. 2), identified as volatile components of orange essence oil (Högnadóttir & Rouseff, 2003). This is the first time these compounds have been found in citrus honeys, and they have not been reported in honeys of other floral origins. Both isomers may therefore be considered, along with other compounds, as excellent floral markers enabling citrus honeys to be differentiated from honeys from other floral sources.

Methyl anthranilate (peak 62), which has been suggested as a floral marker for citrus honeys (Ferreles, Giner, & Tomás Barberán, 1994; Serra Bonvehí, 1988), was present in all samples, although in some cases at concentrations below those of other terpene compounds, such as terpineol.

In order to assess the influence of the compounds studied on overall honey aroma, odour activity values (OAV) were calculated by dividing the concentration of each com-

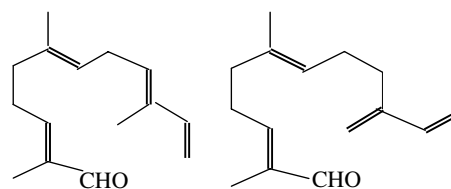


Fig. 2. Chemical structures of α and β sinensal.

Table 2
Aroma-active compounds found in citrus honey

Compounds	OAV		Odour descriptor	Odour threshold (ppb)	
	Min	Max			
(Z)-Linalool oxide	29.4	86.1	Fresh, sweet, floral	6.0–7.0	Serra Bonvehí (2005)
(E)-Linalool oxide	19.1	38.0	Fresh, sweet, floral	6.0–7.0	
Furfural	1.1	3.1	Almod, sweet, bread	776	Shimoda et al. (1996)
Benzaldehyde	1.6	6.3	Almod, sweet, fruit	41.7	
Linalool	9.8	30.1	Fresh, floral	6	
α -Terpineol	0.4	1.6	Green, floral	46	
Lilac aldehyde (isomer I)	4.3	23.4	Fresh, flowery	0.2–20	www.leffingwell.com
Lilac aldehyde (isomer II)	12.9	858	Fresh, flowery	0.2–20	
Lilac aldehyde (isomer III)	73.8	26.8	Fresh, flowery	0.2–20	
Lilac aldehyde (isomer IV)	5.5	36.6	Fresh, flowery	0.2–20	
Lilac alcohol (isomer I)	0.5	3.3	Fresh,sweet, flowery	4.0–74	
Lilac alcohol (isomer II)	1.2	4.1	Fresh,sweet, flowery	4.0–74	
Lilac alcohol (isomer III)	1.3	4.2	Fresh,sweet, flowery	4.0–74	
Lilac alcohol (isomer IV)	0.3	1.1	Fresh,sweet, flowery	4.0–74	
Hotrienol	0.7	1.7	Fresh, floral, fruity	110	Ribereau-Gayon et al. (2000)
Phenylacetaldehyde	151	339	Honey-like	4	Buttery et al. (1988)
β -damascenone	575	1800	Fruity, Sweet, honey	0.004	Ohloff (1978)
Sinensal (isomer I)	1346	5042	Sweet, orange	0.05	
Sinensal (isomer II)	2352	7810	Sweet, orange	0.05	
Methyl anthranilate	53.7	232	Grape, fruity	10	Sawall et al. (2004)

Odour activity values (OAV): compound concentrations ($\mu\text{g/l}$) divided by odour threshold.

pound by its perception threshold. Of all the compounds analyzed, only those displaying OAVs greater than 1 were deemed to contribute to honey aroma.

Table 2 shows OAVs for compounds displaying values greater than 1, together with sensory descriptors and perception thresholds taken from the literature.

The highest OAVs were found for the sinensal isomers. Given the low odour threshold of these compounds, it may be surmised that the “orange-like” aroma of citrus honeys is due mainly to the presence of sinensal isomers, probably derived from orange-blossom nectar.

The next most important compounds in terms of OAVs were β -damascenone and phenylacetaldehyde, compounds with a characteristic sweet aroma, often considered the most characteristic components of the “honey-like” aroma.

Methyl anthranilate also contributes strongly to honey aroma with essential fruity aromas. The remaining compounds listed in Table 2, with the exception of the small contributions of furfural and benzaldehyde, are terpene compounds, mostly derived from linalool. The chief contribution in terms of OAVs was from lilac aldehydes (mainly isomer II) and linalool oxides. Due to their low odour thresholds, these compounds are largely responsible for the “fresh”, “sweet” and “floral” aromas characteristic of these honeys.

To conclude, the volatile composition of citrus honeys was characterized by the presence of terpene compound concentrations, mainly linalool and derivatives (linalool oxides and lilac aldehydes and alcohols), and α -terpineol, higher than those reported in other kinds of honeys, and

by the presence of methyl anthranilate. Sinensal isomers, identified here for the first time in honeys, are suggested as the best floral markers since they are only present in citrus honeys. These isomers, together with linalool derivatives, contributed most strongly to the “floral”, “fresh” and “orange-like” aroma of citrus honeys.

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