

## Volatile Composition and Contribution to the Aroma of Spanish Honeydew Honeys. Identification of a New Chemical Marker

LUCIA CASTRO-VÁZQUEZ, M. CONSUELO DÍAZ-MAROTO, AND  
 M. SOLEDAD PÉREZ-COELLO\*

Área de Tecnología de los Alimentos, Facultad de Ciencias Químicas, Campus Universitario, 10,  
 Universidad de Castilla-La Mancha, 13071 Ciudad Real, Spain

Honeydew honeys from holm-oak, oak, and forest were isolated for aroma compounds by simultaneous distillation-extraction and analyzed by gas chromatography–mass spectrometry. In all, 66 volatile components were identified and quantified. *trans*-Oak lactone, a characteristic volatile component of oak wood, is proposed as a new chemical marker for the plant origin of honeydew honeys. Other compounds, such as aminoacetophenone and propylanisol, could be considered characteristic of holm-oak honeydew honeys. A total of 15 volatile compounds presented odor activity values (OAVs) greater than 1, with phenylacetaldehyde and  $\beta$ -damascenone being those with the highest OAVs.

**KEYWORDS:** Honey; honeydew honey; aroma; volatile compounds; gas chromatography

### INTRODUCTION

Aroma is one of the most sought-after properties in honey. The volatile compounds in honey may be derived from various origins: from the plant or nectar, from the transformation of plant compounds by the metabolism of a bee, from heating or handling during honey processing and storage, or from microbial or environmental contamination (1–6).

Unlike floral honeys, which derive from the nectar of flowering plants, honeydew honey is obtained from honeydew honey secreted by the living parts of plants or excreted onto them by sap-sucking insects. The main Spanish honeydew honey-producing plants are holm-oak and oak. Honeydew honey is increasingly valued by consumers and the food industry because of its strong and characteristic flavor that is different from floral honeys.

Unifloral honeys differ from each other in terms of their volatile-compound composition, which undoubtedly influences the sensory characteristics of individual honey types (7–11). Honeydew honeys differ in chemical composition from nectar honeys; they are darker and also have a higher pH and greater acidity (12). Although their aroma is characteristic and differs from that of nectar honeys, little information is available regarding the volatile fraction in honeydew honeys.

The determination of the botanical origin is more difficult in honeydew honeys, because determination of the honeydew type for a given honey cannot be carried out by palynological analysis. The search for compounds that differentiate these types of honey is of particular interest.

With regard to the potential role of volatile compounds in distinguishing honeys as a function of origin, a number of

authors suggest that certain specific compounds (terpenes, norisoprenoids, benzene compounds, and their derivatives) are characteristic of a given floral origin; because these compounds are found only in certain types of honey, they may in some cases be considered “floral markers” (1, 13–17). In other cases, the floral origin is determined by a greater concentration of certain compounds in some types of honey than in others or by the absence of determined compounds (11, 18–21).

There is some disagreement over which compounds serve as markers for a given type of honey, perhaps because of differences between plant varieties, geographical origins, or beekeeping practices. Moreover, methods of extracting the volatile fraction may display a varying degree of selectivity and effectiveness depending upon the compounds involved (22, 23).

Liquid–liquid extraction with a range of solvents has successfully been used to identify floral markers in Australian and German honeys of various floral origins (3, 14, 15, 24–27). Other authors have used simultaneous distillation-extraction (SDE) to characterize floral origin and establish markers in European chestnut, lime-tree, and heather honeys (16, 19) and more recently to distinguish between French and Portuguese lavender honeys (6). Headspace analysis techniques such as solid-phase microextraction (SPME) have yielded interesting qualitative data but have proven less effective for quantitative analysis (10, 28).

Suggested compounds for discriminating between nectar and honeydew honeys include 1-(2-furanyl)-ethanone, 2,3-butane-diol, 3-hydroxy-2-butanone, and 1-hydroxy-2-propanone (12); high acetic acid concentrations are also reported to be characteristic of honeydew honeys (29), although the formation of this compound by microbial metabolism cannot be ruled out.

The aim of this study is to determine the components of the volatile fraction of Spanish honeydew honeys and their contribution to the aroma of these honeys and also to find if these

\* To whom correspondence should be addressed. Telephone: 00-34-926-295300, ext. 3421. Fax: 00-34-926-295318. E-mail: soledad.perez@uclm.es.

**Table 1.** Mean Concentration ( $\mu\text{g}/\text{kg}$ ), Relative Standard Deviation (%), and Retention Index (RI) of Volatile Compounds in Honeydew Honey Extracts

peak	compounds	RI	holm-oak ( $n = 3$ )		oak ( $n = 2$ )		forest ( $n = 4$ )	
			mean concn	SD	mean concn	SD	mean concn	SD
1	dimethyl disulfide	1061	114.1	30.8	30.2	24.8	25.9	9.5
2	3-methyl-1-butanol	1141	212.0	57.3	206.4	65.7	186.2	44.2
3	3-methyl-3-buten-1-ol	1179	83.8	65.5	54.3	15.5	81.4	24.2
4	2-methyl-3-[2H]-dihydrofuranone <sup>a</sup>	1200	58.4	19.1	82.3	101.9	77.4	18.1
5	3-hydroxy-2-butanone	1213	238.2	163.8	639.9	16.8	272.6	88.5
6	1-hydroxy-2-propanone <sup>a</sup>	1227	47.6	3.8	120.6	31.7	66.8	19.0
7	2-methyl-2-buten-1-ol	1242	125.7	117.7	69.3	17.3	95.2	41.7
8	3-hydroxy-2-pentanone <sup>a</sup>	1261	62.2	35.0	71.1	6.6	56.6	10.5
9	1-hexanol	1269	12.4	5.9	17.8	0.6	15.2	3.0
10	1-hydroxy-2-butanone	1291	42.9	16.9	116.3	32.0	65.1	17.3
11	(Z)-3-hexen-1-ol	1298	33.5	16.6	22.3	5.9	31.6	10.3
12	nonanal	1299	31.7	15.5	38.9	16.5	32.7	16.6
13	5-methyl-2 [3H]-furanone <sup>a</sup>	1346	15.1	5.8	12.4	5.5	17.8	7.0
14	(Z)-linalool oxide (furan type)	1351	170.3	86.7	188.3	5.9	166.9	26.8
15	acetic acid	1355	39.6	19.9	116.6	1.7	42.1	20.2
16	furfural	1377	2350.1	1014.7	2470.3	585.8	1811.3	276.6
17	1-(2-furyl)-ethanone <sup>a</sup>	1411	162.5	36.0	188.1	58.8	149.0	23.1
18	benzaldehyde	1427	159.7	82.3	92.0	44.8	95.6	5.6
19	2-methyl propanoic acid	1459	106.8	25.1	159.7	20.7	108.6	27.0
20	5-methylfurfural	1474	179.2	88.6	113.8	39.0	142.0	20.3
21	2-cyclopentene-1,4-dione <sup>a</sup>	1483	38.9	14.6	100.3	17.7	48.5	6.0
22	isophorone	1489	162.5	217.6	93.8	77.5	41.2	9.5
23	hotrienol <sup>a</sup>	1504	124.5	56.1	171.4	85.1	267.9	94.0
24	butanoic acid	1514	35.8	16.1	74.9	80.8	54.1	7.3
25	$\gamma$ -butyrolactone	1523	51.3	11.0	124.6	27.0	54.6	12.5
26	phenylacetaldehyde	1541	5372.9	7027.9	2287.8	347.2	3025.7	608.6
27	1-nonanol	1553	30.6	16.5	49.7	20.8	26.2	6.7
28	2-methyl butanoic acid	1553	58.7	22.0	60.5	17.3	49.4	10.5
29	furfuryl alcohol	1553	77.2	39.0	149.8	72.2	886.2	74.9
30	ketoisophorone	1581	210.5	175.9	161.4	73.3	87.9	40.8
31	$\alpha$ -terpineol	1584	19.5	6.8	45.4	33.0	29.0	5.8
32	3-formyl-pyridine <sup>a</sup>	1593	152.1	225.6	28.5	11.3	20.0	5.7
33	2-furancarboxylic acid <sup>a</sup>	1607	76.7	15.8	170.9	43.9	76.9	14.9
34	car-2-en-4-one <sup>a</sup>	1613	33.2	12.5	153.1	43.2	47.4	22.6
35	pentanoic acid	1616	23.7	10.7	14.2	3.7	27.8	3.6
36	epoxylinalool (isomer I) <sup>a</sup>	1619	42.1	31.6	69.7	34.5	68.6	29.4
37	epoxylinalool (isomer II) <sup>a</sup>	1643	0.0		93.3	21.0	0.0	
38	heptadecane	1651	268.1	45.0	140.0	0.1	169.4	33.2
39	1-phenylethanol+1-phenyl-1,2-propanedione <sup>a</sup>	1689	54.1	25.5	116.7	19.2	45.0	5.1
40	$\beta$ -damascenone	1698	15.4	4.8	21.6	1.3	31.4	11.2
41	hexanoic acid	1714	54.1	25.5	117.3	30.9	269.7	109.5
42	<i>p</i> -cymen-8-ol <sup>a</sup>	1723	15.4	4.8	33.3	14.3	40.3	13.6
43	2-hydroxycineol <sup>a</sup>	1730	0.0		78.8	66.3	27.8	17.1
44	guaiaicol	1734	19.1	14.1	21.9	2.6	17.5	9.8
45	benzyl alcohol	1747	492.7	435.3	320.8	95.2	284.6	101.5
46	2-phenylethanol	1779	1588.0	451.9	567.8	1.5	315.3	71.7
47	heptanoic acid	1814	36.9	10.2	61.4	23.5	86.0	38.5
48	<i>trans</i> -oak lactone	1824	0.0		74.4	56.3	10.9	15.4
49	2-acetylpyrrol <sup>a</sup>	1836	21.0	9.6	162.8	35.4	43.6	14.0
50	nonadecane	1844	248.7	242.4	207.9	134.7	80.7	18.5
51	<i>p</i> -anisaldehyde	1888	74.2	126.8	47.4	48.8	16.3	19.9
52	octanoic acid	1913	311.8	165.1	235.8	43.5	439.1	186.4
53	propylanisol	1927	1047.6	1789.2	196.2	160.4	143.1	160.4
54	<i>p</i> -cresol	1939	17.2	3.6	26.3	12.2	10.3	4.6
55	3-hydroxy-acetophenone	1975	30.9	5.8	72.0	36.4	120.6	62.6
56	nonanoic acid	2010	93.7	51.1	114.6	16.3	296.0	96.1
57	eugenol	2015	7.4	11.1	65.5	6.6	34.3	10.6
58	thymol	2032	6.5	1.1	4.7	1.3	1.0	0.0
59	vinylguaiaicol	2044	76.8	37.3	112.5	38.9	64.5	16.5
60	carvacrol	2059	10.0	3.7	5.4	1.3	1.0	0.0
61	2-aminoacetophenone	2064	186.1	228.2	0.0		109.5	157.5
62	decanoic acid	2108	43.8	10.7	94.1	77.4	201.4	83.8
63	3-phenyl-2-propen-1-ol	2125	70.7	88.0	77.0	24.5	26.2	8.0
64	3,4,5-trimethylphenol	2201	545.7	221.6	482.1	113.4	754.4	314.2
65	dodecanoic acid	2305	40.8	9.5	54.1	4.0	404.6	114.6
66	tetradecanoic acid	2514	59.2	12.8	5.1	2.6	529.0	242.2

<sup>a</sup> Compounds identified using Wiley Library.

compounds can be used to distinguish among the different honeydew honey types.

## MATERIAL AND METHODS

**Samples.** The study was carried out on 9 Spanish honeydew honey samples obtained in the autumn from the Castilla La-Mancha and

Extremadura regions. Honeys were selected from holm-oak, oak, and forest (3, 2, and 4 samples, respectively). Forest honeys are a mixture of holm-oak and oak honeydew honeys.

**SDE.** To obtain the volatile extracts, a microscale SDE apparatus (Chrompack, Middelburg, The Netherlands) was used as previously described by Godefroot et al. (30). A total of 15 g of honeydew honey

**Table 2.** Most Potent Odorants Found in Honeydew Honeys (Holm-Oak, Oak, and Forest Honeys)

compounds	OAV		odor descriptor	odor threshold (ppb)	reference
	min	max			
$\gamma$ -butyrolactone	1.5	3.6	caramel, sweet	35 <sup>a</sup>	36
$\beta$ -damascenone	1709	3489	fruity, sweet	0.009 <sup>b</sup>	37
eugenol	1.2	10.9	clove, spice, honey-like	6 <sup>a</sup>	37
<i>trans</i> -oak lactone	0.5	3.7	oak, coconut	20 <sup>a</sup>	42
acetophenone	1.7	2.9	sweet, pungent, jasmine	65 <sup>a</sup>	43
vinylguaiaicol	21.5	37.5	spice, clove, curry	3 <sup>a</sup>	43
hotrienol	1.1	2.4	floral, fresh, fruity	110 <sup>a</sup>	44
isophorone	0.2	0.8	peppermint-like	200 <sup>a</sup>	46
<i>p</i> -cresol	22.3	57.2	medicine, phenolic, smoke	0.46 <sup>b</sup>	47
furfural	0.4	3.2	almond, sweet, bread	776 <sup>b</sup>	48
benzaldehyde	2.2	3.8	almond, sweet, fruit	41.7 <sup>b</sup>	48
phenylacetaldehyde	572	1343	honey-like	4 <sup>a</sup>	48
$\alpha$ -terpineol	0.4	1.0	green, floral	46 <sup>b</sup>	48
guaiaicol	17.5	21.9	smoke, sweet, medicine	1 <sup>b</sup>	48
<i>p</i> -anisaldehyde	0.5	2.2	herbaceous, infusion, anise	33.1 <sup>b</sup>	48

<sup>a</sup> Threshold value in water. <sup>b</sup> Threshold value in air.

dissolved in 40 mL of Milli-Q water and 15  $\mu$ L of 2-pentanol (1.0 g/L) as an internal standard was extracted under atmospheric conditions for 2 h using dichloromethane as an extractive solvent. The extracts obtained were concentrated to 200  $\mu$ L under nitrogen flow. This method was previously tested using a model solution containing sugar and amino acids at identical concentrations to that in honeys (22, 23).

**Chromatographic Conditions for the Analysis of Volatiles.** All volatile extracts were analyzed using a Hewlett–Packard G 1800 B GCD System (Hewlett–Packard, Palo Alto, CA), which consists of a gas chromatograph, an electron ionization detector (EID, 70 eV), and a data system. Samples (2  $\mu$ L) were injected in splitless mode during 0.6 min (split ratio of 1:20) on a BP-21 capillary column (50 m  $\times$  0.32 mm  $\times$  0.32  $\mu$ m of film thickness). The temperature program of the column was 60 °C (3 min), 2 °C/min, and 200 °C (30 min). The carrier gas was helium at a flow of 0.8 mL/min. Inlet and transfer line temperatures were 250 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.

Identification of the volatile components was performed comparing their gas chromatography (GC) retention indices and mass spectra with authentic standards from Sigma–Aldrich. The tentative identification of compounds for which it was not possible to find reference volatiles was carried out by a comparison of their mass spectra with spectral data from the Wiley G 1035 A library. Semiquantitative analysis of the compounds was performed assuming that the internal standard and honey volatiles had the same response factor. The concentration of each compound was calculated in  $\mu$ g/kg.

## RESULTS AND DISCUSSION

A total of 66 volatile compounds were identified by gas chromatography–mass spectrometry (GC–MS) in honey samples for holm-oak, oak, and forest honeydews. Mean concentrations in  $\mu$ g/kg and standard deviations are shown in **Table 1**.

Compounds identified included alcohols, aldehydes, and aliphatic acids usually found in honeys, some of which have been associated with microbial metabolism (9).

All samples contained various furan derivatives (furfural, furfuryl alcohol, 5-methylfurfural) formed by the degradation of sugars, as well as compounds formed by Maillard reactions (acetylpyrrol) induced by heat processing or prolonged storage of honeys. Although their odor detection thresholds are high, at elevated concentrations, these compounds may influence honey aroma by giving rise to toasted or burnt notes, with an attendant deterioration in quality (31–33).

The SDE procedure could generate artifacts, for instance, furfural and others. For this reason, we have previously tested the production of artifacts using a model solution containing

sugar and amino acids at identical concentrations to that in honeys. When the SDE methods were applied, only little quantities of furfural and phenylacetaldehyde were formed (22). On the other hand, the recovery of compounds related to the plant, such as terpenic or norisoprenoid compounds, was better using SDE than other methods, such as liquid–liquid or solid-phase extraction (23).

Clearly, any search for compounds to discriminate among floral origins must be focused on plant-derived compounds and their metabolites, such as terpenes and terpene derivatives, benzene derivatives, and norisoprenoids.

Some benzene derivatives are found at high concentrations in all honeys; these include benzaldehyde, benzyl alcohol, 2-phenylethanol, and phenylacetaldehyde (21, 34, 35), which was the most abundant compound in the honeys analyzed in this study.

Others benzene and phenolic compounds identified, including guaiaicol, *p*-anisaldehyde, and propylanisol, could be considered characteristic of honeydew honeys because they have not been previously reported in floral honeys from eucalyptus, citrus, rosemary, thyme, and lavender (39).

Honeydew honeys studied contained terpene compounds including linalool oxide, hotrienol,  $\alpha$ -terpineol, eugenol, car-2-en-4-one, two isomers of epoxylinool, *p*-cymen-8-ol, and 2-hydroxycineol, most of which are also found in unifloral citrus, lavender, and eucalyptus honeys (10, 17, 28, 40).

Certain norisoprenoids, such as isophorone and ketoisophorone, were abundant in holm-oak and oak honeydew honeys and are also reported as characteristic components of eucalyptus honeys, particularly in Australia (10, 34). Aminoacetophenone deriving from amino acid metabolism together with propylanisol were found in high concentrations in holm-oak honeys. The former has been previously suggested as a discriminating compound for chestnut honeys (1).

A previous study highlights high concentrations of 2,3-butanediol and 1-hydroxy-2-propanone in honeydew honeys (12). Actually, hydroxyketones and particularly 3-hydroxy-2-butanone were detected in our volatile extracts but at lower concentrations than those found in eucalyptus honeys (35).

Oak honey samples and some of the forest honeys contained *trans*- $\beta$ -methyl- $\gamma$ -octalactone, also known as oak-lactone because this compound is found only in oak wood. This compound is familiar in winemaking, because it is responsible for the oak aroma of barrel-aged wines (41). It may be found in two isomeric forms that have very low odor thresholds: 1 ppb for the *cis* isomer and 20 ppb for the *trans* isomer (42).

As far as the authors are aware, this compound has not previously been reported in honeys, and because it is present only in oak wood, it could be an excellent marker for oak honeys. Its presence would indicate that a given honey consists partly or wholly of oak honey. Two of the four forest honey samples studied contained oak-lactone and can therefore confidently be described as oak honey blends.

To assess the influence of the compounds studied on overall honey aroma, odor activity values (OAVs) were calculated by dividing the concentration of each compound by its perception thresholds. Of all of the compounds analyzed, only those displaying OAVs greater than 1 were deemed to contribute to honey aroma (38).

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

The highest OAV values were recorded for phenylacetaldehyde and  $\beta$ -damascenone, which, together with  $\gamma$ -butyrolactone, are responsible for sweet and honey-like aromas (13, 36, 37).  $\beta$ -Damascenone has a low odor threshold (0.009 ppb) and a characteristic sweet and fruity aroma (13, 31). The concentrations detected exceeded the odor threshold in all of the honeydew honeys analyzed.

Other compounds, including guaiacol, vinyl-guaiacol, eugenol, and *p*-cresol, displaying characteristic spicy, wood, and smoky aromas, may influence honeydew honey sensory properties (43).

*trans*-Oak lactone, with its characteristic oaky aroma, was found in all oak and two forest honeys in amounts exceeding its odor threshold and can therefore be regarded as having a marked impact on oak honey aromas.

Fresh and floral notes are mainly presented by  $\alpha$ -terpineol, hotrienol, and acetophenone (9, 43, 44). *p*-Anisaldehyde has a herbaceous and anise odor (45), while isophorone is responsible for the peppermint-like impression (46).

In conclusion, from the results of the qualitative and quantitative analysis of our samples, high concentrations of benzene and phenolic compounds could be considered characteristic of honeydew honeys. *trans*-Oak lactone is proposed as an excellent marker for oak honeydew honeys, because it is found only in oak wood.

The characteristic aroma of honeydew honeys may be ascribed to the synergic contribution of 15 volatile compounds found at levels exceeding the odor perception threshold in the honeys studied. The highest OAV values were recorded for phenylacetaldehyde and  $\beta$ -damascenone, followed by phenolic compounds, which, in quantitative and sensory terms, were the most characteristic of honeydew honeys.

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