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To cite this article: Amina Bouseta, Sonia Collin & Jean-Pierre Dufour (1992) Characteristic aroma profiles of unifloral honeys obtained with a dynamic headspace GC-MS system, Journal of Apicultural Research, 31:2, 96-109, DOI: [10.1080/00218839.1992.11101268](https://doi.org/10.1080/00218839.1992.11101268)

To link to this article: <http://dx.doi.org/10.1080/00218839.1992.11101268>



Published online: 24 Mar 2015.



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# Characteristic aroma profiles of unifloral honeys obtained with a dynamic headspace GC-MS system

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(Received 26 May 1992,  
accepted subject to revision 17 November 1992,  
accepted for publication 16 December 1992)

## SUMMARY

The volatile compounds of 84 unifloral honeys (from 14 unifloral sources in 10 countries) were studied with a dynamic headspace gas chromatograph coupled directly to a mass spectrometer system. The average concentrations of the 47 compounds identified are tabulated, together with relevant chromatographic data. Some compounds appeared to be characteristic of the floral source, particularly in lavender (caproaldehyde (hexanal) and heptanal), fir (acetone), eucalyptus (diketones, sulphur compounds, alkanes) and dandelion and rape (three unidentified compounds) honeys. It is concluded that further studies on less volatile compounds are needed in order to further characterize aromas of unifloral honeys and to differentiate honeys derived from floral sources such as chestnut, orange, lime and robinia. Some compounds, such as alcohols, branched aldehydes and furan derivatives, reflected the microbiological purity and processing and storage conditions of the honeys, rather than their floral origins.

**Keywords:** honey, botanical composition, aroma, volatile compounds, gas chromatography, mass spectrometry

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## INTRODUCTION

The floral origin of honeys is routinely authenticated by pollen analyses and physico-chemical and organoleptic determinations (Accorti *et al.*, 1986; Apimondia, undated; Crane *et al.*, 1984; Gonnet & Vache, 1984; Maurizio, 1979). Although such data have been used to index several unifloral honeys (Barth, 1990; Feller-Demalsy *et al.*, 1987a, 1987b, 1989; Ricciardelli D'Albore, 1988), the characteristic parameters used to certify some floral origins are very often limited. The authenticity of lavender (*Lavandula angustifolia*), citrus (*Citrus* spp.), and rosemary (*Rosmarinus officinalis*) honeys, for instance, is based solely on a minimum percentage of the specific pollen, the respective criteria being 10–13%, 15%, and 20% (Serra Bonvehi *et al.*, 1987, 1988b). In some cases, when the floral pollen yield is extremely low or when the plant is sterile (like hybrid lavender), organoleptic analyses alone are used to identify the floral origin.

In ascertaining a honey's floral origin and assessing its overall flavour quality, it would be most useful to have a reliable range of markers. Unifloral honeys possess highly characteristic aromas due to the presence of various particular volatile constituents probably derived from the original nectar sources. Citrus honeys (e.g. orange and lemon) are known to contain methyl anthranilate, a compound which other honeys virtually lack, at a concentration above 0.5 ppm (2–4 ppm for orange samples) (Serra Bonvehi, 1988a; White, 1975). In linden (*Tilia* spp.) honey, the alcohol 8-*p*-menthen-1,2-diol is the primary volatile compound among the 27 compounds identified by Tsuneya *et al.* (1974). Steeg and Montag (1987, 1988a, 1988b, 1988c) have studied the aromatic carboxylic acids and glycosidically bound aromatic compounds of honeys. While high amounts of phenyl lactic acid (above 200 mg/kg) and phenyl propionic acid characterize heather (*Calluna vulgaris*) and rape (*Brassica napus* var. *oleifera*) honeys respectively, buckwheat (*Fagopyrum esculentum*) honey can be identified by the absence of phenyl acetic acid. An aroma study of 7 unifloral Australian honeys revealed the presence of a range of hydrocarbons and oxygenated compounds, some of which were suggested to be unique to the floral sources (Graddon *et al.*, 1979). Among these, high concentrations of acetoin and other hydroxyketones were characteristic of *Eucalyptus* spp. and *Banksia* spp. honeys. Tan *et al.* (1988, 1989, 1990) identified compounds (aliphatic and aromatic acids, diacids, phenols and/or degraded carotenoids) which appear to characterize thyme (*Thymus vulgaris*), willow (*Salix* spp.), heather (*Calluna vulgaris*), white clover (*Trifolium repens*), manuka (*Leptospermum scoparium*), and kanuka (*L. ericoides*) honey floral sources. Manuka and kanuka honeys contain much higher aromatic acid concentrations than honeys derived from white clover. The occurrence of degraded carotenoids (3,5,5-

trimethyl-cyclohex-2-ene derivatives) characterize heather honeys. In thyme honey, 1-(3-oxo-*trans*-1-butenyl)-2,6,6-trimethylcyclohexane-*trans,cis*-1,2,4-triols were found at levels above 40 mg/g honey. Recently, Häusler *et al.* (1990) attempted to characterize the specific honey flavour and floral origin of the honeys from different floral sources on the basis of differences in the occurrence and quantity of aromatic aldehydes and acetophenone.

In the present work, we have investigated the headspace volatile compounds of several honeys (84 samples from 14 unifloral sources in 10 countries), using mild conditions and a dynamic headspace technique. Qualitative and quantitative compositions of headspace volatile fractions clearly show it is feasible to distinguish volatile compounds derived from the floral source from ones which appear during processing and storage. These data, combined with the analysis of solvent-extracted-aroma compounds (Bouseta *et al.*, in preparation) contribute to a better understanding of the factors that cause flavour differences between honeys.

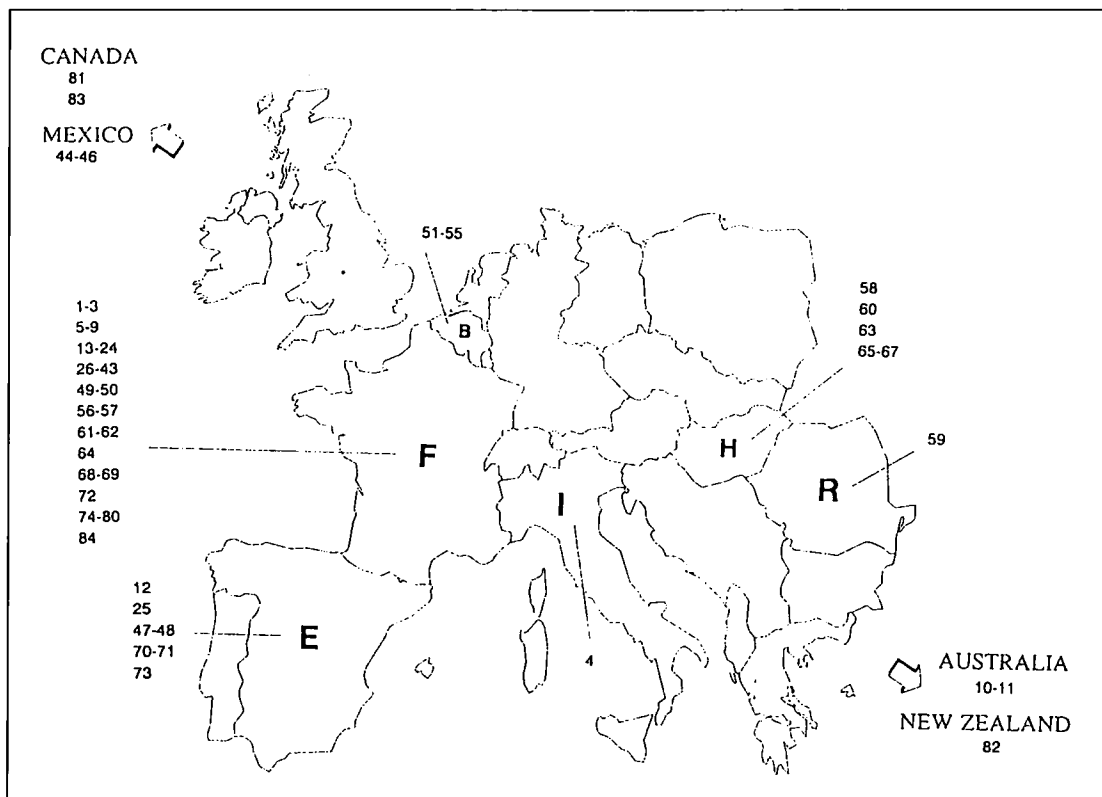
## MATERIALS AND METHODS

### Honey

Eighty-four unifloral honeys from various countries were selected from 151 samples (fig. 1). Screening for floral purity was based on pollen analyses (Louveaux *et al.*, 1978), an organoleptic test, conductivity, pH, titratable acidity (Journal officiel, 1977), and sugar composition (Pourtallier & Rognone, 1977). All samples used in this study and regarded as unifloral honeys met the requirements defined by various authors (Accorti *et al.*, 1986; Apimondia, undated; Crane *et al.*, 1984; Gonnet & Vache, 1984; Maurizio, 1979).

### Chemicals used

Acetaldehyde (> 99.5%), butanal (> 99%), caproaldehyde (98%), diacetyl (> 99.5%), dimethylsulphide (97%), ethyl propionate (> 99%), 3-methylbutanone-2 (99%), pentanedione-2,3 (> 99%), pentanone-2 (> 99%), and valeraldehyde (> 98%) were from Fluka Chemika (Buchs, Switzerland). Benzaldehyde (99+%), ethyl formate (97%), heptanal (95%), 2-methylbutanal (98%), methyl formate (99+%), 2-methylpentanal (98%), pentane (99+%),  $\alpha$ -pinene (98%), *m*-xylene (99%), *p*-xylene (99+%), and *o*-xylene (98%) were from Aldrich Chemie (Steinheim, Germany). Acetone (99.5%), butanone-2 (99+%), furan (99+%), dimethyldisulphide (p.a.), ethyl acetate (99.5+%), heptanone-2 (98%), isobutanol (98%), limonene (97%), 3-methylbutanal (98%), 2-methylbutanol (98%), 3-methylbutanol (98%), 2-methylfuran (99%), nonane (99%), octane (99+%), and styrene (p.a.) were from Janssen Chimica (Geel, Belgium). Butanol (p.a.), 1,3-



**FIG. 1. Geographical origin of the 84 unifloral honeys. 1–8, chestnut; 9, dandelion; 10–12, eucalyptus; 13–19, fir; 20–32, lavender; 33–40, lime; 41, lucerne; 42–50, orange; 51–56, rape; 57–58, rhododendron; 59–70, *Robinia*; 71–76, rosemary; 77–80, sunflower; 81–84, white clover.**

dichlorobenzene (99%), propanol (p.a), and tetrahydrofuran (99.8%) were from Merck (Darmstadt, Germany). Dichloromethane (99.9%) was from Romil Chemicals (Leicester, UK). Isobutanol (p.a) and toluene (99%) were from UCB Chemical (Leuven, Belgium). Hexane was from BDH Chemical (Poole, UK). Ethanol (99.7%) was from Analyticals CARLO ERBA (Milan, Italy).

### Sample preparation

Honey (5 g) was dissolved in 15 ml cold ultrapure water (Milli-Q water purification system, Millipore). Nine ml of this solution were poured into the cold purge vessel. As an internal standard, 9 ppm 2-methyl-pentanal in water (25  $\mu$ l) was added.

### Dynamic headspace injector operating conditions

A purge-and-trap injector from Chrompack (Chrompack Belgium NV, Antwerp, Belgium) was used. Chromatographic injection was achieved in three steps:

**Precooling of the cold trap (metal capillary):** the trap was cooled for 1 min with a stream of liquid nitrogen.

**Purge of the sample:** the temperature of the purge vessel was set at 70°C and the sample directly purged with nitrogen gas (10 ml/min) for 15 minutes. The gas stream was passed through a condenser maintained at –15°C by means of a cryostat (Colora WK 15) to remove water vapour, then through an oven at 200°C. The honey volatiles were finally concentrated in the cold trap maintained at –95°C (liquid nitrogen).

**Desorption of the volatiles:** cooling was stopped and the surrounding metal capillary immediately heated to 220°C for 5 minutes. The carrier gas swept the trapped compounds into the analytical column.

### Gas chromatography analytical conditions

The apparatus used was a Hewlett Packard Model 5890 gas chromatograph equipped with a flame ionization detector and an integrator (Shimadzu CR3A). Analysis of honey volatile compounds was carried out on a 50 m x 0.32 mm, wall-coated, open tubular (WCOT) apolar CP-SIL5 CB capillary column (film thickness, 1.2  $\mu$ m). Oven temperature, initially kept at 30°C for 15 min, was programmed to rise from

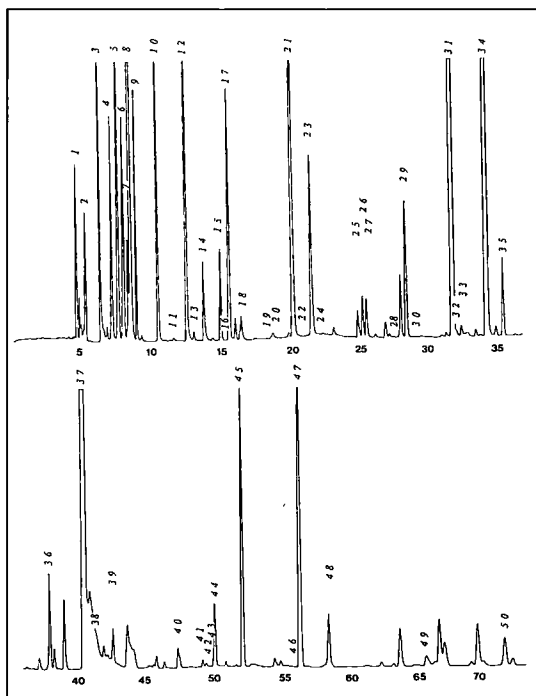
**TABLE 1. Compounds identified in 84 unifloral honeys using a purge-and-trap injector.**

Peak numbering (PN) refers to peak numbers as in figure 2; RT = column retention time (min); min., max. and average = minimum, maximum and average concentrations in honeys (ppb); *n* = number of calibrated points in the given range; *r* = correlation coefficient; GC = gas chromatographic retention data (peak enhancement on GC by coinjection with authentic standard compounds); MS = mass spectral data compared with those of the library and/or standard compounds.

PN	RT	Concentration (ppb)		Calibration parameters		Identification by			
		min.	max.	average	slope		<i>r</i>	<i>n</i>	range
<b>Aldehydes</b>									
1	4.88	0	6671	925	16.1	0.988	8	0-3250	GC,MS
10	10.71	4	695	25	406	0.989	12	0-155	GC,MS
13	13.22	0	2	0	301	0.995	12	0-160	GC,MS
21	20.34	0	252	22	424	0.993	12	0-200	GC,MS
23	21.64	0	482	18	512	0.985	12	0-135	GC,MS
27	25.45	0	14	0	388	0.986	12	0-155	GC,MS
29	28.48	-	-	-	-	-	-	-	MS
34	33.93	-	-	-	-	-	-	-	-
36	38.27	0	922	90	332	0.997	7	0-180	GC,MS
43	49.86	0	241	25	260	0.996	7	0-160	GC,MS
46	56.07	0	14	3	615	0.990	5	0-140	GC,MS
<b>Ketones</b>									
4	7.33	0	749	230	137	0.993	7	0-475	GC,MS
12	12.77	0	2642	238	26.4	0.974	8	0-275	GC,MS
14	13.83	0	191	29	116	0.963	8	0-215	GC,MS
22	21.43	0	36	0	256	0.992	7	0-160	GC,MS
25	24.65	0	14	0	134	0.999	7	0-325	GC,MS
26	25.22	0	158	4	45.1	0.989	7	0-250	GC,MS
41	48.88	0	25	0	271	0.991	7	0-175	GC,MS
<b>Cyclic compounds</b>									
5	7.93	0	256	14	533	0.998	8	0-225	GC,MS
15	15.33	0	29	0	486	0.990	8	0-260	GC,MS
19	18.26	0	252	4	140	0.996	8	0-140	GC,MS

TABLE 1 continued.

	PN	RT	Concentration (ppb)		Calibration parameters		Identification by
			min.	max.	slope	r	
<b>Alcohols</b>							
ethanol	3	6.51	1534	673672	2.23	0.987	0-6500
propanol	11	11.52	0	194	10.3	0.994	0 230
isobutanol	20	18.53	0	310	22.1	0.986	0-250
butanol	24	21.93	-	-	-	-	-
3-methyl-butanol	32	31.70	0	1469	15.3	0.993	0-325
2-methyl-butanol	33	32.20	0	194	30.9	0.989	0-500
<b>Esters</b>							
methyl formate	2	5.57	0	688	85.0	0.988	0-160
ethyl formate	7	8.51	0	1753	135	0.992	0-180
ethyl acetate	18	16.30	0	173	173	0.990	0-280
ethyl propanoate	30	28.68	5	14	343	0.996	0-170
<b>Sulphur compounds</b>							
dimethylsulphide	8	8.81	4	1537	365	0.999	0-170
dimethyldisulphide	31	31.78	0	328	372	0.997	0-200
dimethyltrisulphide	48	58.45	-	-	-	-	-
<b>Hydrocarbons</b>							
pentane	6	8.32	-	-	-	-	-
hexane	17	15.99	0	842	91.3	0.991	0-375
toluene	35	35.27	0	54	654	0.994	0-250
octane	37	40.83	10	12469	37.3	0.998	0-1200
9-C alkane	39	42.84	-	-	-	-	-
p- and m-xylene	40	47.39	0	54	233	0.985	0-450
styrene	42	49.35	0	61	590	0.998	0-200
o-xylene	44	50.73	0	7	318	0.996	0-425
nonane	45	52.03	0	133	109	0.990	0-230
α-pinene	47	56.33	0	8	1636	0.996	0-117
limonene	50	71.27	0	4	2046	0.988	0-112
<b>Chlorinated compounds</b>							
dichloromethane	9	9.20	0	6494	218	0.990	0-240
1,3-dichlorobenzene	49	65.50	0	601	117	0.999	0-600



**FIG. 2.** GC trace obtained for eucalyptus honey (no. 11) volatile compounds. Numbering is as in table 1.

30°C to 100°C at 2°C/min, remaining at the maximum temperature for 30 min thereafter. Nitrogen carrier gas was used at a flow rate of 1.5 ml/min. Injection and detection temperatures were 200°C and 220°C respectively. All analyses were done in duplicate and results averaged. The minimum peak area was set at 500 µV.

**Gas chromatography–mass spectrometry analytical conditions**

The column (see above) was directly connected to an HP5988 quadrupole mass spectrometer. The carrier gas was helium. Electron impact mass spectra were recorded at 70 eV. Spectral recording throughout elution was automatically performed with the HP59970C MS ChemStation analytical workstation. Volatile compounds were identified on the basis of mass spectra (NBS/EPA/NIH library) and peak enhancement by coinjection with authentic standard compounds.

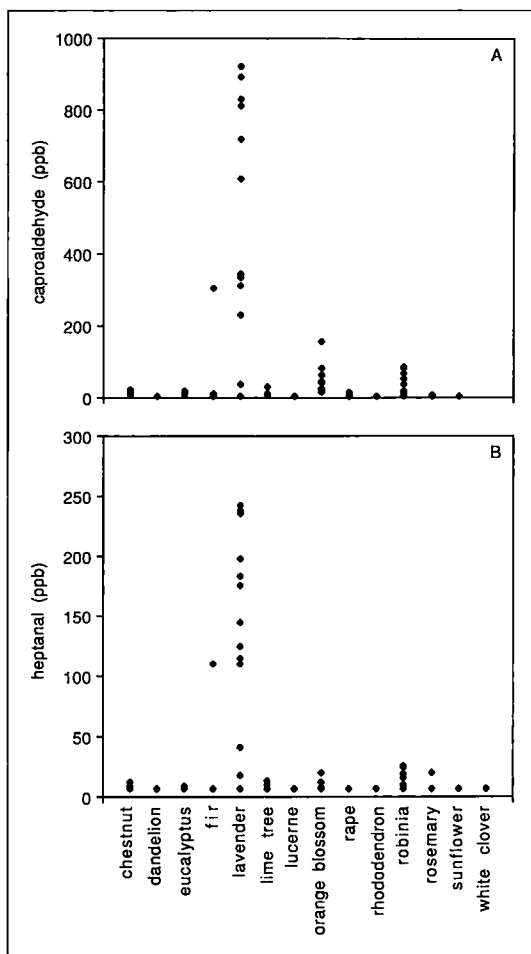
**Calibration**

Response factors for the various volatile constituents were determined by adding standard amounts of pure compounds to the honey samples, from stock solutions in ultrapure water (except for benzaldehyde, pinene and limonene whose stock solutions were prepared in acetone).

**RESULTS AND DISCUSSION**

**Analysis of honey volatile compounds**

The honey volatile compounds (ppb and sub-ppb) were analysed without any pretreatment and under mild conditions using a dynamic headspace injector. No change in honey volatile composition was observed during the time of analysis (data not shown) under the chosen analytical conditions (see Materials and methods section). Figure 2 presents a typical chromatogram of honey volatile compounds. Over 60 compounds were clearly separated, 47 of which could be identified with varying degrees of certainty. Identification and calibration parameters for most of them are summarized in table 1. The compounds belonged to eight major groups: aldehydes, ketones, ethers, alcohols, esters, sulphur compounds, hydrocarbons, and chlorinated compounds.



**FIG. 3.** Distribution of caproaldehyde and heptanal in the 84 honeys.

**TABLE 2. Concentration of some aldehydes, alcohols and oxygen heterocyclic compounds in the 84 honeys.**

-- = not detected

Honey Source	Sample number	Isobutanal (ppb)	2-methylbutanal (ppb)	3-methylbutanal (ppb)	Ethanol (ppm)	Isobutanol (ppm)	Furan (ppb)	2-methylfuran (ppb)
<b>Chestnut</b>	1	60	32	48	674	309	68	14
	2	21	8	16	300	121	142	10
	3	694	483	252	149	--	256	29
	4	47	33	33	83	--	81	11
	5	50	30	41	46	--	123	9
	6	59	35	34	85	--	110	6
	7	33	19	27	58	--	30	6
	8	35	21	19	39	--	60	4
<b>Dandelion</b>	9	6	10	4	19	--	--	--
<b>Eucalyptus</b>	10	41	20	69	63	--	15	--
	11	30	17	60	70	--	22	--
	12	38	21	34	71	86	11	--
<b>Fir</b>	13	44	27	28	11	--	29	8
	14	23	13	14	11	--	22	8
	15	42	28	31	31	--	17	5
	16	17	10	17	9	--	41	8
	17	57	43	44	391	180	28	4
	18	41	26	22	52	81	24	--
	19	23	12	9	25	--	15	--
<b>Lavender</b>	20	11	6	6	10	--	3	--
	21	10	7	8	21	--	5	--
	22	8	5	5	15	--	4	--
	23	14	10	29	13	--	7	--
	24	14	11	7	14	--	6	--
	25	9	7	5	10	--	7	--
	26	25	16	19	9	--	10	--
	27	22	17	18	7	--	--	--
	28	8	5	4	3	--	3	--
	29	10	7	6	5	--	4	--
	30	9	6	4	206	118	6	--
	31	10	6	5	9	--	--	--
	32	16	10	9	10	--	--	--
<b>Lime</b>	33	8	4	4	71	--	6	--
	34	30	20	28	30	--	11	--
	35	8	5	9	44	--	--	--
	36	13	10	17	460	189	5	--
	37	12	9	31	50	81	4	--
	38	19	19	48	387	162	6	--
	39	15	11	40	6	--	5	--
	40	7	4	7	124	--	--	--
<b>Lucerne</b>	41	4	4	4	10	--	--	--



TABLE 2 continued.

Honey Source	Sample number	Isobutanal (ppb)	2-methylbutanal (ppb)	3-methylbutanal (ppb)	Ethanol (ppm)	Isobutanol (ppm)	Furan (ppb)	2-methylfuran (ppb)
<b>Orange</b>	42	26	10	19	36	—	4	—
	43	29	14	43	46	—	8	—
	44	10	4	11	32	—	—	—
	45	22	13	23	80	—	6	—
	46	13	7	12	77	—	4	—
	47	18	11	22	66	174	5	—
	48	20	10	18	328	270	5	—
	49	12	6	12	16	—	—	—
	50	6	4	7	14	—	—	—
	<b>Rape</b>	51	4	4	7	27	—	—
52		40	26	24	14	—	4	—
53		5	4	7	44	—	—	4
54		9	6	7	10	—	—	—
55		9	9	7	11	—	—	—
56		6	5	7	5	—	7	—
<b>Rhododendron</b>	57	4	4	4	27	—	—	—
	58	4	4	4	14	—	—	—
<b>Robinia</b>	59	13	11	32	590	182	—	—
	60	13	10	21	195	—	4	—
	61	16	15	30	172	181	5	—
	62	9	8	15	147	118	4	—
	63	13	9	11	20	—	30	—
	64	31	25	36	65	134	7	—
	65	23	14	15	137	200	6	—
	66	15	8	9	124	95	7	—
	67	4	4	8	8	—	4	—
	68	8	7	16	2	—	4	—
	69	7	4	4	67	—	4	—
	70	6	4	5	8	—	—	—
<b>Rosemary</b>	71	54	29	45	235	201	14	—
	72	24	19	25	206	—	13	—
	73	7	6	8	92	—	4	—
	74	25	18	25	166	84	8	—
	75	12	7	13	69	—	9	—
	76	8	5	4	84	—	—	—
<b>Sunflower</b>	77	8	6	10	46	—	—	—
	78	4	4	4	22	—	—	4
	79	8	8	13	141	—	4	—
	80	4	4	4	8	—	—	—
<b>White clover</b>	81	7	7	17	483	238	6	—
	82	10	9	16	134	107	5	—
	83	12	10	25	131	—	6	—
	84	4	4	6	14	—	4	—

**TABLE 3. Average amounts of volatile components obtained from unifloral honeys using a purge-and-trap injector.**

che (chestnut), dan (dandelion), euc (eucalyptus), lav (lavender), lim (lime), luc (lucerne), ora (orange), rap (rape), rho (rhododendron), rob (robinia), ros (rosemary), sun (sunflower), w.clo (white clover); figures in brackets represent the number of honey samples for each floral source; – = < 0.5 ppb; a, b = concentrations were calculated using the respective response factors for dimethylsulphide and nonane.

	Concentration (ppb) or (ppm)*													
	che (8)	dan (1)	euc (3)	fir (7)	lav (13)	lim (8)	luc (1)	ora (9)	rap (6)	rho (2)	rob (12)	ros (6)	sun (4)	w.clo (4)
<b>Aldehydes</b>														
acetaldehyde	652	127	524	947	495	1700	187	1539	152	234	1256	1338	525	646
isobutanal	125	6	36	35	13	14	4	17	12	4	13	22	2	8
butanal	–	–	–	–	–	–	–	–	–	–	–	–	–	1
3-methyl-butanal	59	4	55	24	9	23	4	19	10	4	17	40	8	16
2-methyl-butanal	83	10	19	23	9	10	–	9	9	4	9	20	5	7
valeraldehyde	–	–	2	1	5	2	–	–	–	–	3	–	–	–
caproaldehyde	11	–	3	48	491	10	–	53	4	–	25	17	1	1
heptanal	5	–	3	16	140	3	–	5	–	–	9	2	–	2
benzaldehyde	6	3	5	3	1	3	–	–	7	–	1	3	–	2
<b>Ketones</b>														
acetone	339	158	165	582	121	267	188	209	224	107	163	195	135	229
diacetyl	330	92	2220	194	177	89	–	230	173	39	103	160	93	116
butanone-2	11	32	46	83	12	19	17	17	39	9	12	52	32	63
3-methyl-butanone-2	–	–	–	–	–	5	–	–	–	–	–	–	–	–
pentanone-2	–	–	–	–	–	–	–	2	–	–	–	–	–	–
pentanedione-2,3	–	–	72	–	–	–	–	6	–	–	–	–	–	–
heptanone-2	–	–	–	–	–	–	–	–	4	–	–	–	–	–
<b>Cyclic compounds</b>														
furan	109	–	16	25	4	5	–	4	2	–	6	8	1	5
2-methyl-furan	11	–	–	5	–	–	–	–	1	–	–	–	1	–
tetrahydrofuran	–	–	–	–	–	–	–	30	–	–	6	3	–	–
<b>Alcohols</b>														
ethanol*	179	190	147	76	26	146	10	77	18	4	128	148	54	190
propanol	–	–	–	–	–	–	–	–	–	–	16	–	–	–
isobutanol	54	–	29	37	9	54	–	49	–	–	76	57	–	86
3-methyl-butanol	189	–	–	222	82	335	–	73	64	–	180	–	14	126
2-methyl-butanol	9	–	–	–	–	–	–	–	–	–	16	8	–	–
<b>Esters</b>														
methyl formate	–	–	–	–	–	–	–	–	133	–	6	–	–	–
ethyl formate	185	344	87	348	139	119	60	159	120	79	132	176	95	570
ethyl acetate	75	26	19	45	18	65	23	10	28	48	40	40	22	21
ethyl propionate	–	–	–	–	8	–	–	–	–	–	–	–	–	–
<b>Sulphur compounds</b>														
dimethylsulphide	179	49	204	193	54	76	5	132	85	16	101	353	62	100
dimethylsulphide	–	–	123	–	1	–	–	–	2	–	3	22	–	–
dimethyltrisulphide*	–	–	12	–	–	–	–	–	–	–	–	–	–	–
<b>Hydrocarbons</b>														
hexane	244	20	117	72	18	3	–	12	40	24	7	6	–	18
toluene	6	3	8	12	4	2	–	5	11	–	3	8	–	3
octane	247	768	7440	212	970	680	261	82	805	827	1038	1158	222	392
9-C alkane <sup>b</sup>	–	–	61	–	–	–	–	–	–	–	–	–	–	–
p- and m-xylene	2	–	–	–	8	–	–	2	–	–	8	8	–	9
styrene	8	–	–	–	1	1	–	–	10	–	–	–	–	–
o-xylene	–	–	–	–	–	–	–	–	–	–	–	–	–	–
nonane	58	33	106	46	35	15	–	49	23	–	43	40	–	29
α-pinene	3	1	11	–	1	1	2	–	–	11	–	2	14	–
limonene	–	–	1	1	1	–	–	1	–	1	1	2	–	–
<b>Chlorinated compounds</b>														
dichloromethane	4376	24	4519	221	836	57	105	597	1729	106	1761	103	50	23
1,3-dichlorobenzene	140	89	333	128	–	97	116	126	122	93	113	82	12	75

**TABLE 4. Characteristic compounds and their concentrations (parts per billion) of some unifloral honeys.**

-- = not detected; a, b = concentrations were calculated using the response factors of dimethylsulphide and nonane respectively. The three unidentified peaks are reported as peak height measured as microvolts.

Compound	Lavender	Fir	Eucalyptus	Rosemary	Dandelion	Rape
Valeraldehyde	0-14	-	0-4	-	-	-
Caproaldehyde	0-922	0-302	7-18	0-86	-	0-14
Heptanal	0-241	0-112	0-7	0-18	-	-
Ethyl propionate	5-14	-	-	-	-	-
Acetone	22-425	410-749	140-202	76-310	158	101-338
Diacetyl	0-468	94-504	1508-2642	90-385	94	0-410
Pentanedione-2,3	-	-	0-158	-	-	-
Dimethylsulphide	0-11	-	14-328	0-61	-	0-4
Dimethyltrisulphide <sup>a</sup>	-	-	0-23	-	-	-
Octane	493-1786	119-328	4694-12469	623-1494	767	288-1109
Nonane	0-65	22-61	90-133	29-61	32	0-36
9-C alkane <sup>b</sup>	-	-	35-105	-	-	-
<b>Peak height</b>						
Peak 16	-	-	0-925	0-767	8966	1928-7411
Peak 28	-	-	-	0-572	8396	963-6579
Peak 38	-	0-4147	-	0-23236	194286	1280-111279

**The presence of some compounds reflects the microbial quality and thermal treatment of honey**

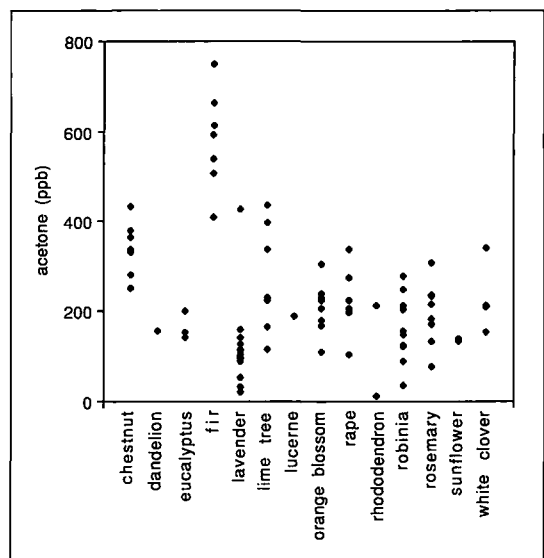
Table 2 shows the detected amounts of some branched aldehydes, alcohols, and oxygen-heterocyclic compounds in the 84 analysed samples.

A good correlation was observed between the concentrations of all branched aldehydes: 2-methylbutanal/isobutanal ( $r = 0.998, n = 84$ ), 3-methylbutanal/isobutanal ( $r = 0.928, n = 84$ ). The highest concentrations were found in chestnut honey (*Castanea sativa*)(no. 3) with 694 ppb isobutanal, 483 ppb 2-methylbutanal and 252 ppb 3-methylbutanal. The results suggest similar production mechanisms for these aldehydes: possibly fermentation and/or amino acid metabolism (valine, leucine, isoleucine) by contaminating yeasts. An additional synthetic route might be the Maillard reactions (Strecker pathway) occurring during extraction and/or storage of the honey.

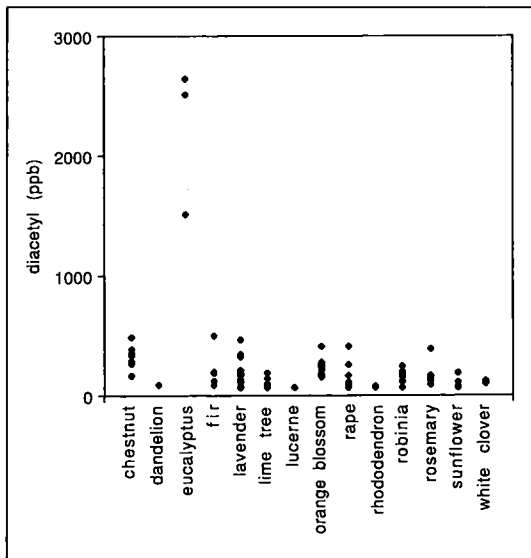
The correlation between ethanol and isobutanol concentrations was also good ( $r = 0.814, n = 84$ ). The highest alcohol content was observed in chestnut honey (no. 1) (674 ppm and 309 ppb for ethanol and isobutanol respectively). Honeys from dandelion (*Taraxacum officinale*), eucalyptus, lavender, lucerne (*Medicago sativa*), rape, and *Rhododendron* spp. were particularly poor in these alcohols (the levels of ethanol and isobutanol were below 71 ppm and 86 ppb respectively), with the exception of one lavender honey (no. 30). The high correlation coef-

ficient among alcohol concentrations points to a similar mechanism for alcohol synthesis, presumably reduction, by contaminating yeasts, of the corresponding aldehydes produced via the Ehrlich catabolic route and/or the Genevoix anabolic pathway.

Given the origin of these alcohols and aldehydes, they cannot be considered characteristic compounds for classifying unifloral honeys. As high



**FIG. 4. Distribution of acetone in the 84 honeys.**

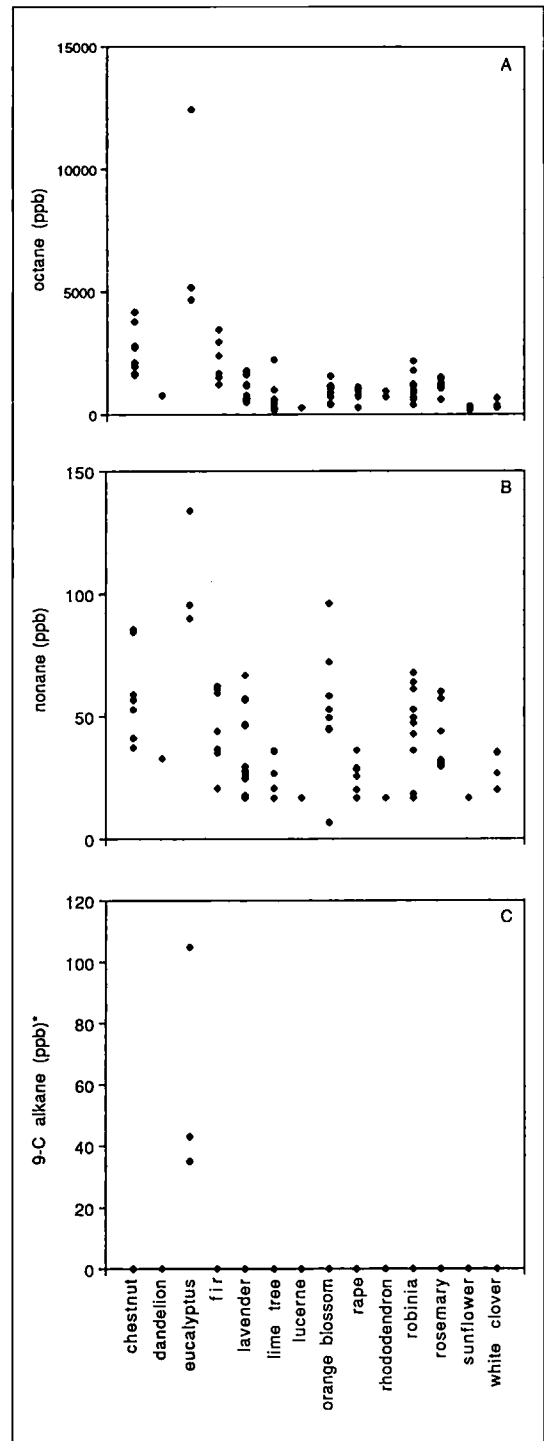


**FIG. 5. Distribution of diacetyl in the 84 honeys.**

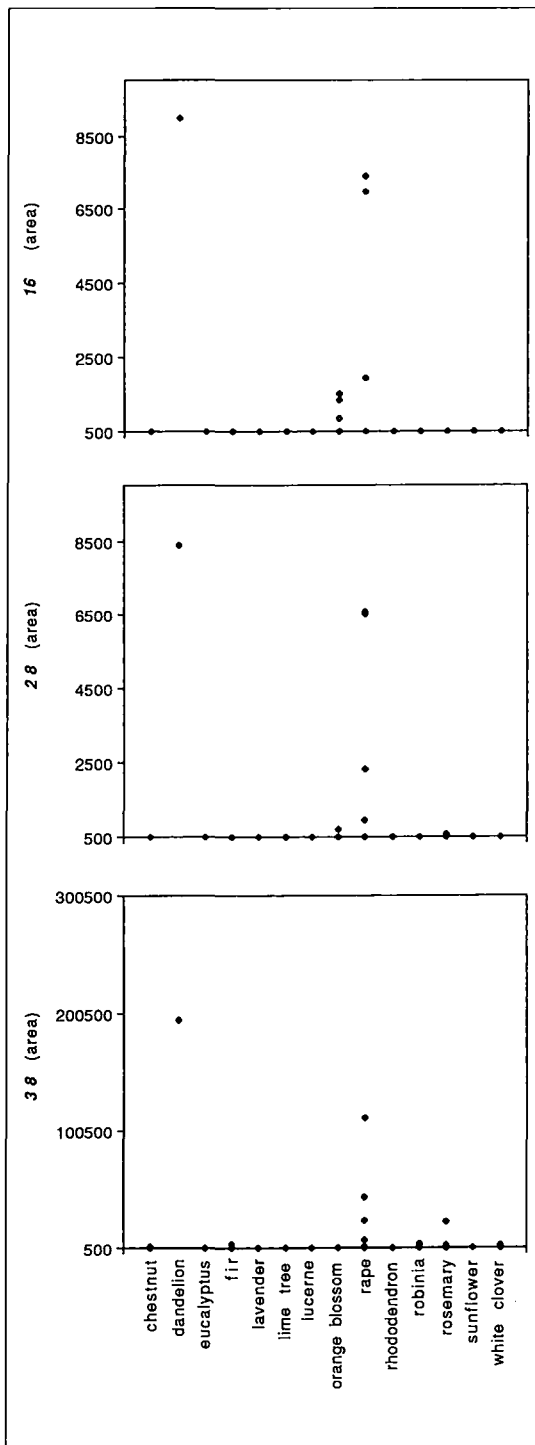
aldehyde levels sometimes coexist with small quantities of ethanol and higher alcohols (table 2), the ethanol concentration might be seen primarily as a microbiological quality index and the Strecker aldehydes as a quality index reflecting both microbiological activity and heat damage during processing and storage of the honey. These conclusions are further supported by the large amounts of furan and methylfuran (also produced by non-enzymatic browning of sugars) detected specifically in honeys with high levels of Strecker aldehydes (table 2). Chestnut honey (no. 3), for instance, presented the highest branched aldehyde and oxygen-heterocyclic compound levels. The correlation coefficient between these two cyclic compounds was 0.894 ( $n = 84$ ). It is noteworthy that chestnut honey and fir honeydew honey, often referred to as dark honeys, are especially rich in these reaction products. It is important to investigate the effects of storage and heating conditions on these compounds, in order to understand and control the flavour changes that result from such handling.

### Characteristic aroma profiles of unifloral honeys

Qualitative and quantitative compositions of the honey volatile fractions clearly varied from one unifloral honey to another, probably contributing to the distinctive bouquets of the different honeys. Table 3 presents the average values obtained for each compound in the investigated unifloral honeys. Unlike the branched aldehydes which appear to reflect product quality, the linear aldehydes proved characteristic of certain unifloral honeys, as shown in figure 3: caproaldehyde (hexanal) and heptanal were detected in abundance in the lavender honeys



**FIG. 6. Distribution of octane, nonane and an unidentified compound (no. 39) in the 84 honeys. \*signifies concentration expressed as nonane equivalents.**



**FIG. 7.** Distribution of unidentified compounds (nos 16, 28 and 38) in the 84 honeys.

only (up to 922 ppb and 241 ppb respectively). A correlation coefficient of 0.966 ( $n = 84$ ) between the concentration of the two compounds (data not shown) suggests a similar synthetic pathway, presumably linked with plant lipid metabolism. As for valeraldehyde, concentrations above 7 ppb (up to 14 ppb) were detected in only 12 samples, seven of which were lavender honeys (tables 3 and 4). Another typical compound of the lavender honeys was ethyl propionate: from 5 ppb to 14 ppb (tables 3 and 4).

As shown in figure 4, acetone concentrations above 410 ppb characterized the fir honeys. The levels of other methylketones (butanone-2, pentanone-2 or heptanone-2) were not characteristically high in fir honeys (table 3).

Diketones were particularly characteristic of the eucalyptus honeys, as shown in figure 5 for diacetyl. Diacetyl concentrations above 1.5 ppm were recorded. Similarly, 2,3-pentanedione was detected in only three samples which included two eucalyptus honeys (eucalyptus honeys nos. 10 and 11 and orange honey no. 46 with 58 ppb, 158 ppb, and 54 ppb respectively) (data not shown). These results are in line with the detection by Graddon *et al.* (1979) of high amounts of acetoin and other hydroxyketones (not analysed by our method) in eucalyptus honeys.

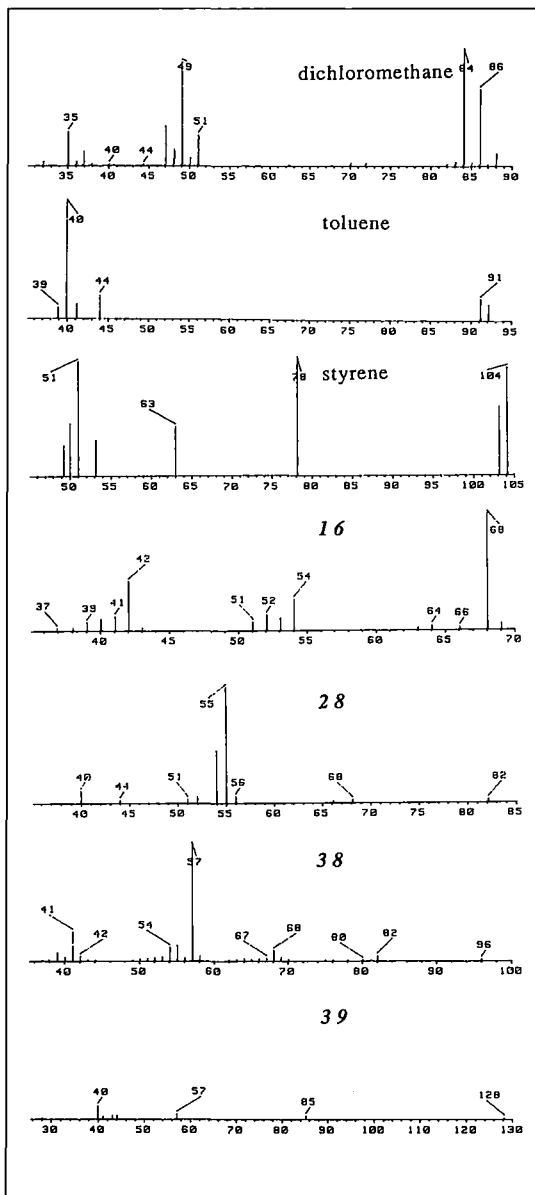
The presence of sulphur compounds was another typical feature of eucalyptus honeys. Dimethyldisulphide and dimethyltrisulphide were found together in this type of honey only (table 3). Dimethyldisulphide was also widely present in rosemary honeys. However, the absence of dimethyltrisulphide, the low diacetyl level (fig. 5), and the absence of the unidentified nine-carbon alkane (fig. 6C) enabled us to discriminate between these two unifloral sources.

Alkanes were also abundant in the eucalyptus honeys. Octane and nonane were present at concentrations above 4.7 ppm and 90 ppb respectively, in all three eucalyptus samples (figs. 6A, 6B). An additional unidentified compound (probably a 9-carbon hydrocarbon) (fig. 8) was also detected exclusively in these three samples (fig. 6C). These various results agree with the data of Graddon *et al.* (1979) who described the eucalyptus honeys as very rich in higher alkanes.

Three unidentified compounds (peak numbers 16, 28, and 38 in fig. 2) seemed characteristic of dandelion honey (only one sample) and, to a lesser extent, rape honey (fig. 7).

$\alpha$ -pinene and limonene were present in all samples. However, no monoterpenes appeared to be characteristic of floral sources. Analyses of less volatile compounds including sesquiterpenes and oxygenated derivatives are in progress.

Although unlikely to reflect the identity of the unifloral



**FIG. 8. Mass spectra of styrene, dichloromethane, toluene and unidentified compounds (nos 16, 28, 38 and 39) recorded in honey samples.**

source, several compounds not originating from natural sources are worth mentioning (table 1 and fig. 8). Styrene (released from the packaging) was detected mainly in chestnut honey samples (up to 9 ppb in chestnut honey no. 3). Dichloromethane was often present in chestnut and eucalyptus honeys (up to 15 ppm). Finally, toluene (54 ppb) was detected in a smoky flavoured fir honey (no. 15).

It will be necessary to extract some less volatile flavour compounds in order to further characterize

unifloral honey aroma and to differentiate unifloral honeys derived from floral sources such as chestnut, orange, lime or robinia. This laboratory has recently developed an optimized extraction protocol using dichloromethane.

## ACKNOWLEDGEMENTS

The authors are indebted to the Centre Apicole de Recherche et d'Information (CARI) for sample screening. SC is Chercheur Qualifié from the Fonds National de la Recherche Scientifique. This work was partly supported by a grant from the Fonds National de la Recherche Scientifique (FRFC 2.4538.90).

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