Floral Origin Markers of Chestnut and Lime Tree Honeys

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The apiculture industry is more and more interested in finding typical markers to authenticate floral origin of honeys. With this aim, some reliable volatile compounds were proposed to identify origin of lime tree and chestnut samples. A dichloromethane extraction followed by a Likens−Nickerson simultaneous steam distillation/solvent extraction led to representative honey extracts. About 400 volatile compounds were separated by gas chromatography, but only a few authenticated the floral origin of honeys. Chestnut honeys are distinguishable from other origins by high concentrations of acetophenone, 1-phenylethanol (>88 ppb), and 2-aminoacetophenone (>154 ppb). Lime tree honeys are characterized by enhanced amounts of shikimate pathway derivatives (ethylmethylphenol isomer (>31 ppb), 4-*tert*-butylphenol, estragole (>51 ppb), and *p*-methylacetophenone but also by high concentrations of monoterpene-derived compounds (menthol, thymol, 8-*p*-menthene-1,2-diol, and carvacrol (>76 ppb)) and methyl(1-methylethenyl)benzene.

Keywords: *Honey; chestnut; lime tree; flavor*

INTRODUCTION

Finding reliable markers to ascertain floral origin of honey is of prime importance in the apiculture industry. European legislation specifies that the term "honey" may be completed by a reference to the origin, whether blossom or plant, provided the product comes predominantly from the indicated source and has the appropriate organoleptic, physicochemical, and microscopic properties corresponding to that origin (Codex Alimentarius Commission, 1970; Council Directive 74/409/EEC, 1974). The interpretation of "predominantly" remains ambiguous even though extensive studies on pollen analyses, physicochemical, and sensory determinations have been carried out (Crane et al., 1984; Oddo et al., 1995; Serra Bonvehi and Ventura Coll, 1995). It is now currently accepted that physicochemical parameters or pollen analysis alone is not sufficient to authenticate floral origin of honey (Oddo et al., 1995). As for sensory analysis, it is still subjective even though references of organoleptic properties have been quite well developed (Gonnet and Vache, 1984).

Specific volatile compounds derived from original nectar sources are in all likelihood responsible for the specific aroma of unifloral honeys. These volatile compounds have been proven to be adequate to authenticate floral origin of honeys, and consequently identifications of such molecules have already been published for a few unifloral types (Steeg and Montag, 1988a,b; Tan et al., 1988; Wilkins et al., 1993; Bouseta et al., 1996).

As previously shown by Bouseta et al. (1992), chestnut and lime tree honeys cannot be easily characterized by their headspace volatile composition. On the other hand, less volatile compounds, such as 3-aminoacetophenone, obtained from a simultaneous distillation extraction procedure, and reported as the main compound in the volatile fraction of chestnut honey, could be used as floral markers (Bonaga and Giumanini, 1986). From an ether extract of 116 kg of lime tree honey, 8-*p*-menthene-1,2-diol was evidenced by Tsuneya et al. (1974) as the major volatile compound of such sample. The *trans*-isomer of this compound, but also linden ether (3,9-epoxy-1,4(8)-*p*-menthadiene) and *cis*-rose oxide, were further proposed as indicators for lime tree honeys (Blank et al., 1989).

In the present work, our objective was to use an optimized Likens−Nickerson methodology, which leads to organoleptically highly representative extracts (Bouseta and Collin, 1995), in order to (i) investigate the volatile compounds of 10 chestnut and 10 lime tree honeys and (ii) find new reliable markers to authenticate their floral origin, in comparison with nine other honey types.

MATERIALS AND METHODS

Honey Samples. Ten chestnut (*Castanea sativa*) and ten lime tree (*Tilia* spp.) unifloral honeys were selected from various countries (chestnut, samples from France and Italy; lime tree, samples from France). Screening for floral purity was based on pollen analyses (Louveaux et al., 1978), sensory tests, electrical conductivity, pH, titratable acidity (Journal Officiel, 1977), and sugar composition (Pourtallier and Rognone, 1977). All samples regarded as unifloral honeys met requirements defined in other studies (Accorti et al., 1986; Crane et al., 1984; Gonnet and Vache, 1984; Maurizio, 1979). The 90 samples of other unifloral origins (*Eucalyptus* from Australia, Italy, and Spain; fir from France; lavender from France and Spain; orange blossom from France, Mexico, and Spain; rape from Belgium and France; robinia from Canada, France, Hungary, Russia, and Spain; rosemary from France and Spain; sunflower from Belgium and France; whiteclover from Canada and New Zealand; 10 of each) were also tested by the usual available physicochemical and sensory tests. All the samples were stored at 4 °C until analysis.

Reagents. Acetoin (>99%) and hexanal (98%) were from Fluka Chemika (Buchs, Switzerland). 2-Aminoacetophenone

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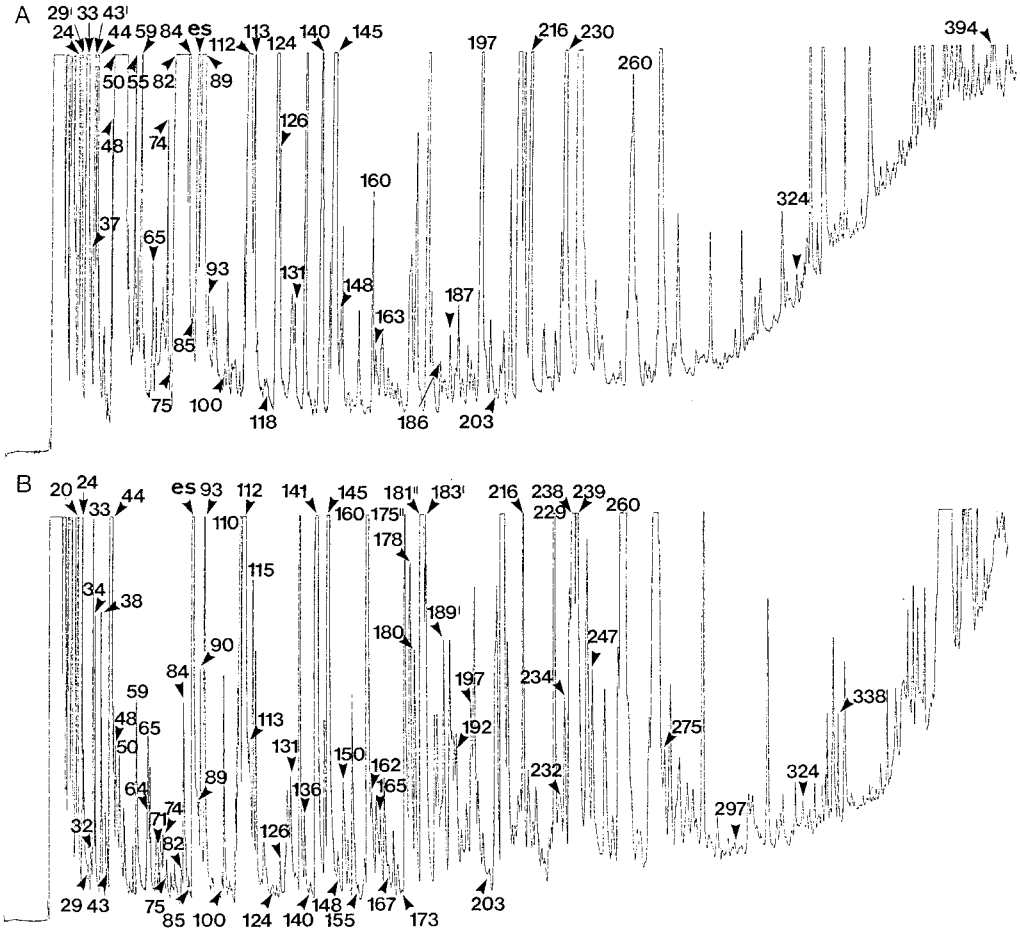

Figure 1. GC/FID chromatograms of chestnut (A) and lime tree honeys (B). Numbering as in Tables 2 and 3. es = external standard.

Table 1. Pollen Density and Pollen Percentage in the Monofloral Honeys

monofloral honey	pollen density		pollen percentage (%)	
	min	max	min	max
chestnut (*Castanea sativa*)	125 600	185 444	90	98
eucalyptus (*Eucalyptus* spp.) fir[a]	40 200	135 000	54	99
lavander (*Lavandula* spp.)	5640	48 800	1	20
lime tree (*Tilia* spp.)	12 100	160 000	5	23
orange blossom (*Citrus aurantinum*)	12 156	40 000	10	69
rape (*Brassica napus*)	22 650	60 320	80	96
robinia (*Robinia pseudacacia*)	16 040	82 040	28	53
rosemary (*Rosmarinus officinalis*)	17 200	75 300	22	54
sunflower (*Helianthus annuus*)	18 620	60 320	46	81
white clover (*Trifolium repens*)	40 000	152 500	80	97

[a] Honey from honeydew.

(98%), benzaldehyde (99+%), butanoic acid (99.5%), 4-*tert*-butylphenol (97%), carvacrol (99%), coumarin, *p*-cymene (99%), decane (>99%), 2,3-dimethyl-2-cyclopenten-1-one (99%), 2,3-dimethylphenol, 3,5-dimethylphenol (99%), guaiacol (98%), heptadecane (99%), heptanal (95%), 3-hexanol (97%), 5-(hydroxymethyl)furfural (99%), isophorone (97%), menthol (99%), 2-methoxy-6-methylpyrazine (99%), 3-methoxypyridine, *p*-methylacetophenone (95%), 5-methylfurfural (99%), octanoic acid (99%), pentadecane (99%), phenylacetaldehyde (95%), 2-phenylethanol (99%), α-pinene (98%), *β*-pinene (98%), 3-pyridinecarboxaldehyde (98%), thymol (98%), and 2,3,5-trimethylphenol (99%) were from Aldrich Chemie (Steinheim, West Germany). Acetophenone (99%), 2-acetylfuran, benzyl alcohol (99+%), 1-chloroheptane (97%), dimethyl disulfide (pa), estragole, 2-furaldehyde (99%), furfuryl alcohol (99%), hexanoic acid (99%), 3-methyl-2-buten-1-ol (99%), 3-methyl-3-buten-1-ol (97%), 2-methyl-1-butanol (98%), nonane (99%), octane (99+%), *γ*-terpinene (95%), and *γ*-valerolactone (98%) were from Janssen Chimica (Geel, Belgium). 3,4-Hexanedione and 1-phenylethanol (98%) were from Merck (Darmstadt, Germany). Dichloromethane (99.9%) was from Romil Chemicals (Leics, England). Benzoic acid and α-humulene were from Sigma Chemical (St. Louis, MO). *n*-Hexanol (>97%), 1-pentanol, phenylacetic acid, and toluene (99%) were from UCB Chemical (Leuven, Belgium). Phenol was from US Biochemical (Cleveland, OH). 2-Methylbutanoic acid was from Alltech Associates, Inc. (Deerfield, IL). Pyridine was from Analyticals Carlo Erba (Milano, Italy).

Honey Flavor Extraction. Aroma compounds isolation was performed by a dichloromethane solubilization, followed by a Likens−Nickerson steam distillation/solvent extraction according to the procedure described by Bouseta and Collin (1995). For each sample, two replicates were achieved.

Gas Chromatography−FID Analytical Conditions. A Hewlett Packard model 5890 gas chromatograph was used, equipped with a Hewlett Packard model 7673 automatic sampler, a cold on-column injector, a flame ionization detector, and a Shimadzu CR4A integrator. Analysis of honey volatile compounds was carried out on a 50 m × 0.32 mm, wall-coated, open tubular (WCOT) CP-SIL5 CB (Chrompack, Antwerpen, Belgium) capillary column (film thickness, 1.2 *μ*m). The oven temperature was programmed to rise from 30 to 85 °C at 55 °C/min and then to 145 °C at 1 °C/min and to 250 °C at 3 °C/min. The carrier gas was He at 1.5 mL/min. The injector temperature was maintained at 3 °C above the oven temperature. The detector temperature was 275 °C. The minimum peak area for data acquisition was set at 500 *μ*V·s. Olfacto-

Table 2. Volatile Compounds in Chestnut and Lime Tree Honeys[a]

			chestnut			lime tree					
compounds	*N*	RI	min	max	avg	min	max	avg	id	odor	FM
acetoin	20			173	17		199	43	GC−MS		
3-methyl-3-buten-1-ol	24	702	147	791	253	29	124	74	GC−MS		
2-methyl-1-butanol	25	706		106	49	13	106	46	GC−MS		
pyridine	26	711		237	84		63	13	GC−MS		
dimethyl disulfide	29	723				3	218	52	GC−MS	cabbage	
1-pentanol	29′	723	6	49	34				GC−MS		
butanoic acid	32	736		70	9	8	78	43	GC−MS	fatty-spicy	
3-methyl-2-buten-1-ol	33	743	125	309	170	29	86	55	GC−MS		
toluene	34	751	2	37	22	9	79	29	GC−MS		
3-hexanol	37	769	2	424	132		54	10	GC−MS	solvent-spicy	
hexanal	38	772		33	9		27	5	GC−MS		
octane	43	800				20	332	116	GC−MS		
2-furaldehyde	44	804	170	1512	717	232	1476	670	GC−MS	spicy-irritant	
2-methylbutanoic acid	48	818	175	1625	657	39	726	234	GC−MS		
furfuryl alcohol	50	823	113	3314	1203	9	414	122	GC−MS	burned	
n-hexanol	55	843	9	55	31	1	79	17	GC−MS	cut grass	
2(3*H*)-furanone[b] $M^+ = 84$	59	857	32	173	81	21	55	39	MS		
heptanal	64	876				4	40	13	GC−MS		
2-acetylfuran	65	880	23	289	117	9	59	36	GC−MS		
nonane	71	900		132	47	11	43	26	GC−MS		
2,5-hexanediol[c] $M^+ = 118$	74	907	9	47	29	7	43	18	MS	fatty	
γ-valerolactone	75	912	5	33	21	5	27	13	GC−MS		
5-methylfurfural	82	935	9	3229	434		27	3	GC−MS	phenolic	
benzaldehyde	84	939	28	204	88	31	727	203	GC−MS	bitter almond	
α-pinene	85	942		36	9		18	2	GC−MS	resin	
phenol	89	962		291	110	1	286	44	GC−MS	phenolic-spicy	
hexanoic acid	90	965					76	18	GC−MS	fatty-cheese	
3-pyridinecarboxaldehyde	91	968					44	22	GC−MS		
2,3-dimethyl-2-cyclopenten-1-one	93	972	3	18	8	16	187	82	GC−MS	herbaceous	
3-methoxypyridine	99	993		1	0		4	2	GC−MS		
β-pinene	100	998	3	11	8	2	9	5	GC−MS	resin	
benzyl alcohol	112	1018	482	1712	1255	211	1520	784	GC−MS		
phenylacetaldehyde	113	1023	10	119	47	6	243	84	GC−MS	floral	
p-cymene	115	1026	0	12	5	17	78	39	GC−MS		
2-methoxy-6-methylpyrazine	118	1032	1	151	44		31	5	GC−MS	cut grass	
1-phenylethanol	124	1042	88	218	148	3	13	7	GC−MS	floral	CH
acetophenone	126	1045	28	121	66	3	11	5	GC−MS		CH
γ-terpinene	131	1058		27	10	18	62	40	GC−MS	resin	
guaiacol	136	1071				1	26	14	GC−MS	camphor-spicy	
1-cyclopentyl-2-propanone[b] $M^+ = 126$	140	1075	43	153	99		633	113	MS	burned tree	
methyl(1-methylethenyl)benzene[d] $M^+ = 132$	141	1081		20	5	113	971	531	MS	vegetable	LT
2-phenylethanol	145	1091	262	691	532	238	5928	1413	GC−MS	floral	
isophorone	148	1099		40	10	3	33	11	GC−MS	spicy	
2,6,6-trimethyl-2-vinyltetrahydropyranne[b] $M^+ = 154$	150	1103				6	83	34	MS	floral	
2,3-dimethyl-4-isopropenyl-1-cyclopentanone[b] $M^+ = 152$	155	1113					63	14	MS		
trimethoxybenzene isomer[e] $M^+ = 168$	160	1121	9	66	26	134	975	536	MS	spicy	
2,3-dimethyl-4-isopropyl-1-cyclopentanone[b] $M^+ = 154$	162	1126		19	5		32	9	MS	fatty-cheese	
4-butyl-1,3-cyclopentanedione[b] $M^+ = 154$	163	1129	11	23	16		83	31	MS		
2,3-dimethylphenol	165	1132		7	3	9	166	44	GC−MS	phenolic	
benzoic acid	167	1135				13	118	57	GC−MS		
3,5-dimethylphenol	173	1145		11	2	4	69	20	GC−MS	phenolic	
4-ethyl-3,4-dimethyl-2-cyclohexen-1-one[b] $M^+ = 152$	175″	1150				37	193	115	MS	mentholated	
octanoic acid	177	1153					112	20	GC−MS	fatty-cheese	
p-methylacetophenone	180	1158		5	1	14	90	45	GC−MS	hay	LT
menthol	183′	1166				7	252	112	GC−MS	mentholated	LT
5-(hydroxymethyl)furfural	186	1173	18	253	52				GC−MS	spicy	
1,3-diphenyl-2-propanone[b] $M^+ = 210$	187	1166	7	46	16				MS	cut grass	
estragole	189′	1180				51	236	113	GC−MS	anise-fennel	LT
3-phenyl-1-propanol[b] $M^+ = 136$	197	1202	35	135	81	9	72	37	MS	herbaceous	
phenylacetic acid	203	1212		9	4		31	9	GC−MS	floral	
3-methoxybenzeneethanol[b] $M^+ = 152$	216	1235	5	175	29	16	766	276	MS	floral	
1-methoxy-4-propylbenzene[e] $M^+ = 150$	229	1257				85	825	350	MS	fennel	
2-aminoacetophenone	230	1260	154	544	300		68	16	GC−MS	vegetable	CH
4-*tert*-butylphenol	232	1261					107	26	GC−MS	phenolic	LT
thymol	234	1263		6	1	18	161	83	GC−MS	spicy-thyme	LT
carvacrol	239	1271		33	6	76	393	210	GC−MS	spicy-thyme	LT
ethylmethylphenol isomer[f] $M^+ = 136$	247	1281				31	155	80	MS	phenolic	LT
8-*p*-menthene-1,2-diol[b] $M^+ = 170$	260	1304	15	106	40	15	1317	632	MS	spicy	LT
coumarin	297	1392					49	17	GC−MS	floral	
α-humulene	324	1463	10	88	34	5	50	22	GC−MS	hop	
pentadecane	338	1500					226	53	GC−MS		
heptadecane	394	1700	7	53	20		174	40	GC−MS		

[a] *N* = peak number gives order of elution through column; the signs ′ or ″ following some peak number appear when different compounds found either in chestnut, in lime tree honeys, or in another monofloral honey exhibit the same retention indice. RI = retention indice. Min, max, avg = minimal, maximal, average concentrations (ppb) in the honeys. [b] Concentrations given in ppb decane equivalent. [c] Concentrations given in ppb 3,4-hexanedione equivalent. [d] Concentrations given in ppb *p*-cymene equivalent. [e] Concentrations given in ppb estragole equivalent. [f] Concentrations given in ppb 2,3,5-trimethylphenol equivalent. M^+ = molecular weight determined by chemical ionization with methane. id = identification. GC = identification by gas chromatography (peak enhancement by coinjection of reference compound). MS = identification by mass spectroscopy (compared with NBS/EPA/NIH library). Odor = results from the sniffing experiment. FM = floral marker of chestnut (CH) or lime tree (LT) honey.

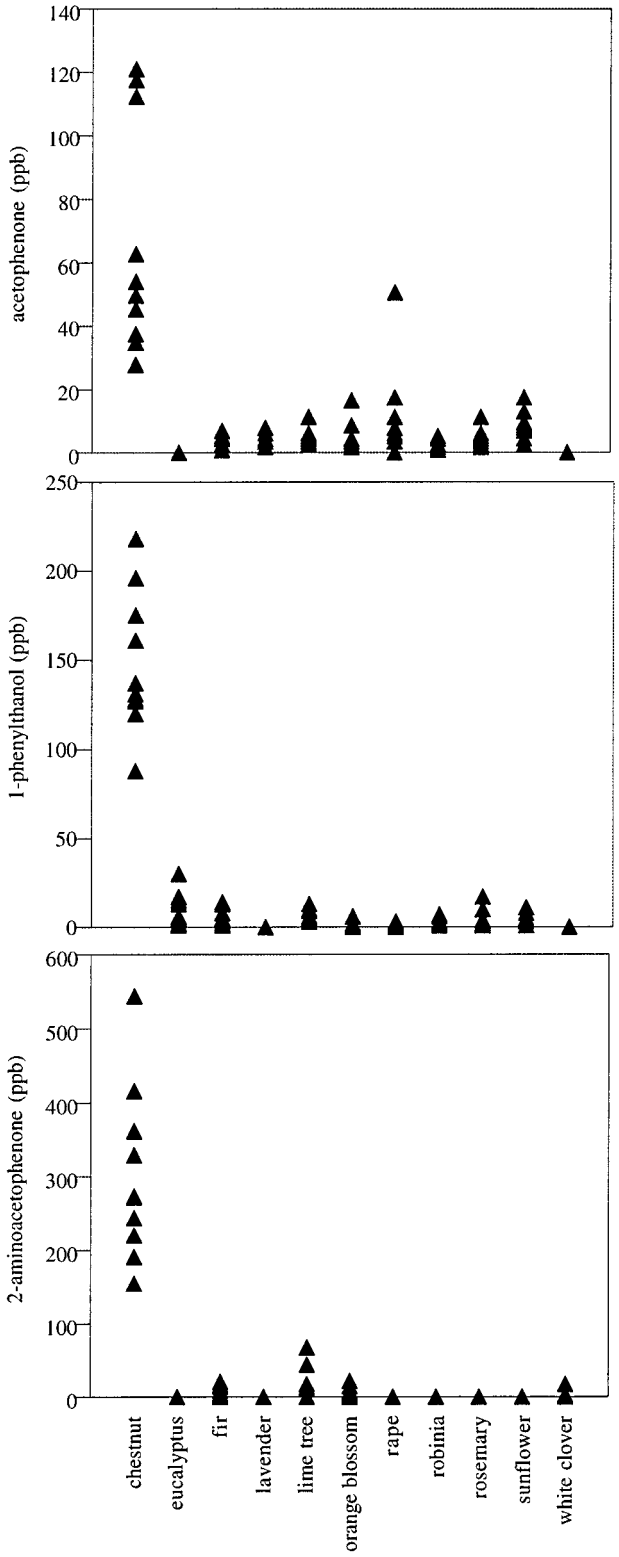

Figure 2. Distribution of acetophenone, 1-phenylethanol, and 2-aminoacetophenone in the 110 honey samples.

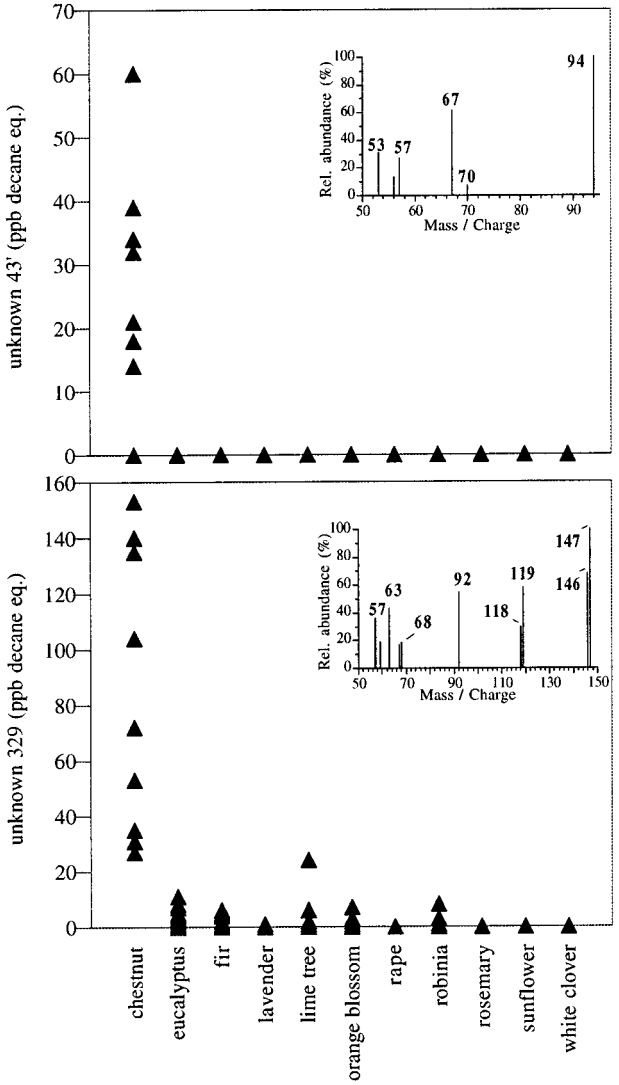

Figure 3. Distribution of unknown compounds 43′ and 329 in the 110 honey samples.

metric analyses were operated under the same conditions, by means of a sniffing outlet. Retention indices were determined by GC/FID by interpolation of the retention times of a *n*-alkanes (C_6−C_{19}) mixture analyzed under identical conditions.

Gas Chromatography−Mass Spectrometry Conditions. Chromatographic conditions were the same as those used for FID detection. The column was directly connected to an HP 5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV (filament current, 300 mA; electron multiplier voltage, 2500; scan rate, 4 s^{-1}; *m*/*z* range, 40−300). Spectral recording throughout elution was automatic using HP59970C software. Identification was on the basis of peak enhancement by coinjection with authentic standard compounds and comparison with the NBS/EPA/NIH mass spectra library. Chemical ionization with methane was also achieved in order to determine the molecular weight of unidentified compounds.

Quantification of Volatile Compounds. Previous analyses have shown that the extraction recovery factors were >70% for most of the chemicals mentioned (Bouseta and Collin; 1995). For this reason, concentration of each compound in the honey samples was calculated with a recovery factor equal to 100% with respect to the one of the external standard, according to the equation

$$C_i/C_e = (S_i/S_e)/400K_i \quad (1)$$

where the suffixes i and e refer, respectively, to the quantified compound and the external standard; *S* refers to the surface obtained in GC; *C* refers to the concentration; 400 is the concentration factor calculated on the basis of volume ratio; and K_i is the response factor at the FID detector of the compound i with regard to the external standard.

In the case of unidentified and most of the tentatively identified compounds, quantifications were performed with regard to decane. Compounds 74, 141, 160, 229, and 247 were quantified with regard to 3,4-hexanedione, *p*-cymene, estragole, estragole, and 2,3,5-trimethylphenol, respectively.

Table 3. Unidentified Markers To Authenticate Floral Origin of Chestnut and Lime Tree Honeys[a]

			chestnut			lime tree				
unidentified compds	*N*	RI	min	max	avg	min	max	avg	odor	FM
(94 M^+, 67, 53, 57)	43′	800		60	28					CH
(109, 69, 67, 81) $M^+ = 152$	110	1014		6	2	71	292	176	floral	LT
(168 M^+, 70, 43, 83)	178	1154				50	343	134	mentholated	LT
(135, 91, 65, 150 M^+)	181″	1164				438	1771	1023	floral	LT
(84, 83, 56, 77) $M^+ = 168$	192	1189		17	8	13	132	53		LT
(93, 136 M^+, 91, 79)	238	1270				172	589	362	balsamic	LT
(95, 153, 91, 67) M^+=166	275	1336		8	3	2	68	24	cumin	LT
(147, 146, 119, 92) $M^+ = 177$	329	1478	31	153	78		24	3	vegetable	CH

[a] The prominent ions in the electronic impact MS are given in decreasing order in parentheses for each unknown compound. M^+ = molecular weight determined by chemical ionization with methane. *N* = peak number gives order of elution through column. An ′ or ″ following some peak number appears when different compounds found either in chestnut, in lime tree, or in another monofloral honey exhibit the same retention indice. RI = retention indice. Min, max, avg = minimal, maximal, average concentrations (in ppb decane equivalent) in the honeys. Odor = results from the sniffing experiment. FM = floral marker of chestnut (CH) or lime tree (LT) honey.

Statistical Analyses. All the statistical analyses were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, NC). An analysis of variance (ANOVA) was used in order to determine significant differences among honeys. A Student−Newman−Keuls test was used to perform a multiple comparison of means.

RESULTS AND DISCUSSION

Pollen Analysis. Pollen analyses of chestnut and lime tree honeys are reported in Table 1. Floral origin of chestnut honeys is confirmed by a high pollen percentage (>90%), in agreement with the observations of Gonnet and Vache (1984). On the contrary, lime tree samples exhibit lower pollen percentages (>5%) and for this reason, their authentication had to be completed by sensory assessments (data not shown).

Volatile Compound Analysis. About 400 aroma compounds have been separated by gas chromatography (Figure 1), but only 72 were well identified by gas chromatography and/or mass spectrometry in chestnut and lime tree honey extracts (Table 2). Most of the peaks cannot be used as reliable markers, due to their presence in honeys of other origins. However, chestnut or lime tree samples can be authenticated on the basis of a few discriminant flavoring compounds, of which contents were proved to be significantly different from those of the other honeys by mean of an analysis of variance ($p < 0.0001$).

Chestnut Honeys. Chestnut honeys are characterized by high amounts of acetophenone, 1-phenylethanol, and 2-aminoacetophenone with concentrations varying, respectively, from 28 to 121 ppb, from 88 to 218 ppb, and from 154 to 544 ppb (Figure 2). In the other honeys, the concentrations of acetophenone, 1-phenylethanol, and 2-aminoacetophenone remain below 20, 30, and 70 ppb, respectively (except one rape sample contained 51 ppb of acetophenone).

Considered as a product related to the shikimic acid pathway (Devon and Scott, 1975), acetophenone is formed during the phenylpropane metabolism by enzymatic reactions from hydroxy-substituted aromatic acids (Häusler and Montag, 1990a). This compound has been previously reported by Bonaga and Giumanini (1986) in chestnut honeys. However, this molecule was not considered by the authors as a specific marker for the floral source, and no quantitative determination was achieved. Paradoxically, a subsequent study of different unifloral honeys performed by Häusler and Montag (1990b) did not evidence acetophenone in chestnut samples. In spite of an odor threshold in aqueous solution equal to 65 ppb (Buttery and Ling, 1995), acetophenone appeared odorless (Table 2) during the sniffing experiments.

1-Phenylethanol was never reported as a floral marker of chestnut honey or even as a honey constituent. According to its chemical structure, this compound is suspected to result from acetophenone reduction. 1-Phenylethanol was described with a floral odor (Table 2) and may therefore contribute to the characteristic floral aroma of chestnut honey. Nevertheless, as aromatic alcohols have high flavor thresholds (100−500 ppm) (Meilgaard, 1975), 1-phenylethanol acts in all likelihood in synergy with other compounds, such as phenylacetaldehyde or 2-phenylethanol (Table 2). The origin of chestnut samples could be authenticated with a 1-phenylethanol concentration above 50 ppb.

Floral origin of chestnut honey can be also ascertained by a third marker: 2-aminoacetophenone (Figure 2). This compound was never evidenced in honey, contrary to its isomer, 3-aminoacetophenone, previously reported by Bonaga and Giumanini (1986) as the main volatile constituent of chestnut samples and consequently considered as a marker of the floral origin. This latter compound, characterized by a retention time 10 min higher, has not been evidenced in our samples. Contrary to all expectations, 2-aminoacetophenone is probably not issued from the shikimate pathway but might result from tryptophane degradation, as shown by Rapp et al. (1995) in the case of fermented model wine solutions. Tryptophane was, however, not evidenced in our samples (sensitivity in the ppm range; data not shown). With an odor threshold in aqueous solution equal to 0.2 ppb (Buttery and Ling, 1994), 2-aminoacetophenone is a powerful odorant, suspected to contribute to the aroma of chestnut honey. Usually described with an "animal-floral, faintly reminiscent of tobacco leaf" odor (Arctander, 1969), this compound was perceived with a "vegetable" note during our sniffing experiments (Table 2). As shown by Figure 2, a 2-aminoacetophenone concentration above 70 ppb certificates the floral origin of chestnut samples.

Two unidentified compounds complete the characterization of chestnut honey (Table 3 and Figure 3). The first one (peak number 43′) is odorless and was not evidenced in the other origins. The second one (peak number 329), which was described by vegetable descriptors, has been found in chestnut samples at concentrations varying from 31 to 153 ppb decane equivalent. Chemical ionization with methane led to molecular weights of 94 and 177 Da, respectively, indicating that

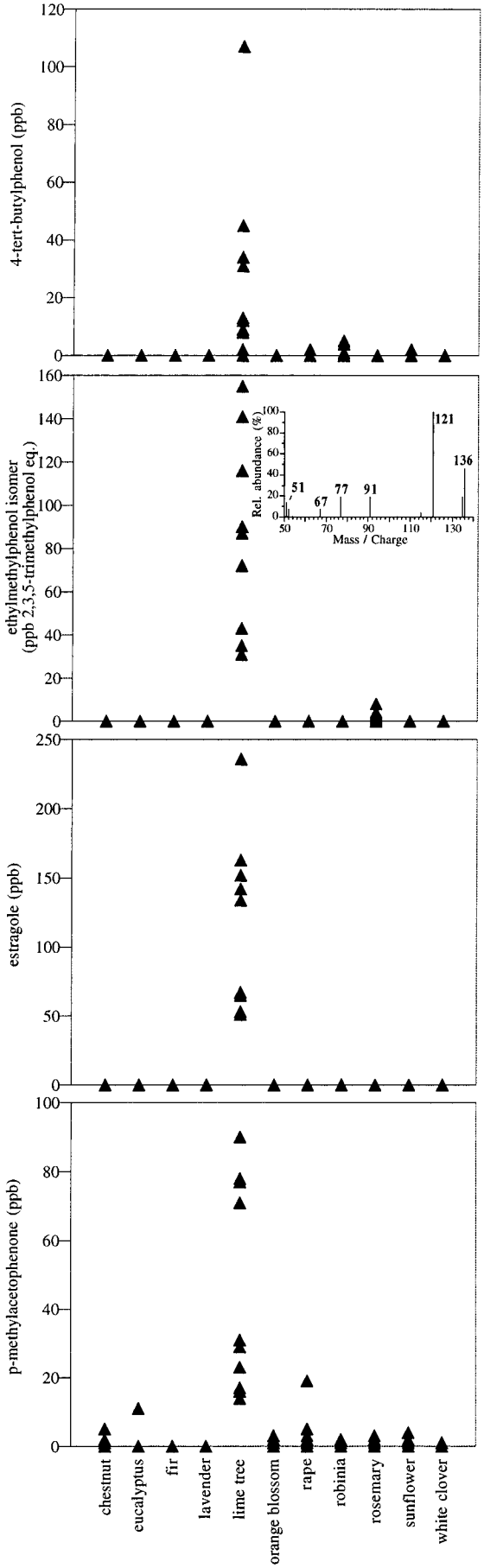

Figure 4. Distribution of 4-*tert*-butylphenol, ethylmethylphenol isomer, estragole, and *p*-methylacetophenone in the 110 honey samples.

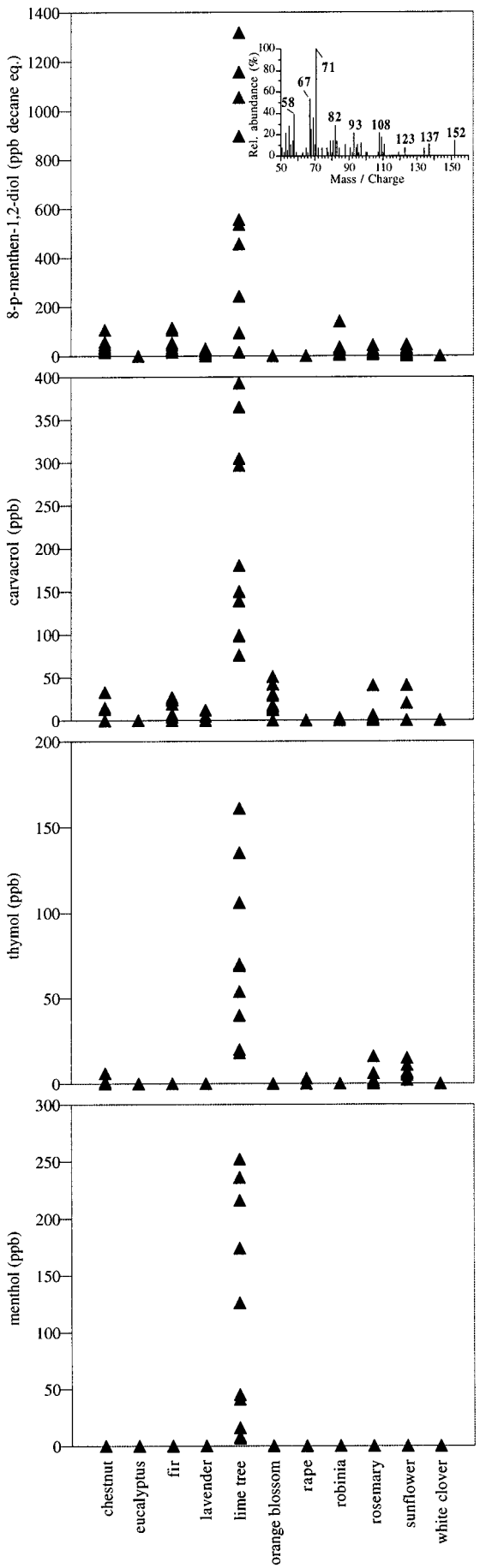

Figure 5. Distribution of 8-*p*-menthene-1,2-diol, carvacrol, thymol, and menthol in the 110 honey samples.

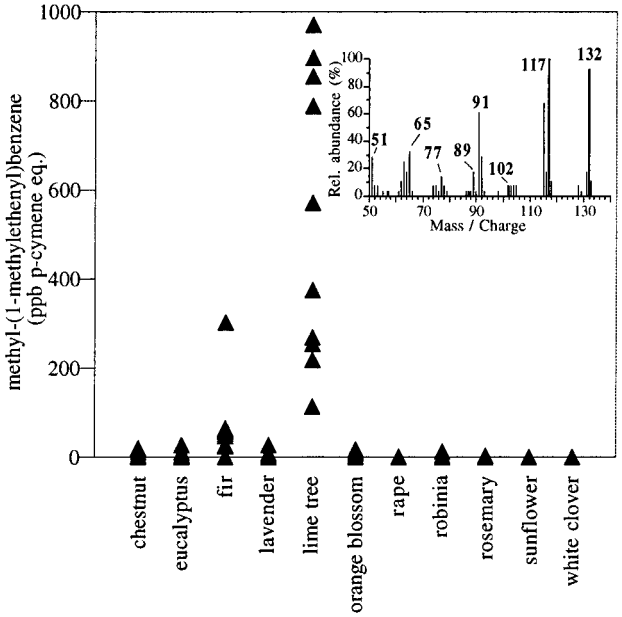

Figure 6. Distribution of methyl(1-methylethenyl)benzene in the 110 honey samples.

the latter is a nitrogenous compound (Table 3). On the basis of the MS fragmentation, it could be an aminopropane acetophenone derivative (Figure 3).

Many other nitrogenous compounds were detected in chestnut honeys with an NPD detector (data not shown). Moreover, high concentrations of products issued from nonenzymatic browning of sugars such as 2-acetylfuran, 5-methylfurfural, and 5-(hydroxymethyl)furfural were evidenced (Table 2). These results fit in with the large amounts of furan and methylfuran previously measured by Bouseta et al. (1992) with a headspace dynamic method in the same origin. These furan derivatives are usually indicators of thermal treatment and storage conditions and cannot be therefore considered as good floral markers (Bouseta et al., 1992). Nevertheless, they can be used as additional proofs to authenticate origin of chestnut honeys.

Lime Tree Honeys. Four compounds, originated in all likelihood from the shikimic acid pathway, can be selected as floral markers of lime tree honeys.

Phenolic compounds such as 4-*tert*-butylphenol (2−107 ppb) and an unidentified ethylmethylphenol isomer (31−155 ppb 2,3,5-trimethylphenol equivalent) were evidenced in high content in lime tree samples (Figure 4). In spite of the lack of information about the position of the substituents, a concentration of ethylmethylphenol isomer above 30 ppb would allow us to easily discriminate lime tree honeys from the other origins. As far as 4-*tert*-butylphenol is concerned, Steeg and Montag (1988b) unfortunately reported it as a constituent of buckwheat, dandelion, rape, heath, forest, and conifer honeys, without, however, further quantification. These two potent odorants were olfactometrically detected in our samples with a phenolic note (Table 2).

Estragole (51−236 ppb), the allyl isomer of anethole, is a discriminant methoxylated derivative previously isolated by Tsuneya et al. (1974) in lime tree honeys (Figure 4). Sniffing experiments indicated in that case "anise" and "fennel" notes (Table 2).

Surprisingly, *p*-methylacetophenone was evidenced in high concentration in lime tree honeys (14−90 ppb) (Figure 4). This acetophenone derivative was previously

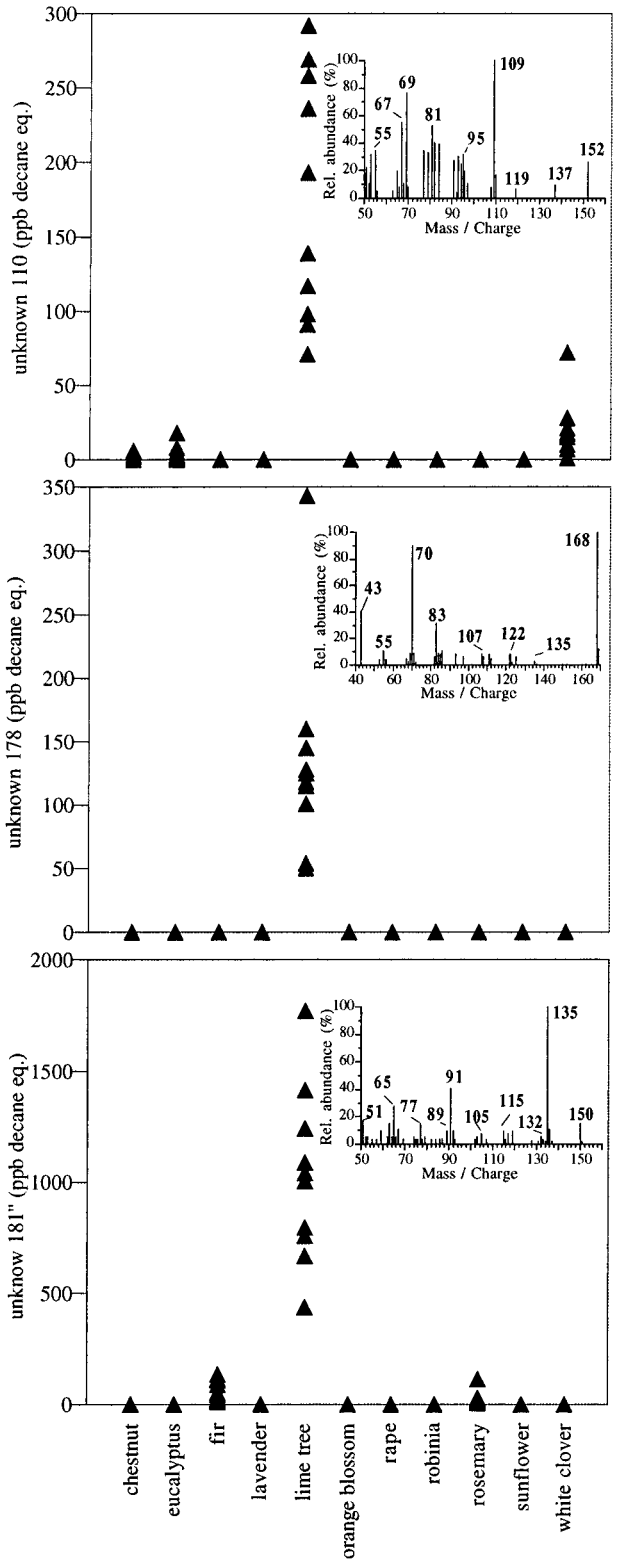

Figure 7. Distribution of unidentified compounds 110, 178, and 181″ in the 110 honey samples.

isolated by Tsuneya et al. (1974) from the same origin, but was not reported as a floral marker. From a sensory point of view, *p*-methylacetophenone was considered as an intensive odorant of lime tree honeys by means of an aroma extract dilution analysis (Blank et al., 1989). Described with "spicy-almond-like" notes, its odor threshold was found equal to 2−3 ng/L air (Blank et al., 1989). In our study, this odorant was characterized by hay notes (Table 2).

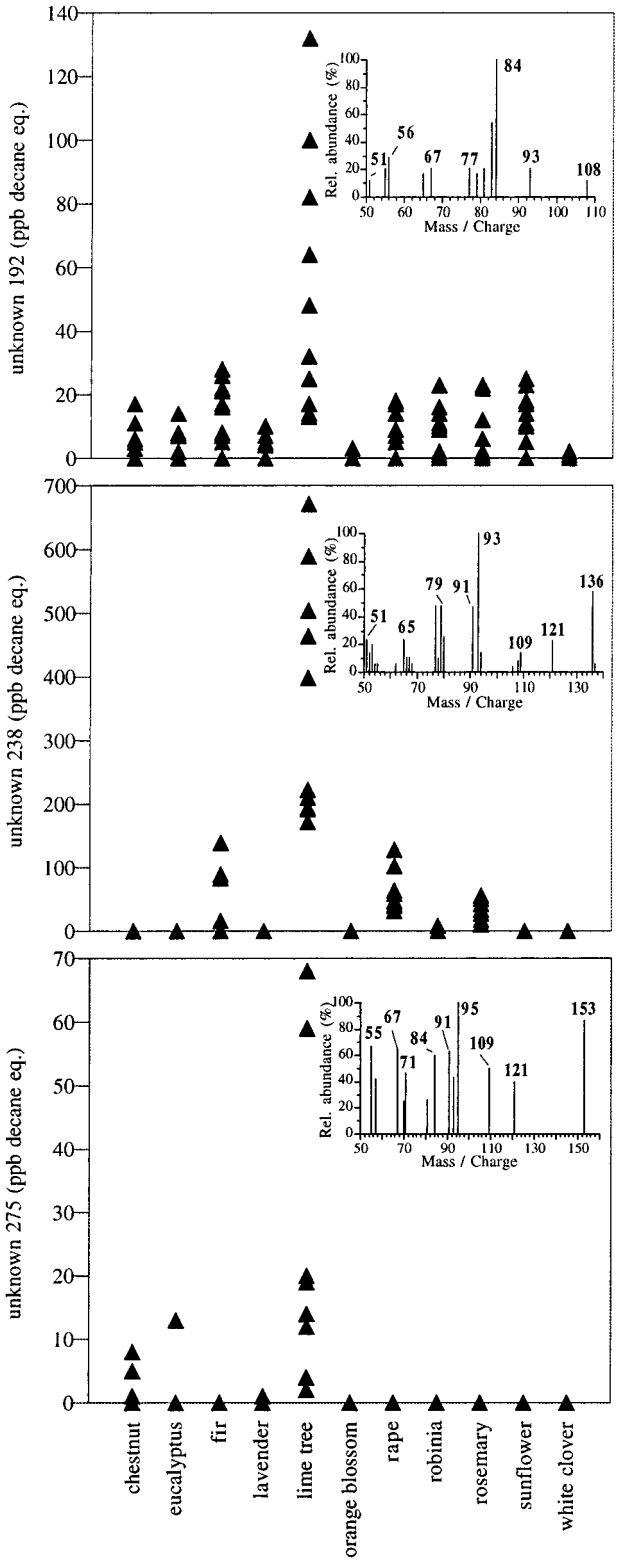

Figure 8. Distribution of unidentified compounds 192, 238, and 275 in the 110 honey samples.

Contrary to lavender (Bouseta et al., 1996) honeys that are especially authenticated with shikimate derivatives, lime tree origin can also be established with compounds issued from other metabolic pathways. Four compounds issued from the terpenic metabolism (Devon and Scott, 1972) were evidenced in larger amounts in lime tree honeys (Figure 5). 8-*p*-Menthene-1,2-diol (15−1317 ppb decane equivalent in our samples) was previously reported by Tsuneya et al. (1974) as a predominant compound of lime tree honeys. Sniffing experiments depicted it as spicy. Its odorless *trans*-isomer was also proposed as an indicator of this origin by Blank et al. (1989). According to these authors, this compound might be synthesized by the bee through limonene hydroxylation. Carvacrol (76−393 ppb), which was also previously reported by Tsuneya et al. (1974), appears in our study as the most interesting marker, with all the other origins showing a concentration far under 60 ppb. This quantitative marker was found a potent odorant with a spicy-thyme odor (Table 2). Thymol (18−161 ppb) and menthol (7−252 ppb), which are evidenced for the first time in lime tree honeys, could be also considered as reliable indicators. However, the latter can also occur in honeys derived from mint plants (Li et al., 1993). Despite a 36 ppm taste threshold in honey (Li et al., 1993), this odorant is logically suspected to contribute to the characteristic mentholated aroma of lime tree honey samples. L-Menthol was recently used by the United States and Canada to treat Tracheal mite infection in bees (Li et al., 1993).

In spite of the lack of knowledge about its metabolic origin, methyl(1-methylethenyl)benzene, an aromatic hydrocarbon olfactometrically detected with vegetable notes (Table 2), can also be considered as an important quantitative marker of lime tree honeys, with concentrations varying from 113 to 971 ppb *p*-cymene equivalent (Figure 6). This compound was previously reported by Overton and Manura (1994) as a minor constituent of wildflower and alfalfa honeys.

Several compounds unidentified until this time also occur as significant components in lime tree honeys (Figures 7 and 8). Most of them are potent odorants with floral, mentholated, balsamic, spicy, and cumin notes (Table 3). Further isolation and identification studies of such compounds could be interesting to use them as floral markers, especially compounds number 178 and 181″ (Figure 7), which are found only in lime tree samples. On the basis of MS fragmentation, the latter, with a 135 prominent ion in the electronic impact spectrum (Figure 7), could be linden ether (3,9-epoxy-1,4(8)-*p*-menthadiene), previously reported by Blank et al. (1989) as floral marker of lime tree honey.

CONCLUSION

From these results, it appears that floral origin of chestnut and lime tree honeys can be easily authenticated with a few aroma compounds.

1-Phenylethanol, in all likehood derived from acetophenone by reduction, and 2-aminoacetophenone could be regarded as the only selection criterions to characterize chestnut honeys. This honey is also suspected of containing other specific aroma constituents and more particularly, nitrogenous compounds.

In the case of lime tree honeys, a large number of odorants could be used for authentication. The quantification of three very discriminant compounds could, however, be advised: ethylmethylphenol, estragole, and carvacrol.

In the context of fraud detection, this work could be reiterated on other unifloral origins. However, further studies would be necessary to elucidate the effect of climatic conditions or the influence of geographic origin on aroma composition of honeys and, particularly, on marker concentrations.

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