Cromathographic Profile of Volatiles of Multifloral and Unifloral Honey Collected by *Apis Mellifera* from Transilvania, Romania

IRINA CIOTLAUS[1], ANA BALEA[1]*, MARIA POJAR-FENESAN[1], IOAN PETEAN[2]
[1]Babes-Bolyai University, "Raluca Ripan" Institute of Research in Chemistry, 30 Fantanele Str., 400294, Cluj-Napoca, Romania
[2]Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos, 400028, Cluj-Napoca, Romania

Chromatographic profile of volatiles organic compounds (VOCs) from several varieties of multifloral and unifloral honey produced in Transylvania, Romania, was analysed in order to determine the differences between them. VOCs collected using solid phase microextraction (SPME) technique were analysed by gas-chromatography coupled with mass-spectrometry (GC-MS). The fiber used was Carboxen / PDMS (polydimethylsiloxane) 75µm. By qualitative analysis, a number of 98 volatiles in unifloral honey and 52 volatiles in multifloral honey were identified. The differences regarding volatile compounds of multifloral honey were observed to be between varieties from different areas of Transylvania (mountain, plain, hill and urban area) while in the case of unifloral assortments (rapeseed, acacia, sunflower and linden) differences were observed at specific compounds. Compounds identified in our samples were grouped into main classes of substances: hydrocarbons, alcohols, aldehydes, ketones, organic acids and their esters, furan and pyran derivatives and terpenes.

Keywords: multifloral honey, unifloral honey, SPME/GC-MS, volatile compound

The amazing flowers varieties growing on plains, hills and mountains in Transylvania offer large number of volatiles compounds (VOCs) in nectar collected by worker bees *Apis mellifera*. Some aspects of floral chemistry are transferred to honey, making from chemical markers an important technique to identify the botanical and geographical origins of the honey. The flavour of honey is due to volatile components contained in it. [1, 2]. Unifloral honey is produced from nectar that is derived either wholly or mainly from a single species or plant and therefore is comprised of specific VOCs [3]. Multifloral honey, from the spontaneous flora harvested, has more VOCs in agreement with the specific flora from that area.

Other authors have studied various types of honey by SPME / GC-MS technique in order to obtain information regarding the geographical and botanical origin [4-7], biomedical properties [8,9], environmental pollution and pesticides accumulation [10-12].

The aim of the present paper is to identify the honey volatiles profile with different geographical and botanical origins collected from Transylvania area, Romania using SPME/GC/MS technique. Floral markers from these honey varieties were identified for the first time in accordance with the melliferous flora of provenance.

Certification of botanical origin of honey was done by Polarized Light Microscopy (PLM). The microscope may be used as a tool, for determination of the botanical origin of pollen found in natural honey. The pollen granules from honey were compared with the images of the pollen from the fresh melliferrous flowers recorded by the same type of microscopy as PLM, as well as the scientific literature. According to the studies of melisopalinological analysis made by authors from Romania, it was found that *Apis mellifera* honey bees from Romania collects pollen mainly from *Brassica napus*, *Tilia*, *Heliantus* and *Robinia pseudocacia* [13]. In other paper [14] we identified by SPME/GC-MS, the volatiles from melliferous plants: acacia, rape, linden and sun flower. This was the reason we have studied honey produced from these plants. Aroma compounds are present in honey at very low concentrations as complex mixtures of volatile components of different classes and low molecular weight. These substances are present in honey, and have been described as characteristics of the floral source, such as terpenes, alcohols, aldehydes, esters, furfural derivatives and other compounds. SPME method has the advantage of being fast, simple and uses samples without chemical processing, don't use of organic solvents, allows the quantification of a large number of molecules, little or no manipulation of samples, substantially shortens the time of analysis and moreover, covers a wide range of sampling techniques including in situ and air sampling [15,16]. Moreover, it can be easily coupled to various analytical instruments, e.g., GC, GC-MS, HPLC, LC-MS and GC-O (GC-olfactometry) [17],

email: ana.balea@yahoo.com

NMR, HPTLC [18]. However, the efficiency of SPME technique is influenced by the following parameters: fiber coating, sample amount, matrix modification by water and sodium chloride addition, agitation of sample matrix, extraction temperature, extraction time and analyte desorption [19].

Experimental part

Materials and methods

The honey analysed are multifloral honey from 2015 with 4 geographical origin: Mures plain, Rodna Mountains, Lapus Subcarpathians and outskirts of Cluj-Napoca city, but also 4 (four) types of unifloral honey: acacia (*Robinia pseudoacacia*), sunflower (*Helianthus annuus*), linden (*Tilia*) and rapeseed (*Brassica napus*) honey. The samples of honey were obtained from apiaries run by independent producers from each area type. The sample honey were stored directly in a refrigerator at 4°C. For unifloral samples, the melisopalinological analysis was performed in order to confirm unifloral origin. Certification of botanical origin of unifloral honey was done in addition to melisopalinological analysis by Polarized Light Microscopy (PLM).

Polarized Light Microscopy (PLM):

The each honey sample with an average size of honey drop, was placed on a glass slide 5 cm x 2.5 cm, clean and degreased with 96% alcohol. The resulting dispersion was coated with a glass lamella and the preparation was studied under the optical microscope. Observations regarding shapes and sizes of pollen was made under transmitted light. PLM was made by using an Laboval 2 Karl Zeiss Jena mineralogical microscope equipped with a Kodak 10 MPx digital imaging acquisition system. The obtained images were processed in a standard manner using Image J specific soft.

Collection of volatiles using SPME method:

SPME sampling was performed in HS (headspace extraction) mode with matrix modification by sodium chloride addition. Sampling preparation was performed according to the method reported previously by *Verzera et al* slightly modified. In each vial, with a volume of 20 ml, 8 grams honey and 3,5 mL of water, together with 1g of NaCl were placed [20]. The closed vials were placed in a water bath with a temperature of 40-45°C and magnetic stirring for 30 minutes to disperse and release the volatiles from the matrix. Then the fiber attachment needle was inserted and exposed SPME fiber in the headspace. Heating and stirring of the sample continue 30 min during collection/ adsorption. At the end of time, the fiber is retracted, holder with the saturated fiber was injected in GC port. The fiber used was Carboxen / PDMS 75µm. Before used, the fiber was preconditioned at 300 °C during 60 minutes in the GC injector port. All parameters involved in using SPME technique (fiber coating, sample amount, matrix modification by water and sodium chloride addition, temperature and time extraction, extraction and desorption time) was optimised to ensure efficient extraction in order to obtain high recoveries of honey volatiles.

GC-MS analysis

The analysis were performed on instrument Model Agilent 7890 & 5975 Series MSD, equipped with a HP-5MS column (30 m x 0.25 mm x 0.25 µM,), using helium as the carrier gas. Volatile compounds adsorbed on the SPME fibre were immediately thermally desorbed in the injector port of a GC and then separated on the GC column. Injections were done using the splitless system. The temperature program was the following: Oven temperature was programmed as 50˚C for 5 min and an increase by 8 ˚C /min to 220 ˚C. From 220 ˚C to 280 ˚C, increase with 20 ˚C /min. It is maintained at 280 ° C for 5 minutes. Injector temperature was 260˚C, detector: 230˚C, splitless. All analyses were carried out in duplicate. NIST library was used for identification/ confirmation of the structure components In addition, a C_8-C_{20} standards alkanes (Alkane Standard Solution C_8-C_{20}, Sigma Aldrich) was used for calculation of the linear retention index (RI), and matching the experimental values with those reported in the literature for similar chromatographic columns, in the same condition. For compounds with RT < 5.690 and RT > 29.978, KI was reported from Nist Library Spectra.

Results and discussions

The unifloral honey samples and pollen from melliferous fresh flower was visualized by the PLM technique are shown in the figures below. These pictures certify honey samples belonging to the botanical origin indicated (Fig.1) comparative with pollen samples from fresh flower indicated (Figure 2).

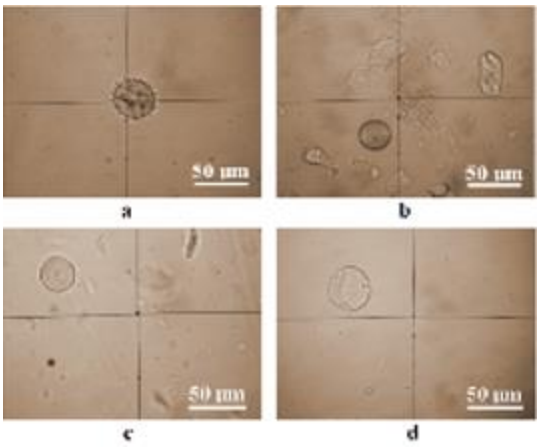

Fig. 1. Pollen grains identified in honey samples: [a) *Helianthus annuus*, b) *Brassica napus*, c) *Robinia pseudoacaccia* and d) *Tillia cordata.*]

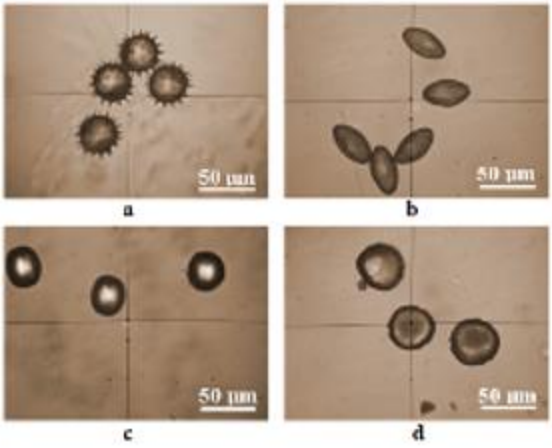

Fig. 2. Pollen grains identified in fresh flower

The average diameter of *sunflower pollen* particles observed in liquid honey is around 37 µm, according to the literature data [21].

In the Rapeseed honey sample only the *Brassica napus* pollen grains were identified without pollen from other plant species. Their resulting average dimensions are 33 µm long and 28 µm in diameter, which is in line with the size of the fresh pollen from the rape flowers. The diameter is significantly higher due to the fact that the pollen granules of the liquid honey are slightly swollen due to the long contact with the honey.

In the *Robinia pseudoacacia* honey have been identified as the spheroidal particles with sizes close to those of the pollen granules collected from the fresh acacia flower. The *Robinia pseudoacacia* pollen grains have a spheroidal shape with an average diameter of 30 micrometres with the values and descriptions of the specialized databases [22].

In the Linden honey**,** from a dimensional viewpoint, the observed *Tilia* pollen granules have a diameter between 32 and 40 µm, which required some additional information. These have been found in the Palodat Palynological Database. In this way, we have pollen granules of the *Tilia tomentosa* and *Tilia cordata*. Pollen granules in the liquid linden honey have the sizes between 26 - 50 µm depending on the positioning of the particle on the glass slide for microscope. Dimensions we get are within the reference range [23, 24].

Multifloral honey volatiles

All the volatile compounds identified in the samples analysed were presented in Table 1.

Table 1
VOLATILES COMPOSITION OF MULTIFLORAL HONEY BY SPME

No.	Compounds	RT	KI	Plain %	Hill %	Mountains %	Urban %
1	Dimethyl sulfide	1.763	512	-	1.8	-	-
2	3-Hidroxy butanal	1.898	755	-	1.59	-	-
3	2-Furfural aldehyde	3.526	830	1.05	12.32	-	1.75
4	3-Furfural aldehyde	3.648	831	3.20	-	-	3.12
5	Benzaldehyde	7.441	957	0.70	-	-	0.50
6	2-Furancarboxaldehyde, 5-methyl	7.528	960	-	-	1.66	2.35
7	Hexanoic acid	7.888	971	-	0.95	0.32	-
8	1-Octen-3-ol	8.096	977	0.11	-	-	-
9	1-octen- 3-one	8.178	980	0.01	-	-	-
10	d-Limonene	9.734	1031	2.72	-	-	-
11	p-Cymene	9.804	1033	0.74	-	3.36	1.17

12	Benzyl alcohol	9.834	1034	-	0.34	-	1.88
13	Benzenacetaldehyde	10.060	1042	1.36	0.80	1.69	4.84
14	Acetophenone	10.766	1065	-	-	-	0.17
15	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	10.823	1067	0.17	-	-	-
16	Cis-linalooloxide (furanoid)	10.983	1075	33.21	5.62	4.80	16.02
17	Trans –linalool oxide (furanoid)	11.447	1088	16.29	2.16	-	9.38
18	Bicyclo[3.2.0]heptan-3-ol,2-methylene-6,6-dimethyl	11.707	1097	-	-	0.87	-
19	Linalool	11.793	1101	-	0.42	2.08	1.90
20	Hotrienol	11.902	1102	14.15	4.91	6.10	27.38
21	Lilac aldehyde A	12.873	1139	-	-	-	3.90
22	Isopulegol	13.090	1140	2.03	-	-	-
23	Lilac aldehyde B	13.234	1148	1.72	-	-	-
24	Lilac aldehyde C	13.241	1154	-	0.31	1.43	5.10
25	4H-Pyran-4-one,2,3-dihydro-3,5-dihydro-3,5-dihydroxy-6-methyl	13.281	1155	-	-	8.68	-
26	Lilac aldehyde D	13.664	1163	-	0.91	1.84	1.59
27	Isoborneol	13.668	1164	-	0.25	1.25	-
28	1-Nonanol	13.827	1168	-	2.59	0.77	-
29	Citronellal	13.849	1169	2.91	-	1.76	3.46
30	Octanoic acid	13.867	1170	-	0.59	-	-
31	Isohydro lavandulyl aldehyde	13.971	1178	-	0.67	-	-
32	Terpinen-4-ol	14.291	1182	0.07	0.52	0.85	2.34
33	Benzenemethanol, α,α,4-trimethyl-	14.352	1187	-	-	-	0.40
34	Myrtenal	14.451	1198	0.32	-	-	0.39
35	Myrtenol	14.496	1199	-	1.52	-	-
36	Decanal	14.664	1206	0.41	-	-	0.83
37	Verbenone	14.828	1208	-	0.22	0.54	-
38	5-Hydroxymethylfurfural	15.227	1229	-	-	7.99	-
39	Nonanoic acid	16.241	1270	-	-	0.30	0.25
40	Thymol	17.044	1303	-	-	-	0.15
41	Cittronelic acid	17.421	1320	-	-	0.26	-
42	n-Decanoic acid	18.570	1371	-	-	0.45	-
43	Beta-damascenone	18.986	1389	-	0.08	0.41	0.25
44	Beta-elemene	19.172	1398	0.13	-	-	-
45	Beta -Calarene	20.083	1441	0.28	0.08	-	-
46	Furan,2-methyl-5-(1,1,5-trimethyl-5-hexenyl)	20.330	1452	-	-	0.26	-
47	1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene	21.084	1488	-	-	-	0.13
48	2,4-Di-tert-butylphenol	21.645	1525	-	0.08	-	-
49	Trans-calamenene	21.960	1531	0.06	-	-	-
50	Pentanoic acid 2 2 4-trimethyl-3-carboxy isopropyl isobutyl ester	23.364	1602	-	-	-	0.08
51	2 2 4-Trimethyl-1 3-pentanediol diisobutyrate	23.378	1603	-	-	0.30	-
52	Dimethyl palmitamine	28.762	1907	-	-	-	0.08

In the multifloral honey case, depending on the geographic area and therefore by the spontaneous flora abundance specific to the relief: mountain, hill, valley, urban, a certain class of chemical compounds predominates (Figure 3).

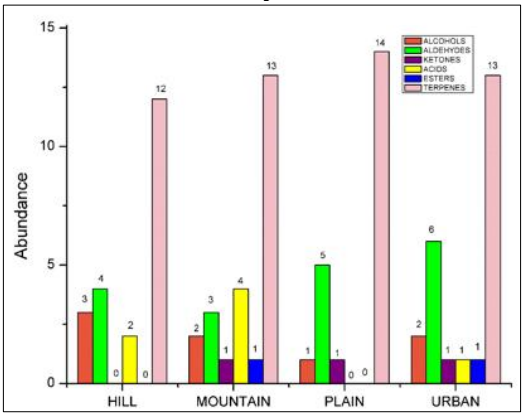

Fig. 3. The classes of chemical compounds from multifloral honey

Four common compounds were identified in all the samples: hotrienol, terpinen-4-ol, cis-linalooloxide and benzeneacetaldehyde. Regarding the possible biosynthetic origins of the Hotrienol, it can be obtained during honey ripening or by other compounds degradation (eg.8-hydroxy linalool) [25]. Hotrienol aroma has been described as sweet and floral [26].

(1) Plain multifloral honey is characterized by a high amount of cis- Linalool oxide, trans- linalool oxide and hotrienol. *The markers are*: isopulegol; d-limonene; 1-octen-3-ol; 1-octen-3-one; lilac aldehyde B; trans-calamenene; β-elemene

(2) Hill multifloral honey is richer in*:* 2-furfural aldehyde; cis-linalool oxide; hotrienol. The markers *are*: 3-hydroxy-butanal; dimethyl sulphide; isodihydro lavandulyl aldehyde; myrtenol; octanoic acid; 2,4-di-tert-bytylphenol.

(3) Mountains multifloral honey is richer in 4H-pyran-4-one-2,3-dihydro-3,5-dihydro-3,5-dihydroxy-6-methyl; 5-hydroxy-methyl-furfural; hotrienol; cis-linalool-oxide. *The markers are:* 4H-pyran-4-one-2,3-dihydro-3,5-dihydro-3,5-dihydroxy-6-methyl; 5-hydroxy-methyl-furfural; 2,2,4-Trimethyl-1, 3-pentanediol diisobutyrate.

(4) Urban multifloral honey – is richer in: hotrienol; cis-linalool oxide; benzene acetaldehyde. The markers are: acetophenone; lilac aldehyde A; benzenemethanol,α,α,4-trimethyl; thymol; dimethyl palmitamine; 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene; pentanoic acid 2, 2, 4-trimethyl-3-carboxy isopropyl isobutyl ester.

Unifloral honey volatiles

All the volatile compounds identified in the samples analyzed were presented in Table 2.

Table 2
VOLATILES COMPOSITION OF UNIFLORAL HONEY BY SPME

No.	Compounds	RT	KI	Acacia %	Sunflower %	Linden %	Rape %
1	Ethanol	1.187	427	-	-	-	1.33
2	Ethyl acetate	1.421	605	-	-	-	1.55
3	3-hydroxy butanal	1.898	755	2.85	1.65	-	-
4	1-butanol-3-methyl	2.166	760	-	-	-	2.63
5	Butanoic acid	2.733	822	-	-	0.1	-
6	2-Furfural-aldehyde	3.526	830	3.64	-	-	-
7	3-Furfural aldehyde	3.648	831	-	11.68	-	-
8	1-Pentanol-3-methyl	3.983	850	-	-	-	4.55
9	2-Hexen-1-ol	4.022	874	4.21	-	-	-
10	Dihydroxyacetone	4.853	922	6.60	2.51	-	-
11	2-Cyclopenten-1-one, 2-hydroxy	6.123	924	0.42	-	-	-
12	Isopropyl benzene	6.530	926	-	-	0.20	-
13	Alpha-pinene	6.740	927	-	0.16	-	-
14	Pentanoic acid, 3-methyl	7.213	950	0.20	0.21	-	-
15	Benzaldehyde	7.441	957	1.25	0.27	0.40	0.48
16	Pentanoic acid,-3-methyl-, ethyl ester	7.477	958	-	-	-	0.19
17	Hexanoic acid	7.888	971	-	-	-	0.09
18	Hexanoic acid, ethyl ester	8.851	1002	-	-	-	0.19
19	Acetaldehyde, (3,3-dimethylcyclohexylidene)	8.950	1005	-	0.83	-	-
20	Isoneral	9.067	1009	-	-	15.19	-
21	p-Methylacetophenone	9.595	1026	-	-	2.05	-
22	D-Limonene	9.734	1031	0.10	0.48	-	-
23	p-Cymene	9.804	1033	-	-	1.12	-
24	Benzyl alcohol	9.834	1034	0.21	0.50	1.92	0.81
25	Benzenacetaldehyde	10.060	1042	2.59	1.22	1.74	
26	Pentanoic acid,2-hydroxy-4-methyl,ethyl ester	10.585	1059	-	-	-	0.10
27	Cis linalool oxide	10.983	1075	8.48	7.13	1.51	1.01
28	Trans-linalool oxide	11.447	1088	5.54	1.68		0.40
29	o-Isopropenyltoluene (p-cymenene)	11.469	1089	-	6.05	15.87	-
30	Linalool	11.793	1.101	1.16	-	2.91	-
31	Hotrienol	11.902	1102	10.55	4.01	3.72	1.07
32	Myrtenyl acetate	12.409	1122	-	-	2.25	-
33	Phenylethyl alcohol	12.145	1113	1.10	1.69	2.81	1.61
34	1,3,3-Trimethylcyclohex-1-ene-4-carboxaldehyde	12.863	1138	-	1.92	-	-
35	Lilac aldehyde A	12.873	1139	0.05	0.06	-	-
36	3-tert-butyl-1-methyl-2-pyrazolin-5-one	13.133	1140	-	-	0.33	-
37	Lilac aldehyde B	13.234	1148	3.34	-	0.5	-
38	Ethanone ,1-(1,4-dimethyl-3-cyclohexen-1-yl)	13.238	1153	-	2.87	1.75	-
39	Lilac aldehyde C	13.241	1154	4.70	-	-	-
40	p-Menthan-3-one	13.289	1155	-	3.24	-	-

41	Pinocarvone	13.581	1161	0.26	-	-	-
42	4-methyl-5-decanol	13.607	1162	-	-	-	0.12
43	Lilac aldehyde D	13.664	1163	1.31	-	-	-
44	Octanoic acid	13.867	1170	5.51	-	-	-
45	Endo-Borneol	13.890	1171	-	4.04	-	-
46	Benzofuran 4,5,6,7-tetrahydro-3,6-dimethyl (menthofuran)	13.899	1172	-	-	2.68	-
47	Benzoic acid, ethyl ester	13.911	1173	-	-	-	11.04
48	Menthol	13.923	1174	-	8.67	-	-
49	2-Undecenal	13.944	1179	-	-	7.21	-
50	Benzenemethanol,α,α,4-trimethyl (p-cymen-8-ol)	14.148	1187	-	-	-	0.13
51	Alpha terpineol	14.349	1193	0.20	3.83	-	-
52	Myrtenal	14.451	1197	0.19	-	-	-
53	Octanoic acid, ethyl ester	14.453	1198	-	-	-	0.36
54	Myrtenol	14.496	1199	-	7.39	-	-
55	Decanal	14.664	1206	1.15	-	-	-
56	Benzene , 1-methyl-4-(1-methylpropyl)	14.729	1207	-	-	2.22	-
57	Verbenone	14.828	1208	-	2.01	-	-
58	Lilac alcohol C	14.851	1214	-	-	-	0.20
59	Phenol 2 (2-methyl-2-propenyl) (6-Allyl-o-cresol)	14.965	1218	-	-	1.29	-
60	1-Cyclohexene-1-acetaldehyde,alpha,4-dimethyl	14.968	1219	0.03	-	-	-
61	5-Hydroxymethylfurfural	15.227	1229	0.63	-	-	-
62	3-Phenyl propanol	15.302	1232	-	-	-	0.06
63	p - Cuminaldehyde	15.610	1245	1.04	2.65	4.23	0.05
64	2-Phenyl propenal	15.632	1246	0.09	-	-	-
65	Benzen acetic acid ethyl ester	15.675	1247	-	-	-	0.55
66	Trans-dihydrocarvone	15.778	1252	-	-	0.44	-
67	Isopiperitone	15.912	1257	-	0.74	-	-
68	Teresantalol	16.038	1262	-	-	0.25	-
69	Nonanoic acid	16.241	1270	0.82	1.17	1.76	0.07
70	p-cymen-7-ol (cuminol)	16.662	1287	-	0.32	-	-
71	1,3-p-Menthadien-7-al	16.684	1288	-	-	6.46	-
72	Nonanoic acid ethyl ester	16.906	1298	-	-	-	0.07
73	Thymol	17.044	1303	-	0.30	0.61	-
74	Sabinene	17.182	1310	-	-	1.06	-
75	R-(+)-Citronellic acid	17.308	1315	-	-	0.32	-
76	Allylphenylsulfide	17.557	1327	-	-	0.08	-
77	Anethole	17.867	1340	-	-	0.06	-
78	Limonene glicol	17.945	1343	-	-	0.35	-
79	8-hydroxylinalool	17.997	1346	0.37	-		-
80	Geranic acid	17.257	1357	-	-	0.14	-
81	Benzene propanoic acid ,ethyl ester	18.151	1358	-	-	-	0.61
82	Eugenol	18.344	1361	-	0.41	-	-
83	Oleic acid	18.440	1365	0.30	-	-	-
84	2(3H)-Furanone,dihydro-5-propyl	18.458	1366	-	-	-	0.03
85	2-(4-methoxyphenyl)ethanol	18.552	1370	-	-	0.47	-
86	Beta-damascenone	18.986	1389	0.25	0.46	0.18	0.08
87	Decanoic acid ethyl ester	19.165	1397	-	-	-	0.03
88	Beta-calarene	20.083	1441	-	1.60	-	-
89	Benzene (2-isothiocyanatoethyl)	20.752	1472	0.04	-	-	-
90	2,4-ditertbutylphenol	21.645	1516	0.90	-	0.05	0.01
91	Tridecanoic acid, 3-hydroxy, ethyl ester	22.070	1537	-	-	-	0.01
92	Dodecanoic acid ethyl ester	23.245	1596	-	-	-	0.01
93	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	23.366	1602	0.65	-	0.05	0.01
94	Triciclo[3.2.1.02,7]oct-3-ene,2,3,4,5-tetramethyl	24.388	1657	-	-	0.02	-
95	Dimethyl palmitmine	28.779	1907	0.46	-	-	-
96	Ethyl palmitate	29.938	1997	-	-	-	0.01
97	Morpholyne,4-octadecyl	30.969	2054	0.16	-	-	
98	Ethyl oleate	31.639	2179	-	-	-	0.02

For unifloral honey**,** 98 compounds have been identified. Common volatile compounds identified in all unifloral honey type are: β-damascenone, cis-linalool oxide, hotrienol, p-cuminalaldehyde, nonanoic acid, phenylethyl alcohol, benzyl alcohol, benzaldehyde. This compounds reported as new specific compounds, can be considered as new markers for that type of honey. Specific markers for each type of honey show the authenticity of the floral origin.

In the unifloral honey case, the assortment of honey have different of volatile contents, some or other classes of substances are predominant (Figure 4)

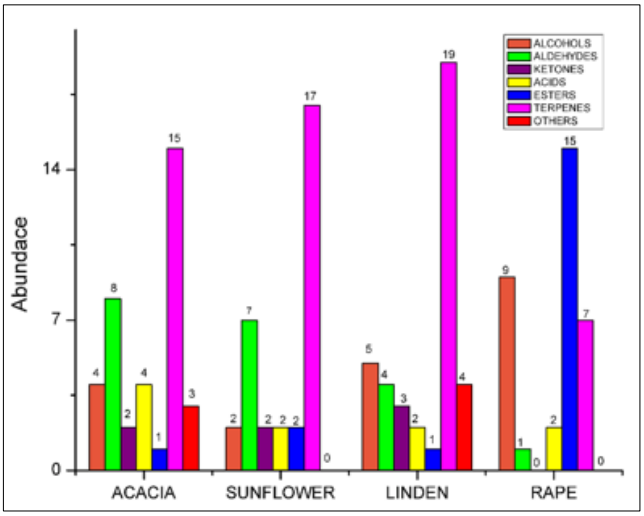

Fig. 4. The classes of chemical compounds from unifloral honey

(1) Acacia honey is richer in terpenes and aldehydes, totalling 37 compounds. The new specific compounds of acacia honey are: 2-furfural-aldehyde; 2-hexen-1-ol; 2-cyclopenten-1-one; 2-hydroxy; lilac aldehyde C; lilac aldehyde D; octanoic acid; pinocarvone; myrtenal; decanal; 5 – hydroxymethylfurfural; 2-phenylpropenal; 8-hydroxylinalool; oleic acid; benzene (2-isothiocyanatoethyl); dimethyl palmitamine. Hotrienol along with linalool oxide, identified in high concentration in the acacia honey, contributes to its specific aroma.

(2) Sunflower honey - is richer in terpenes and aldehydes, totalling 32 compounds. Sunflower honey had the least numbers of VOCs. Interestingly, the *Helianthus annuus* plant contains more menthol than peppermint (*Mentha piperita L*) (*35*). *The new specific compounds of sunflower honey are:* 3-furfural aldehyde; acetaldehyde,(3,3-dimethylcyclo-hexylidene); 1,3,3-trimethylcyclohex-1-ene-4-carboxaldehyde; p-menthan-3-one; endo-borneol; menthol; myrtenol; verbenone; isopiperitone; p-cymen-7-ol; eugenol; beta-calarene. The volatiles that give the odour of sunflower honey are: 3-furfural aldehyde - sweet, woody, almond, fragrant, baked bread; menthol–very cooling, fresh; cis-linalool oxide (furanoid) - sweet floral, green, fruity, myrtenol - warm-herbaceous odour, slightly woody; hotrienol - sweet, tropical and ginger. [27, 28].

(3) Linden honey - is richer in terpenes and aromatic alcohols, totalling 38 compounds. *The new specific compounds of linden honey are*: butanoic acid; isopropyl benzene; isoneral; o-methylacetophenone; benzofuran-4,5,6,7-tetrahydro-3,6-dimethyl; 2-undecenal; benzene, 1-methyl-4-(1-methylpropyl); 6-allyl-o-cresol; trans-dihydrocarvone; teresantalol; sabinene; allylphenylsulfide; geranic acid; myrtenyl acetate. For linden honey, according to the Database of Plant's Aroma Molecules, p-cymenene offers a fragrance of citrus.

(4) Rape honey – *is* richer in esters, totalling 34 compounds. The abundant compounds identified in this sample are the following*:* benzoic acid, ethyl ester; 1-pentanol-3-methyl; 1-butanol-3-methyl. The new specific compounds of rape honey are: 1-pentanol-3-methyl; pentanoic acid, 2-hydroxy-4-methyl,ethyl ester; trans-linalool oxide; benzenemethanol,α,α,4-trimethyl; lilac alcohol C; 3-phenyl propanol, 2(3H)-furanone,dihydro-5-propyl; decanoic acid ethyl ester; tridecanoic acid, 3-hydroxy, ethyl ester; dodecanoic acid ethyl ester; 2,2,4-trimethyl-1,3-pentanediol diisobutyrate; ethyl palmitate; ethyl oleate; The rape honey, with less volatiles has as odorants: benzoic acid ethyl ester -sweet, wintergreen, fruity, medicinal, cherry, grape; 1-pentanol-3-methyl- pungent, fusel, cognac and wine, cocoa, with green fruity undernotes; 1-butanol-3-methyl - alcoholic, pungent, etherial, cognac, fruity, banana and molasses; [29].

Conclusions

Many volatile substances previously identified and reported in the literature were detected in honey but there were also new compounds identified, which did not reported in the literature.

The honeys produced from different floral sources have different aromas and tastes due to differences in volatile composition, which is also dependent on the botanical and geographical origins of the plant, eventually to the flowers from the apiary, variability of weather conditions, proximity to the forest, soil characteristics and beekeeping practices.

For multifloral honey depending on the spontaneous flora specific to the relief: mountain, hill, valley, urban, a certain class of chemical compounds predominates.

For unifloral honey: rapeseed, linden, acacia and sunflower, new floral markers were identified. Specific markers for each type of honey show the authenticity of the floral origin.

Some volatile compounds identified are involved in the antibacterial and antioxidant effect of the honey.

Identification of honey volatiles by hyphenated technique SPME-GC-MS contributes to establish specific markers. These can be included in a database useful in authenticating honey varieties.

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