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Identification of volatile organic compounds in honeydew honeys using comprehensive gas chromatography

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Summary
This work was focused on characterization of volatile organic compounds present in honeydew honeys using solid phase microextraction (SPME) followed by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. Among five commercially available SPME fibres tested for proper sample preparation procedure, divinylbenzene/carboxen/polydimethylsiloxane coating presented the best results as to the total peak areas and higher number of detected compounds. Furthermore, two column setups with diverse orthogonal properties were applied for honeydew honey samples analysis. Consequently, up to 300 compounds were detected at the given signal-to-noise ratio of 200. The identified compounds belonged to various chemical classes (hydrocarbons, alcohols, aldehydes, ketones, terpenes and benzene derivatives). On the basis of the results of the analyses, 2,3-butanediol, 3-hydroxy-2-butanone, acetic acid and methyl ester of 2-hydrobenzoic acid were established as markers of honeydew honey.

Keywords
honeydew honey; volatile organic compounds; comprehensive two-dimensional gas chromatography

Honey was used from the Stone Age till the eighteenth century as the only available concentrated natural sweetener. Codex Alimentarius defines honey as "the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature" [1]. Among various kinds of honeys, a special one is the honeydew honey, which originates from the secretion of insects feeding on plant juices or plant secretion [2]. In general, the chemical composition of honeydew honey differs from that of nectar honey [3] in many parameters, e.g. pH [4], ash content [5], colour and saccharide profile [6], as well as mineral contents [7]. Moreover, while nectar honey is invariably levorotatory, honeydew honey may be also dextrorotatory. Honeydew honey contains lower levels of fructose and glucose, and higher levels of oligosaccharides, and exhibits higher antioxidant and antibacterial activities than most nectar honeys [8].

The growing market for honeydew honey in many European countries requires powerful analytical methods for its differentiation from other floral honey types, especially in the case of adulteration of honey or a partial replacement with similar artificial products [8]. One of the most typical feature of a food product used for the evaluation of both organoleptic quality and authenticity is its characteristic aroma profile, which is mainly represented by the composition of volatile organic substances. Generally, high number of volatile organic compounds (VOC) present in the food product generates its characteristic VOC profile, so-called fingerprint of the given product. VOC fingerprinting was successfully used to distinguish botanical and geographical origin of honey [9–13].

The liquid–liquid extraction, simultaneous distillation–extraction, supercritical fluid extraction, solid-phase extraction and ultrasound extraction are traditional analytical methods employing solvents, which are commonly used. These methods are labour-intensive and time-consuming as well as dangerous, because require large amounts of toxic and expensive solvents [14]. For these reasons,

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another technique, namely, solid-phase microextraction (SPME) has become the most common used technique. SPME is a solvent-free sample pre-treatment technique that integrates sampling, isolation and enrichment of analytes into one step. The technique was developed and implemented in analytical practice in the 1990s by professor Janusz Pawliszyn, University of Waterloo (Ontario, Canada) [15]. SPME shows advantages compared to traditional analytical techniques: eliminates the use of toxic organic solvents, facilitates quantification of a large number of compounds and covers a wide range of sampling techniques, including field, in situ and air sampling. On the other hand, optimization of conditions of its use is time consuming, regarding the type of SPME fibre, sorption temperature, sorption time and desorption temperature [14]. Recently, SPME was widely used for extraction of VOC from honeys [11, 14, 16–20].

Based on simultaneous application of two chromatographic columns, comprehensive two-dimensional gas chromatography with higher separation efficiency has become a preferred technique for analysis of complex matrices. Špánik et al. [21] studied VOC fraction of acacia, lime, rape, sunflower, raspberry, phacelia monofloral honey samples by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detector (GC×GC-TOF-MS). The identified compounds were divided into two important groups, ubiquitous compounds found in all studied honeys and compounds specific for individual type of honeys, depending on their botanical origin. Another study using GC×GC-TOF-MS was devoted to VOC composition of Polish honeys obtained from various geographical regions [22]. Totally, 329 compounds were identified in studied acacia, linden, rapeseed, buckwheat and honeydew honeys. Many VOC identified in Polish honey samples were present also in honeys from other European regions, such as acyclic and cyclic alkanes, acyclic and cyclic alkenes, aromatic hydrocarbons, oxygenated aromatic compounds, alcohols, aldehydes, ketones, esters, ethers, nitriles, organic sulphides, phenolic compounds and terpenes, while some of them were present only in Polish honeys. VOC profiles were also studied in samples of honeys from various countries of Europe, in order to determine geographical markers of Corsican honeys, significant differences being identified in the types of extracted compounds and their concentrations [23]. The GC×GC analysis was also used for identification of extraction artifacts in honeys [24] and separation of disaccharides [25]. Consequently, the purpose of this study was to properly define the VOC profile of Slovak honeydew honeys by GCxGC-TOF-MS technique using different types of SPME fibres for VOC extraction from honey.

MATERIALS AND METHODS

In this work, 35 samples of honeydew honeys were investigated in detail. All studied honeys originated from Slovakia and were collected by local beekeepers in 2010–2012. The honeys originated from regions upper Kysuce and Orava, where spruce forests mixed with pines and firs are predominant.

VOC from honey samples were extracted by the solid phase microextraction (SPME) procedure using a Gerstel MPS2 autosampler (Gerstel, Mülheim, Germany). An amount of 5 g of honey together with 0.5 g of NaCl was dissolved in 1 ml of distilled water in a 20 ml clear glass vial. An amount of 20 μl of benzophenone solution in methanol (10 $\mu g \cdot ml^{-1}$) was then added to the solution as an internal standard. A stirred sample solution at 47 $rad \cdot s^{-1}$ was heated at 60 °C for 30 min. VOC were extracted by five different SPME fibres, namely, polydimethylsiloxane (PDMS, 100μm), divinylbenzene/polydimethylsiloxane (DVB/PDMS, 65 μm), polyacrylate (PA, 85 μm), carboxen/polydimethylsiloxane (CAR/PDMS, 85μm) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CARB/PDMS, 50/30 μm). All five fibres were obtained from Supelco (Bellefonte, Pennsylvania, USA). The fibres were conditioned prior to use by heating under the conditions recommended by the manufacturer. GC-TOF-MS was used to find suitable SPME working conditions: equilibration time 30 min, equilibration temperature 60 °C, extraction time 30 min and extraction temperature 60 °C, while the solution was stirred at 47 $rad \cdot s^{-1}$. Desorption was performed in GC injector in splitless mode at 220 °C for 2 min.

LECO Pegasus 4D (LECO, St. Joseph, Michigan, USA) consisting of an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA) and Gerstel MPS 2 autosampler, equipped with a cooled injection system (CIS) injector, dual-stage thermal modulator and secondary oven connected to a time-of-flight mass spectrometer was used to characterize the VOC honey profiles. Mass spectra at a mass range m/z from 29 to 600 were recorded. Helium was used as a carrier gas at a constant flow of 1.0 $ml \cdot min^{-1}$. The extracted VOC were separated in a 30 m capillary column of 0.25 mm internal diameter and 0.25 μm film thickness of polyethylene glycol modified by nitroterephthalic acid phase (DB-FFAP; Agilent

J&W, Santa Clara, California, USA) in one-dimensional experiments.

For two-dimensional GC experiments, either non-polar × polar column combination consisting of DB-5ms column (30 m × 0.25 mm × 0.25 μm film thickness of (5%-phenyl)-methylpolysiloxane; Agilent Technologies) in the first dimension and 1.2 m Supelcowax-10 column (1.2 m × 0.1 mm × 0.1 μm film thickness of polyethylene glycol; Supelco) in the second dimension, or, in a reversed column setup, polar × medium-polar type containing DB-FFAP in the first and BPX-50 (1.5 m × 0.1 mm × 0.1 μm film thickness of 50% diphenyl – 50% dimethyl polyphenylene-siloxane; SGE Analytical Science, Victoria, Australia) in the second dimension were used.

The primary oven was programmed from 40 °C (1 min) to 220 °C (5 min) at 2.0 °C·min^{-1} rate. The temperature offset between secondary and primary oven was 10 °C, 8 s modulation period, hot pulse duration 1.5 s, temperature offset of modulation 30 °C, temperature of transfer line 240 °C, temperature of ion source 230 °C, electron ionization energy 70 eV. Leco ChromaTof software (version 4.21) was used for instrument control and data evaluation. Data were processed at signal-to-noise ratio of 200. Identification of organic compounds was performed by:

i) comparison of obtained MS spectra with Willey and NIST MS libraries (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). The compound was considered as identified by GC-MS, if the quality match higher than 90% was reached,
ii) GC-MS analysis of standards,
iii) comparison with previously reported compounds found in honeydew honeys by GC-MS,
iv) comparison of calculated linear retention indices (LRI) with LRI libraries.

Only compounds identified by combination of (i) or (ii) were considered as identified. Compounds identified by combination of (i) with (iii) were considered as tentatively identified. Solutions of authentic standard compounds (1 μl) were injected directly into CIS injector in split mode (1:50).

Anhydrous NaCl used in sample treatment procedure was obtained from Mikrochem (Pezinok, Slovakia). The list of available authentic standard compounds is given in Tab. 1. Standard solutions of chemicals (1 mg·kg^{-1}) were prepared in hexane or methanol (Merck, Darmstadt, Germany). The mixture of alkanes (C7-C30) used for calculation of linear retention indices was obtained from Supelco.

RESULTS AND DISCUSSION

Solid-phase microextraction procedure

Although solid phase microextraction is the most commonly used method for extraction of volatile organic compounds from honey, any single solution concerning the appropriate fibre coating has not been presented [26]. For example, Soria et al. [11] showed that CAR/PDMS SPME fibre had satisfactory extraction efficiency, precision and sensitivity for volatile and semi-volatile compounds. In a different study, Čajka et al. [27] recommended DVB/CAR/PDMS fibre due to the best sorption capacity for the broadest range of volatiles extracted from honey samples. In our work, VOC from honeydew honey were extracted by five different SPME fibres, such as non-polar PDMS, semi-polar DVB/PDMS, polar PA, and bipolars CAR/PDMS and DVB/CAR/PDMS. Moreover, due to carboxen and divinylbenzene additives, fibre coatings display porous structure with a wide range of pore sizes. Fig. 1 shows the comparison of relative peak areas for compounds belonging to various chemical classes obtained from honeydew honey by different SPME fibres. Totally, 185 compounds were extracted by triple phase DVB/CAR/PDMS fibres from honeydew honeys, among them mostly alkanes, alcohols, carbonyl compounds, esters and terpenes. It is noteworthy that carbonyl compounds were found at the lowest concentration level, while terpenes at the highest concentration level. Similar results were obtained by DVB/PDMS fibres but relative peak areas and number of extracted VOC were lower. Overall, 158 VOC were extracted by fibres with DVB/PDMS coating, where alcohols and carbonyl compounds were found at lower concentration levels than for the previous fibres, but the opposite situation was in the case of terpenes. On the contrary, PA fibre coating was more effective at extraction of esters and terpenes. Only 80 compounds were extracted by PDMS and CAR/PDMS coatings, most of which being terpenes and alcohols. These results implied that DVB/CAR/PDMS fibres were the most suitable for extraction of VOC from honeydew honey.

The column setups

In general, the orthogonal approach determines an effective selection of column combination in comprehensive two-dimensional gas chromatography but, at the same time, non-orthogonal approach is also capable to provide sufficient separation efficiency [28]. The combination non-polar × polar and polar × medium polar column setups were studied in our work. The

Tab. 1. Volatile organic compounds identified in honeydew honey by GC × GC-TOFMS.

	Name	Area* [%]	DB-FFAP × BPX-50			DB-5ms × SUPELCOWAX-10		
			1st [min]	2nd [s]	*LRI*	1st [min]	2nd [s]	*LRI*
1	Hexane[a]	2.63	4.25	1.23	600	4.93	0.53	600
2	Dimethyl sulfide[b]	1.29	4.86	1.23	744	4.13	0.58	516
3	Furan[b]	0.14	5.09	1.19	789	13.20	5.17	835
4	Octane[c]	1.75	5.14	1.73	800	11.20	0.62	800
5	2,4-Hexadiene[b]	0.13	5.22	1.37	807			
6	Acetic acid, methyl ester[b]	0.37	5.49	1.25	829	4.13	0.68	516
7	5-Methylfuran[b]	0.23	6.23	1.35	886			
8	2-Butanone[b]	2.93	6.51	1.39	905	5.07	0.80	607
9	2,3-Butanedione[b]	2.46	8.27	1.33	985	4.80	0.97	587
10	Decane[b]	0.66	8.65	3.30	1000	27.60	0.69	1000
11	2-Methylbutanoic acid, methyl ester[c]	0.08	8.93	1.83	1010			
12	α-Pinene[b]	0.09	9.22	2.80	1019	24.67	0.86	974
13	2-Butanol[b]	0.07	9.37	1.32	1024			
14	2,3-Pentanedione[b]	0.04	10.83	1.58	1067	7.20	1.18	700
15	Dimethyl disulfide[b]	0.18	11.16	1.83	1076	8.80	1.15	745
16	Pentanoic acid, methyl ester[c]	0.11	11.70	2.04	1090	12.53	1.05	824
17	2-Methyl-1-propanol[b]	0.39	11.85	1.32	1093			
18	Undecane[b]	0.37	12.11	4.50	1100	31.07	0.72	1100
19	2-Methyl-2-butenal[b]	0.32	12.15	1.78	1100			
20	3-Pentanol[a]	0.49	12.46	1.45	1101	7.47	1.37	708
21	2-Pentanol[b]	1.02	13.09	1.44	1102	7.33	1.45	704
22	3-Penten-2-one[b]	0.03	13.67	1.86	1103	47.07	1.44	1333
23	1-Butanol[a]	0.10	14.22	1.41	1103			
24	α-Terpinene[b]	0.03	15.87	3.27	1106			
25	Heptanal[b]	0.05	16.40	2.34	1107	17.07	1.19	890
26	Hexanoic acid, methyl ester[c]	2.37	16.51	2.40	1107	18.67	1.16	910
27	Limonene[b]	0.28	16.76	3.29	1107	26.00	0.93	986
28	3-Methyl-1-butanol[b]	0.54	17.47	1.44	1207			
29	γ-Terpinene[b]	0.02	19.47	3.45	1245			
30	*p*-Cymene[b]	3.08	20.93	2.97	1270	25.73	1.14	984
31	Terpinolene[b]	0.39	21.60	3.54	1281	30.40	0.97	1082
32	2-Octanone[b]	0.69	21.98	2.63	1287	23.33	1.27	961
33	3-Hydroxy-2-butanone[b]	0.21	22.00	1.43	1287			
34	Heptanoic acid, methyl ester[c]	1.40	22.13	2.73	1290	22.00	1.09	948
35	Octanal[b]	0.22	22.17	2.64	1290	24.13	1.27	969
36	3-Heptanol[b]	0.03	22.40	1.76	1294			
37	Tridecane[c]	0.24	22.80	6.08	1300	45.07	0.73	1300
38	4-Methyl-1-pentanol[b]	0.10	23.60	1.55	1315			
39	2-Heptanol[b]	1.70	23.87	1.76	1320	17.33	1.87	894
40	3-Methyl-2-buten-1-ol[b]	0.11	24.13	1.45	1324			
41	6-Methyl-5-hepten-2-one[b]	0.16	25.07	2.35	1341	23.07	1.52	959
42	Rose oxide[b]	0.12	25.73	3.23	1352	32.00	1.11	1114
43	1-Hexanol[b]	0.28	25.87	1.59	1354	15.47	2.43	869
44	3-Hexen-1-ol[b]	0.10	27.81	1.56	1385			
45	Octanoic acid, methyl ester[c]	5.17	28.21	2.99	1391	32.93	1.15	1128
46	3-Octanol[b]	0.10	28.40	1.90	1394	23.87	1.71	967
47	Nonanal[b]	1.22	28.40	2.87	1394	31.47	1.26	1106
48	Tetradecane[c]	0.09	28.80	6.42	1400	51.47	0.75	1400
49	2-Octanol[b]	0.10	30.00	1.85	1422			
50	*p*,α-dimethylstyrene[b]	0.52	30.95	2.71	1438	30.67	1.49	1089
51	1-Octen-3-ol[a]	0.61	31.87	1.70	1453	22.80	2.27	956
52	1-Heptanol[b]	0.07	32.07	1.70	1457			

Tab. 1. continued

	Name	Area* [%]	DB-FFAP × BPX-50			DB-5ms × SUPELCOWAX-10		
			1st [min]	2nd [s]	*LRI*	1st [min]	2nd [s]	*LRI*
53	Acetic acid[b]	0.41	32.29	1.22	1460	6.93	7.73	690
54	6-Methyl-5-hepten-2-ol[b]	0.11	32.56	1.75	1465	23.73	2.25	965
55	Furfural[b]	0.05	33.07	1.57	1473	13.20	5.17	835
56	2-Ethyl-1-hexanol[b]	0.27	34.13	1.77	1490			
57	Nonanoic acid, methyl ester[c]	1.87	34.29	3.20	1492	40.00	1.11	1227
58	Decanal[e]	1.00	34.67	3.01	1498	38.67	1.26	1206
59	Pentadecane[c]	0.19	34.80	6.49	1500	55.33	0.74	1500
60	2-Acetylfuran[b]	1.59	35.33	1.72	1510			
61	2-Nonanol[b]	0.57	35.87	1.98	1519			
62	Benzaldehyde[b]	3.68	36.27	1.91	1526	21.33	3.44	941
63	2,3-Butanediol[b]	0.55	37.20	1.30	1543			
64	Linalool[b]	0.16	37.60	1.94	1550			
65	1-Octanol[b]	0.32	38.13	1.80	1559	29.60	2.30	1059
66	Lilac aldehyde D[b]	0.02	39.73	2.45	1585			
67	α-Isophorone[b]	0.90	39.87	2.37	1587			
68	Decanoic acid, methyl ester[c]	0.25	40.27	3.27	1594	46.67	1.16	1326
69	4-Terpineol[b]	1.62	40.43	2.38	1596	37.07	1.72	1185
70	Dihydrocarvone[b]	0.01	40.80	2.71	1603	38.27	1.71	1200
71	Hotrienol[b]	1.15	41.26	1.85	1611	31.87	2.39	1112
72	γ-Pentalactone[b]	0.65	41.33	1.79	1613			
73	2-Acetyl-5-methylfuran[b]	0.02	41.60	1.96	1618			
74	Benzoic acid, methyl ester[c]	1.30	41.87	2.14	1623	31.07	2.66	1100
75	Butyrolactone[b]	0.15	42.40	1.71	1633	18.40	6.98	907
76	Menthol[b]	0.03	42.65	2.08	1638			
77	Benzeneacetaldehyde[b]	1.92	43.20	1.92	1648	27.33	3.68	998
78	Acetophenone[b]	0.87	43.47	2.02	1653	29.07	3.42	1044
79	1-Nonanol[b]	0.98	43.89	1.90	1660	36.67	2.10	1180
80	Isoborneol[b]	1.10	44.13	2.04	1665	36.40	2.56	1176
81	Benzoic acid, ethyl ester[c]	0.06	44.27	2.37	1667	36.40	2.22	1176
82	α-Terpineol[b]	0.81	45.87	2.10	1695			
83	Verbenone[b]	0.07	46.13	2.48	1700			
84	Carvone[b]	0.07	47.73	2.51	1731	41.60	2.14	1251
85	2-Hydroxybenzoic acid, methyl ester[c]	0.45	50.13	2.13	1776			
86	Cumaldehyde[b]	0.03	50.40	2.32	1781			
87	Myrtenol[b]	0.20	50.93	1.87	1790	46.80	1.46	1328
88	δ-Hexalactone[b]	1.18	51.07	1.95	1793			
89	3-Methylpentanoic acid[b]	0.98	51.20	1.30	1795			
90	Lilac alcohol D[b]	0.13	52.83	1.87	1827	38.67	2.39	1206
91	Carveol [b] (*cis* isomer)	0.02	53.33	1.85	1837	33.87	1.24	1141
92	Benzenepropanoic acid, methyl ester[c]	0.14	53.87	2.29	1847	43.60	2.62	1280
93	Geraniol[a]	0.03	54.00	1.82	1850			
94	Hexanoic acid[b]	0.31	54.00	1.33	1850	24.53	6.37	973
95	P-cymen-8-ol[b]	0.86	54.10	1.78	1852	37.87	3.81	1195
96	Guaiacol[b]	0.02	54.80	1.58	1865			
97	Benzyl alcohol[b]		55.60	1.47	1880	27.60	2.60	1000
98	Phenylethyl alcohol[b]		57.20	1.59	1911	32.80	7.70	1126
99	1-(1H-pyrrol-2-yl)ethanone[b]		60.40	1.47	1979			
100	Phenol[b]		62.00	1.26	2012	8.13	1.18	728

LRI – linear retention indices were calculated on the basis of the elution data from the first dimension.
* – the ratio of the compound peak area related to the internal standard response to total peak areas of identified compounds obtained on DB-FFAP column, where peak area was calculated relative to the internal standard area.
a – Merck (Darmstadt, Germany); b – Sigma–Aldrich (St. Louis, Missouri, USA); c – Supelco (Bellefonte, Pennsylvania, USA); d – SAFC Biosciences (Lenexa, Kansas, USA); e – Firmenich & Cie (Geneva, Switzerland).

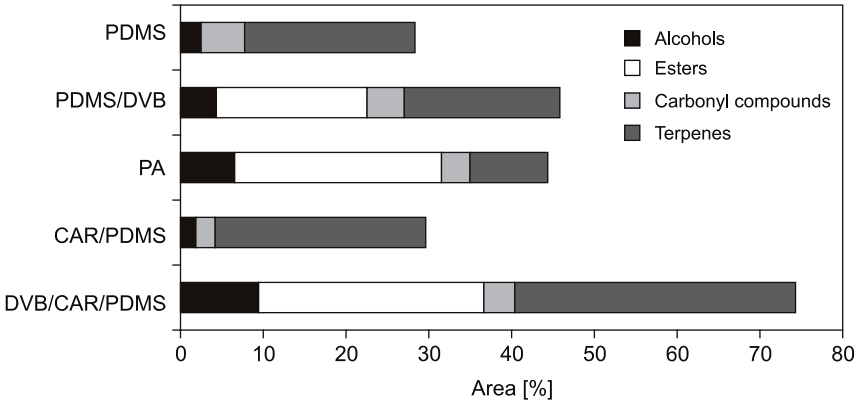

Fig. 1. Comparison of peak areas of volatile organic compounds obtained by different SPME fibres.

non-polar × polar setup was realized by DB-5ms and Supelcowax-10, while DB-FFAP and BPX-50 was used for polar × medium polar column setup. GC×GC-TOF-MS chromatograms of honeydew honey obtained by various column setups are shown in Fig. 2. The combination of non-polar × polar columns provides apparently a better 2D separation space with better distribution of peaks, but the results should be more carefully interpreted. DB-FFAP × BPX-50 column set shows a chromatographic profile with ordered structure for the most common classes of compounds found in honeydew honey, which could simplify the evaluation of results (Fig. 3). This fact suggests that the separation with desired group-type identification was successfully achieved in the case of non-orthogonal approach. However, alkanes, alcohols and furan compounds found at low concentration levels were separated only by DB-5ms × Supelcowax-10 column setup.

Volatile organic compounds profile

Totally, more than 300 various volatile organic compounds (VOC) were identified in honeydew honeys by GC×GC-TOF-MS, while for 100 of them, confirmed by injection of standards, a calculated area percentage greater than 0.01% was found. The list of identified compounds together with retention times, area percentage as well as calculated linear retention indices obtained by both column combinations is presented in Tab. 1.

The identified compounds belonged to various chemical classes (hydrocarbons, alcohols, aldehydes and ketones, terpenes, benzene derivatives and various compounds containing a heteroatom). Since the detected hydrocarbons, such as C8 to C15 linear alkanes, 2,4-hexadiene, α-pinene, *p*-cymene, are known to originate mainly from beewax or plantwax, and honeybee cuticle [29], these compounds could not be defined as the markers of the studied honey type. Regarding alcohols

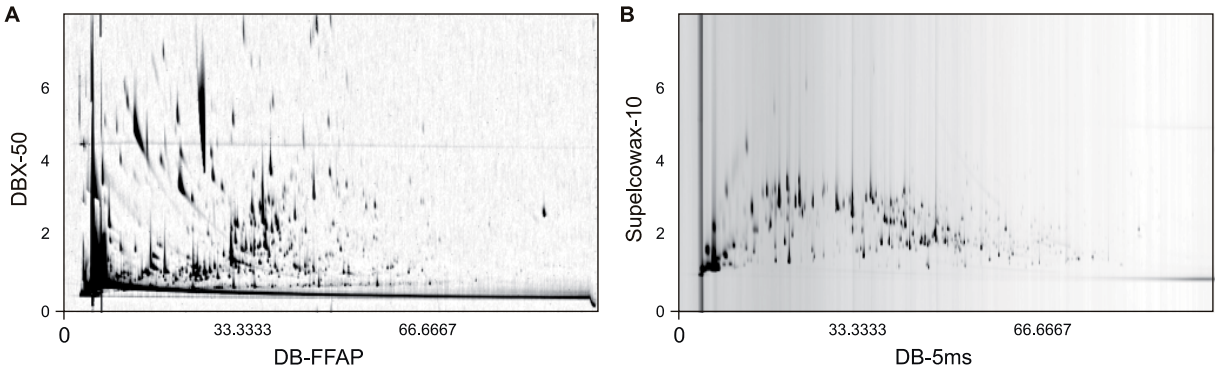

Fig. 2. GC×GC-TOF-MS chromatograms of the studied honeydew honey obtained with the different column combinations.

A – DB-FFAP and BPX-50, B – DB-5ms and Supelcowax-10.

that, similarly to esters, are synthesized by acetic metabolic pathway in plants, 2-heptanol, 1-octen-3-ol, 2,3-butanediol and 2,6-dimethyl-1,7-octadien-3,6-diol were present at higher concentration levels. In addition, 2,3-butanediol was suggested as authentication marker of honeydew honeys by other authors [30–32]. The presence of these diols in honeydew honeys could be related to 3-hydroxy-2-butanone, which is also reported as a typical marker for honeydew honeys [30]. The other carbonyl compounds, in particular aldehydes 3-methyl-1-butanal, octanal and nonanal, were found at higher concentration levels compared to monofloral honeys. Furthermore, all studied honeydew honeys contained a homologous series of 2-ketones, cycloketones, cyclohexanone and 2,6,6-trimethylcyclohexanone. On the other hand, cyclohexanone, which was previously reported as a suitable marker for honeydew honey, was also identified in citrus honey, and therefore is not suitable as a marker of botanical origin [33]. The presence of acetic acid was confirmed by an authentic standard compound. This compound was previously reported as indicative for honeydew honey [30, 34, 35]. Among the large number other organic acids and their esters, methyl esters of C6 and C9 carboxylic acids, and of benzeneacetic acid, were dominant. In honeydew honey, 2-oxooctanoic acid and 4-oxapentanoic acid, allyl ester of acetic acid and methyl ester of 2,6-dihydroxybenzoic acid were identified. These VOC are characteristic unique compounds for honeydew honey, because they were not identified in the most common Slovakian unifloral honeys [21]. Moreover, the detected methyl ester of 2-hydrobenzoic acid was also found to be specific for willow honey [29]. It is supposed that the presence of benzoic and phenolic derivatives mainly connected with the shikimate metabolic pathway. At the same time, some other benzene compounds such as benzofuran, propylbenzene, benzaldehyde and benzeneacetaldehyde benzene derivatives were identified in honeydew honeys. Some authors assumed that benzene and phenolic compounds might be characteristic for honeydew honeys [36], but other authors classified these VOC as “floral markers” [37, 38].

A wide range of compounds containing a furan ring, (2-acetyl-5-methyl-furan, 2-butyltetrahydrofuran, 2,5-dihydro-3,5-dimethyl-2-furanone, 2-hexanoylfuran, 2-methyl-4,5-dihydro-3-(2H)-furanone, 2-n-hexylfuran, 3-methyl-2-(5H)-furanone, 3-pentyldihydro-2-(3H)-furanone, 4,5-dimetyldihydro-2-(3H)-furanone, 4,7-dimetylbenzofuran or hydroxydihydro-4-2-(3H)-furanone) were found in all studied honeydew

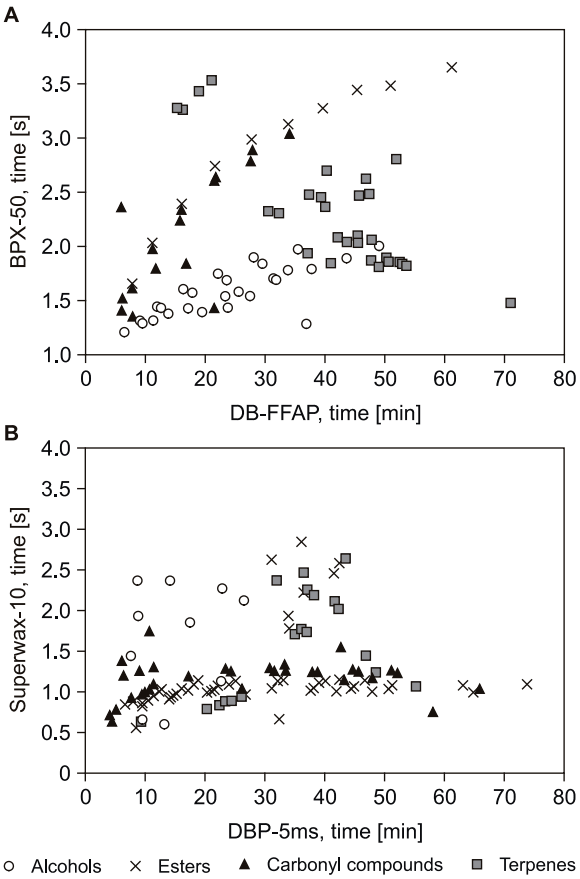

Fig. 3. Chromatographic profile of main classes of compounds detected in honeydew honey.

A – DB-FFAP and BPX-50, B – DB-5ms and Supelcowax-10.

samples. Based on previous studies, furan derivates in honeydew honeys may originate from different processing or storage conditions of this honey type [39]. 5-Methyl-2-(3H)-furanone and 2-methyl-3-(2H)-dihydrofuranone were reported as unique markers for oak honeydew honeys [40]. Another very interesting group of compounds found in honeydew honey are pyrazines: 2,5-dimethylpyrazine and 2,6-dimethylpyrazine; 2-ethylpyrazine; 2-methyl-5-ethylpyrazine and 2-methylpyrazine. Terpenes represent the most abundant chemical class identified in honeydew honeys. Their presence may be associated with the floral nectar or honeydew gathered by honeybees [39]. Many authors indicate certain terpenes such as limonene, hotrienol and linalool oxides as markers of the botanical origin of honeydew honey [31]. Linalool oxides and hotrienol were already found in oak honeydew [40]. Isophorone originating from cartenoid degradation was shown as a compound specific for other honey types [11].

In our previous work, monofloral honeys (rape,

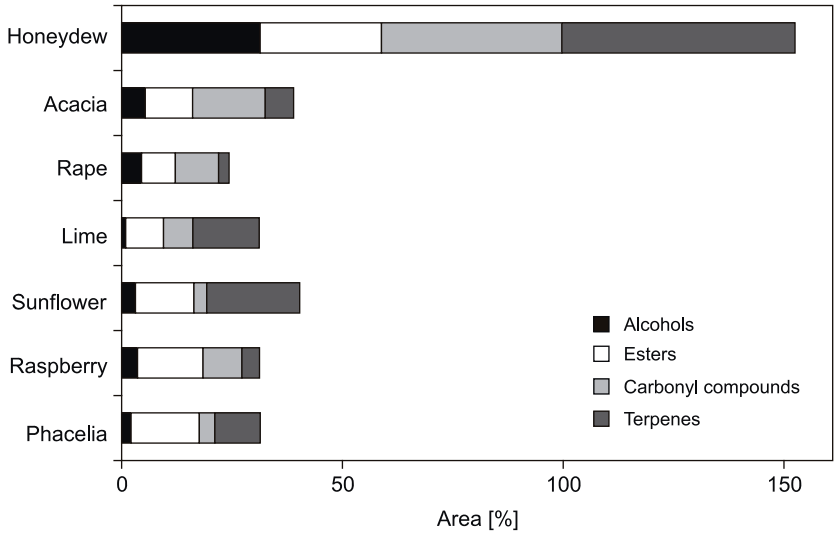

Fig. 4. Comparison of area percentage of volatile organic compounds in honeydew, rape, sunflower, acacia, lime, raspberry and phacelia honeys.

The area percentages of volatile organic compounds in monofloral flower honeys were obtained in our previous work [21].

sunflower, acacia, lime, raspberry, and phacelia) were studied in detail [21]. Fig. 4 shows a comparison of peak areas of selected chemical classes in honeydew, rape, sunflower, acacia, lime, raspberry and phacelia honeys. For estimation of VOC content in different types of honey, only compounds confirmed by authentical reference standards were applied. Overall, honeydew honeys were specified by the presence of a significantly higher number of volatile organic compounds compared to monofloral honeys.

CONCLUSIONS

A complete analysis of VOC content of honeydew honeys was performed by comprehensive two-dimensional chromatography. In the first part of study, DVB/CARB/PDMS, PDMS/DVB, CAR/PDMS, PA and PDMS SPME fibres were compared, where the DVB/CARB/PDMS fibre was found to be more effective for extraction of terpenes, esters and alcohols. In the next part, two column combinations with different separation characteristics were applied, and the "reversed" (polar × medium-polar) setup was found to provide an ordered structure of the chromatogram profile for the most common classes of compounds detected in honey. In general, more than 300 compounds were detected in the samples, whilst 170 compounds were confirmed by authentic reference standards. On the basis of theses results, 2-oxooctanoic acid and 4-oxapentanoic acid, allyl ester of acetic acid, methyl ester of 2,6-dihydroxybenzoic acid were considered to be markers for Slovak honeydew honey. At the same time, the presence of other compounds previously reported as specific for this kind of honey, namely, 2,3-butanediol, 3-hydroxy-2-butanone, acetic acid and methyl ester of 2-hydrobenzoic acid was established.

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REFERENCES

1. CODEX PFV 04/22/3. Codex Committee on Processed Fruits and Vegetables. Rome: FAO/WHO Codex Alimentarius Commission, 2004.
2. European Commission Council Directive 2001/110/CE concerning honey. Official Journal of the European Communities, *L10*, 2002, pp. 47-52.
3. Simova, S. – Atanassov, A. – Shishiniova, M. – Bankova, V.: A rapid differentiation between oak honeydew honey and nectar and other honeydew honeys by NMR spectroscopy. Food Chemistry, *134*, 2012, pp. 1706–1710. DOI: 10.1016/j.foodchem.2012.03.071.
4. Kukurová, K. – Karovičová, J. – Kohajdová, Z. – Biliková, K.: Authentication of honey by multivariate analysis of its physico–chemical parameters. Journal of Food and Nutrition Research, *47*, 2008, pp. 170–180.
5. Sanz, M. L. – Gonzalez, M. – De Lorenzo, C. –

Sanz, J. – Martinez-Castro, I.: A contribution to the differentiation between nectar honey and honeydew honey. Food Chemistry, *91*, 2005, pp. 313–317. DOI: 10.1016/j.foodchem.2004.06.013.
6. Bentabol Manzanares, A. – Hernández García, Z. – Rodríguez Galdón, B. – Rodríguez Rodríguez, E. – Díaz Romero, C.: Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. Food Chemistry, *126*, 2011, pp. 664–672. DOI: 10.1016/j.foodchem.2010.11.003
7. Madejczyk, M. – Baralkiewicz, D.: Characterization of Polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. Analytica Chimica Acta, *617*, 2008, pp. 11–17. DOI: 10.1016/j.aca.2008.01.038.
8. Prodolliet, J. – Hischenhuber, C.: Food authentication by carbohydrate chromatography. Zeitschrift für Lebensmitteluntersuchung und –Forschung A, 207, 1998, pp. 1–12. DOI: 10.1007/s002170050286.
9. Baroni, M. V. – Nores, M. L. – Del Pilar Diaz, M. – Chiabrando, G. A. – Fassano, J. P. – Costa, C. – Wunderlin, D. A.: Determination of volatile organic compound patterns characteristic of five unifloral honey by solid-phase microextraction-gas chromatography-mass spectrometry coupled to chemometrics. Journal of Agricultural and Food Chemistry, *54*, 2006, pp. 7235–7241. DOI: 10.1021/jf061080e.
10. Radovic, B. S. – Careri, M. – Mangia, A. – Musci, M. – Gerboles, M. – Anklam, E.: Contribution of dynamic headspace GC-MS analysis of aroma compounds to authenticity testing of honey. Food Chemistry, *72*, 2001, pp. 511–520. DOI: 10.1016/S0308-8146(00)00263-6.
11. Soria, A. C. – Martínez-Castro, I. – Sanz, J.: Analysis of volatile composition of honey by solid phase microextraction and gas chromatography-mass spectrometry. Journal of Separation Science, *26*, 2003, pp. 793–801. DOI: 10.1002/jssc.200301368.
12. Tezcan, F. – Kolayli, S. – Sahin, H. – Ulusoy, E. – Erim, F. B.: Evaluation of organic acid, saccharide composition and antioxidant properties of some authentic Turkish honeys. Journal of Food and Nutrition Research, *50*, 2011, pp. 33–40.
13. Pažitná, A. – Janáčová, A. – Špánik, I.: The enantiomer distribution of major chiral volatile organic compounds in Slovakian monofloral honeys. Journal of Food and Nutrition Research, *51*, 2012, pp. 236–241.
14. Pontes, M. – Marques, J. C. – Camara, J. S.: Screening of volatile composition from Portuguese multifloral honeys using headspace solid-phase microextraction-gas chromatography–quadrupole mass spectrometry. Talanta, *74*, 2007, pp. 91–103. DOI: 10.1016/j.talanta.2007.05.037.
15. Pawliszyn, J. (Ed.): Handbook of solid phase microextraction. Amsterdam : Elsevier, 2012. ISBN: 978-0-12-416017-0.
16. Kaškoniene, V. – Venskutonis, P. R. – Čeksteryté, V.: Composition of volatile compounds of honey of various floral origin and beebread collected in Lithuania. Food Chemistry, *111*, 2008, pp. 988–997. DOI: 10.1016/j.foodchem.2008.05.021.
17. Soria, A. C. – Sanz, J. – Martínez-Castro, I.: SPME followed by GC–MS: a powerful technique for qualitative analysis of honey volatiles. European Food Research and Technology, *228*, 2009, pp. 579–590. DOI: 10.1007/s00217-008-0966-z.
18. Plutowska, B. – Chmiel, T. – Dymerski, T. – Wardencki, W.: A headspace solid-phase microextraction method development and its application in the determination of volatiles in honeys by gas chromatography. Food Chemistry, *126*, 2010, pp. 1288–1298. DOI: 10.1016/j.foodchem.2010.11.079.
19. Bianchi, F. – Mangia, A. – Mattarozzi, M. – Musci, M.: Characterization of the volatile profile of thistle honey using headspace solid-phase microextraction and gas chromatography–mass spectrometry. Food Chemistry, *129*, 2011, pp. 1030–1036. DOI: 10.1016/j.foodchem.2011.05.070.
20. Čajka, T. – Hajslova, J. – Pudil, F. – Riddellova, K.: Traceability of honey origin based on volatiles pattern processing by artificial neural networks. Journal of Chromatography A, *1216*, 2009, pp. 1458–1462. DOI: 10.1016/j.chroma.2008.12.066.
21. Špánik, I. – Janáčová, A. – Šusterová, Z. – Jakubík, T. – Jánošková, N. – Novák, P. – Chlebo, R.: Characterisation of VOC composition of Slovak monofloral honeys by GCxGC-TOF-MS. Chemical Papers, *67*, 2013, pp. 127–134. DOI: 10.2478/s11696-012-0254-z.
22. Dymerski, T. – Chmiel, T. – Mostafa, A. – Sliwinska, M. – Wisniewska, P. – Wardencki, W. – Namiesnik, J. – Gorecki, T.: Botanical and geographical origin characterization of Polish honeys by headspace SPME-GCxGC-TOF-MS. Current Organic Chemistry, *17*, 2013, pp. 853–870.
23. Čajka, T. – Hajslová, J. – Pudil, F. – Riddellová, K.: Traceability of honey origin based on volatiles pattern processing by artificial neural networks. Journal of Chromatography A, *1216*, 2009, pp. 1458–1462. DOI: 10.1016/j.chroma.2008.12.066.
24. Rivellino, S. R. – Wang Hantao, L. – Risticevic, S. – Carasek, E. – Pawliszyn, J. – Augusto, F.: Detection of extraction artifacts in the analysis of honey volatiles using comprehensive two-dimensional gas chromatography. Food Chemistry, *141*, 2013, pp. 1828–1833. DOI: 10.1016/j.foodchem.2013.05.003.
25. Brokl, M. – Soria, A. C. – Ruiz-Matute, A. I. – Sanz, M. L. – Ramos, L.: Separation of disaccharides by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. Application to honey analysis. Journal of Agricultural and Food Chemistry, *58*, 2010, pp. 11561–11567. DOI: 10.1021/jf102646n
26. Cuevas-Glory, L. F. – Pino, J. A. – Santiago, L. S. – Sauri-Duch, E.: A review of volatile analytic methods for determining the botanic origin of honey. Food Chemistry, *103*, 2007, pp. 1032–1043. DOI: 10.1016/j.foodchem.2006.07.068.
27. Čajka, T. – Hajšlova, J. – Cochran, J. – Holadova, K. – Klimankova, E.: Solid phase microextraction–comprehensive two-dimensional gas chromatography–

time-of-flight mass spectrometry for the analysis of honey volatiles. Journal of Separation Science, *30*, 2007, pp. 534–546.
28. Adahchour, M. – Beens, J. – Vreuls, R. J. J. – Brinkman, U. A. Th.: Recent developments in comprehensive two-dimensional gas chromatography (GC × GC) I. Introduction and instrumental set-up. Trends in Analytical Chemistry, *25*, 2006, pp. 438–454. DOI: 10.1016/j.trac.2006.03.002.
29. Jerkovic, I. – Marijanovic, Z. – Tuberoso, C. I. G. – Bubalo, D. – Kezic, N.: Molecular diversity of volatile compounds in rare willow (*Salix* spp.) honeydew honey: identification of chemical biomarkers. Molecular Diversity, *14*, 2010, pp. 237–248. DOI: 10.1007/s11030-009-9164-6.
30. Soria, A. C. – González, M. – De Lorenzo, C. – Martínez-Castro, I. – Sanz, J.: Estimation of the honeydew ratio in honey samples from their physicochemical data and from their volatile composition obtained by SPME and GC-MS. Journal of the Science of Food and Agriculture, *85*, 2005, pp. 817–824, DOI: 10.1002/jsfa.1890.
31. Escriche, I. – Visquert, M. – Juan-Borrás, M. – Fito, P.: Influence of simulated industrial thermal treatments on the volatile fractions of different varieties of honey. Food Chemistry, *112*, 2009, pp. 329–338. DOI: 10.1016/j.foodchem.2008.05.068.
32. Soria, A. C. – Gonzalez, M. – de Lorenzo, C. – Martinez-Castro, I. – Sanz, J.: Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data. Food Chemistry, *85*, 2004, pp. 121–130. DOI: 10.1016/j.foodchem.2003.06.012.
33. Alissandrakis, E. – Daferera, D. – Taranilis, P. A. – Polissiou, M. – Harizanis, P. C.: Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey. Food Chemistry, 82, 2003, pp. 575–582. DOI: 10.1016/S0308-8146(03)00013-X.
34. Campos, G. – Nappi, G. U. – Raslan, D. S. – Augusti, R.: Volatile substances in floral honey and honeydew honey. Ciencia e Tecnologia de Alimentos, *20*, 2000, pp. 18–22. DOI: 10.1590/S0101-20612000000100004.
35. Plutowska, B. – Chmiel, T. – Dymerski, T. – Wardencki, W.: A headspace solid-phase microextraction method development and its application in the determination of volatiles in honeys by gas chromatography. Food Chemistry, *126*, 2011, pp. 1288–1298. DOI: 10.1016/j.foodchem.2010.11.079.
36. Castro-Vazquez, L. – Diaz-Maroto, M. C. – Pérez-Coello, M. S.: Aroma composition and new chemical markers of Spanish citrus honeys. Food Chemistry, *103*, 2007, pp. 601–606. DOI: 10.1016/j.foodchem.2006.08.031.
37. Fisher, C. – Scott, T. R.: Food flavours. Biology and Chemistry. Cambridge: The Royal Society of Chemistry, 1997. ISBN: 0-85404-538-4.
38. Serra-Bonvehí, J. – Ventura-Coll, F.: Flavour index and aroma profiles of fresh and processed honeys. Journal of the Science of Food Agriculture, *83*, 2003, pp. 275–282. DOI: 10.1002/jsfa.1308.
39. Soria, A. C. – Martínez-Castro, I. – Sanz, J.: Some aspects of dynamic headspace analysis of volatile components in honey. Food Research International, *41*, 2008, pp. 838–848. DOI: 10.1016/j.foodres.2008.07.010.
40. Castro-Vázquez, L. – Díaz-Maroto, M. C. – Pérez-Coello, M. S.: Volatile composition and contribution to the aroma of Spanish honeydew honeys. Identification of a new chemical marker. Journal of Agricultural and Food Chemistry, *54*, 2006, pp. 4809–4813. DOI: 10.1021/jf0604384.

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