Rape honey: determination of botanical origin based on volatile compound profiles

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Summary
Generally, it is very difficult to determine which volatile compounds significantly contribute to the typical rape honey aroma. The aim of this research was to characterize, for the first time, the main volatile compounds present in Czech rape honeys using solid phase microextraction (SPME) followed by gas chromatography coupled to mass spectrometry (GC-MS). The study also explored possibilities to reliably identify and distinguish between rape and multifloral honeys on the basis of the profile of volatile compounds. A total of 28 unifloral rape honeys (*Brassica napus*) from local Czech beekeepers produced in 2014–2016 were analysed. Obtained data were statistically processed using principal component analysis, linear discriminant analysis and analysis of variance. Based on the results, the following compounds were the most abundant in each of the analysed Czech rape honeys (relative representation in percent): hotrienol (7.2–39.3 %), benzaldehyde (0.7–19.0 %), 3-methylbutanenitrile (0.1–15.7 %), 2-phenylethanol (1.0–10.2 %) and nonanoic acid (0.4–7.0 %). Moreover, 3-methylbutanenitrile and nonanoic acid were identified as the characteristic compounds for rape honeys. Nonetheless, the presence of 5–25 % of other botanical species in the honeys had a significant impact on the occurrence and representation of the individual volatile compounds.

Keywords:
rape honey; quality; volatile compound; aroma; origin; solid phase microextraction; gas chromatography–mass spectrometry

In recent years, honey production in EU is declining and has plummeted below 50.0 % in self-sufficiency. This trend has led to a permanent shortage of domestically produced honey [1]. The average consumption of honey per person ranges from 0.5 kg to 1 kg per year. Multifloral and rape honeys are the most commonly consumed honeys in EU. Although multifloral honeys make up for the majority of consumed honey, consumers appreciate unifloral honeys for their specific flavour, therefore heather and honeydew honeys (e.g. from coniferous honeydew) and protected designation of origin (PDO) honeys are becoming increasingly popular [2]. Czech beekeepers obtain pure unifloral honey mostly from rape, acacia, raspberry, clover or honeydew [3–5].

Rape (*Brassica napus* L.) is an oilseed important for the food industry and for technical purposes. It is also an important spring source of nectar and pollen for bees. In the Czech Republic, rape honey is a prominent floral honey and the first unifloral honey bottled in the season. The high glucose content in rape honey causes rapid crystallization with very fine crystals [6]. Pasting turns the crystals into a paste form, creating an easy-to-use paste with increased stability. Rape honey has specific sensory properties, its flavour and aroma are most often described as mild to neutral. The specific descriptors for rape honey aroma are hay-like, cheesy, with sour notes, and for its flavour are sweet, musty, slightly fermented with notes of bitterness. Rape honey has a yellow colour, is in a liquid state under normal conditions [7–9] and its susceptibility to fermentation is an undesirable feature. Tab. 1 shows the characteristic physico-chemical parameters of European rape honeys [6, 9, 10].

Determination of the botanical origin of honey

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Tab. 1. Physico-chemical parameters of European rape honeys [6, 9, 10].

Parameter	Range
Electric conductivity [mS·cm^{-1}]	0.1–0.3
Free acidity [meq·kg^{-1}]	6.2–29.9
Diastase activity (degree of Schade)	7.7–36.8
Fructose content [g·kg^{-1}]	319–417
Glucose content [g·kg^{-1}]	323–456
Fructose/glucose ratio	0.8–1.1
Moisture [g·kg^{-1}]	149–199
pH	3.7–4.4
Hydroxymethylfurfural [mg·kg^{-1}]	0.2–13.0
Brassica pollen grains content [%]	45.2–99.2
Proline content [mg·kg^{-1}]	142–466

is very important for assessing its quality. Honey producers, vendors and inspection authorities recognize the need for reliable methods for determination of the botanical origin of honey and of its botanical purity. According to laboratory analyses of unifloral honeys in scientific studies [6, 11, 12], the following parameters were identified to have the greatest discriminatory power: colour, electrical conductivity, specific rotation, diastase activity, acidity, fructose content and glucose content. These studies also showed that parameters, for each individual honey type, with very high values (glucose content, fructose+glucose content, glucose/water ratio) and very low values (electrical conductivity, free acidity, fructose/glucose ratio) have greater classification power than the ones with medium values [6]. In 2003, Bartáková et al. [13] evaluated seven physico-chemical quality parameters (electrical conductivity, water content, water activity, pH, hydroxymethylfurfural content, invertase activity and diastase activity) and determined the botanical origin of 37 honeys produced in the Czech Republic (South and North Moravia, East and Central Bohemia). Based on the results of this study, rape and acacia honeys showed the lowest values of electrical conductivity, compared to other honey types.

The following methods were applied to determine the botanical origin of honey (including the origin of rape honey) [6, 14]:

Analysis of pollen: melissopalynology (microscopical analysis of pollen) is a traditional method; DNA (meta)barcoding was applied to characterize the taxa of pollen collected either from beehives or isolated from honey;

Mineral elements in honey: the mineral content in honey is determined by botanical, geographical and environmental factors; it is used to classify the botanical and/or geographical provenance of honey; inductively coupled plasma mass spectrometry (ICP-MS, used in Poland for discrimination of honeydew, buckwheat and rape honey), ICP-MS and inductively coupled plasma - optical emission spectrometry ICP-OES (used in Hungary for discrimination of acacia, rape, sunflower and multifloral honeys);

Spectroscopic profiling methods (targeted or untargeted profiling; e.g. nuclear magnetic resonance, Fourier-transform infrared spectroscopy, Fourier transform Raman spectroscopy): were applied in combination with multivariate analysis to detect the addition of sugar syrups or determine the botanical and geographical origin;

Phytochemical markers: phenolic compounds (using high-performance liquid chromatography (HPLC) with a diode-array detector (DAD), e.g. abscisic acid, which was reported as a possible marker for heather honey, was also detected in rape, lime tree and acacia honeys); free amino acids profile (can be determined either by HPLC or gas chromatography combined with chemometric data analysis).

As for other advanced instrumental methods, the use of volatile compound profiles obtained by gas chromatography–mass spectrometry (GC-MS) and chemometric data evaluation have were successfully tested for identification of botanical origin (Tab. 2) [15–19].

Seisonen et al. [15] used correspondence analysis (CA) to map his samples and flavour descriptions with the Pearson correlation coefficient (p = 0.05) to find correlations between the attributes of the studied honey samples. The aroma profiles of the tested heather, rape, raspberry and alder buckthorn honeys proved to be rather similar. The tested rape honey had the poorest profile without many characteristic notes. Presence of dimethyltrisulfide indicated the content of rape pollen in the honey.

Analyzing four rape honeys from Europe, Radovic et al. [20] found that rape honey was characterized by the presence of dimethyldisulfide and absence of 2-methyl-1-propanol. However, their study also showed that other types of honey may have the same profile. Therefore, the presence of dimethyldisulfide and absence of 2-methyl-1-propanol cannot be considered as a reliable marker to classify rape honey.

On the contrary, Kaškonienė et al. [21] detected dimethyldisulfide in only six of their eleven analysed samples of rape honey from Lithuania, while 2-methyl-1-propanol was absent. These find-

Tab. 2. Significant volatile compounds of rape honeys.

n_1	n_2	Geographical origin	Rape honey volatile compounds			Instrumental method of analysis + statistical evaluation method	References
			Abundant compounds and their descriptions	Presence of characteristic and/or unique compounds	Compounds conclusively determined not to occur in rape honeys at all (unlike other types) or occur only at very low representation		
6	2 (raspberry), 3 (heather), 2 (high alder buckthorn pollen content)	Estonia	Rape honey has the poorest profile, without any characteristic peaks.	Dimethyltrisulfide refers to the content of rape pollen in the honey.		SPME coupled to GC-MS and GC-O Agglomerative hierarchical clustering and correspondence analyses	Seisonen et al. [15]
7	33 (6 sunflower, 17 acacia, 6 lime, 2 raspberry and 2 phacelia honeys)	Slovakia	The poorest VOC profiles found for acacia and rape honeys. Hexane, nonane, benzaldehyde, hotrienol, butan-2-one, 3-methylbutanenitrile, dimethylsulfide, methylester of acetic and nonanoic acids	2-Methyl-2-butenoic acid, 1-phenylpropan-2-ol, 2,4-dithiapentane, pentan-3-one, cinnamic alcohol, 2-phenethyl ester of formic acid, perillol, and α-bisabolol	The absence of heptan-1-ol, 2-octenoic acid, methyl ester, pentan-2-one, undecan-2-one, hexanal, and tetradecane	SPME-GC × GC-TOF-MS	Špánik et al. [16]
1	7 (acacia, linden, buckwheat, heather, multifloral and honeydew honeys)	Poland	Rape honey is characterized by the poorest chromatogram without any characteristic peaks. Benzoic acid and benzoic alcohol	The presence of disulfide and the simultaneous absence of isobutyl alcohol is not a typical feature for rape honeys.	Many compounds occurring in the remaining honeys are not found at all or in much lower concentrations, e.g., rose oxides, linalool oxides, furfural and phenylacetaldehyde.	HS-SPME-GC-MS	Plutowska et al. [17]
8	32 (8 samples for each type: acacia, buckwheat, lime, honeydew)	Poland	Benzyl alcohol, furfural		Absence of *p*-cymene in rape honeys; lilac aldehyde lacked in acacia, rape and honeydew honeys.	SPME coupled to GC-MS and GC-O	Wardencki et al. [18]
13	12 (spring turnip rape)	Swiss rape and Finnish spring turnip rape	Benzaldehyde, benzenethanol, 3,5-dimethoxybenzaldehyde, benzoic acid	4-Methylphenol and 1,4-dichlorobenzene	Octanal, pentadecane, α-terpineol, 5-methyl-2-(1-methylethyl)phenol	SPME-GC-FID and SPME-GC-MS LDA	Ruoff [19]

n_1 – number of analysed rape honey samples, n_2 – number of analysed samples of other honey types.
VOC – volatile organic compound, SPME – solid phase microextraction, GC-MS – gas chromatography–mass spectrometry, GC/O – gas chromatography-olfactometry, GC-TOF-MS – gas chromatography coupled to time of flight–mass spectrometry, HS-SPME-GC-MS – headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry, GC-FID – gas chromatography–flame ionization detection, LDA – linear discriminant analysis.

ings suggest that characteristic markers of rape honey may vary, depending on plant's secondary metabolism products. Benzaldehyde and benzeneacetaldehyde were the only compounds they detected in the entire honey samples set. In case of rape honey, other important compounds identified included nonanal and benzylnitrile. The authors evaluated also changes in volatile profiles during a storage period of 3 months. They found that the profile of volatiles changed with the storage time, the content of volatiles decreased in most of the samples, with the exception of three rape honey samples. Changes in the profile of volatiles of honey, during storage, are mainly caused by direct chemical changes in the honey composition and by physical changes, e.g. in consistency or in rheological properties [21].

Ruisinger and Schieberle [22] reported the following compounds to primarily contribute to the rape honey aroma: phenylacetaldehyde, 3-methylbutanal, 2,3-butanedione, 4-allyl-2-methoxyphenol, 2-methoxy-4-vinylphenol and (*E*) β-damascene, the latter being identified as the most important volatile compound in rape honey. The authors identified the following compounds as the important aroma compounds transferred from rape plants to rape honey by bees: 3-phenylpropanoic acid, phenylacetaldehyde, 4-methoxybenzaldehyde and 3-methylbutanoic acid.

Pažitná et al. [23] studied the distribution of selected chiral volatile enantiomers in 45 unifloral honey samples (Slovakian rape, acacia, sunflower basswood and raspberry honeys) using solid phase microextraction (SPME) coupled to GC-MS. The first-eluted *cis*-enantiomer of linalooloxide was found in a wide range of the acacia, rape and sunflower honey samples. The second-eluted enantiomer of lilac aldehyde B was present in higher purity in the acacia honey samples, which could be used to distinguish between acacia and rape honeys (these plants blossom at the same time).

Dymerski et al. [24] found that ethyldecanoate was only present in rape honey and, therefore, can be considered a characteristic marker for this type of honey. The authors also identified eugenol as the discriminant of the botanical origin of rape and buckwheat honeys.

The results of the mentioned studies are ambiguous and mutually different. Generally, it remains unclear which volatiles could be used as markers to identify rape honey and which compounds (whatever their concentrations) significantly contribute to the typical rape honey aroma. Rape honeys from the Czech Republic have not been studied yet. The aim of this work was to characterize volatile compounds in Czech rape honeys and explore the possibilities of using volatile profiles to distinguish rape honeys from multifloral honeys.

MATERIALS AND METHODS

Chemicals

Sodium chloride (p.a.) and methanol (p.a.) were obatined from Penta (Prague, Czech Republic). Benzophenone (99%; internal standard) and C8–C20 alkanes standards (40 $mg \cdot l^{-1}$) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Vials (10 ml), caps and septa were supplied by Agilent Technologies (Santa Clara, California, USA). Helium (purity 99.998 %) was provided by Linde Gas (Prague, Czech Republic).

Samples

In this study, a set of 28 unifloral rape (*Brassica napus*) honeys was analysed. The rape honeys were sourced from local Czech beekeepers and were harvested in 2016 (14 samples; R1–14; location: Velký Beranov 49°24'18.1"N, 15°40'1.2"E; Slavětín 50°21'2.1"N, 13°54'27.7"E; Svitavy 49°45'21.3"N, 16°28'5.8"E; Přerov 49°27'17.4"N, 17°26'56.3"E), in 2015 (5 samples; R15–19; location: Jaroměř 50°21'22.3"N, 15°55'16.9"E; Teplá 49°58'54.6"N, 12°51'46.9"E; Olbramov 49°50'36.1"N, 12°52'0.3"E; Měřín 49°23'35.5"N, 15°53'1.7"E; Třešť 49°17'27.3"N, 15°28'55.6"E) and in 2014 (9 samples; R20–28; Chodová Planá 49°53'35.5"N, 12°43'48.5"E; Pacov 49°28'14.7"N, 15°0'6.0"E; Tulešice 49°2'18.7"N, 16°12'25.6"E; Krhov 49°27'36.9"N, 16°35'1.7"E). All the rape honey samples were stored at 20 °C and analysed within 6 months after being harvested.

Volatile profiles of 28 multifloral honeys obtained in a previous study by Kružík et al. [25], which had been analysed under the same isolation and chromatographic conditions, were also included in our statistical analysis. These honeys were from various origins (Czech Republic, EU and non-EU countries; 28 samples; M29–56; exact location unknown) and were purchased in the Czech market in 2012.

Melissopalynological analysis

Pollen grains were analysed according to the Von der Ohe et al. [26] to confirm the botanical origin of the rape honey samples. Microscopical analysis of honey sediment and comparison of specific pollen grain shapes were conducted. Individual pollen species were identified and quantified. For each individual sample, pollen grains were counted in 20 fields of view, 500 to 1000 pollen

grains being counted to determine relative frequencies.

Sample preparation and isolation of volatile compounds

Volatile compounds were extracted from the honey samples using SPME with Autosampler Combi PAL (Agilent Technologies). Each of the honey samples (1 g) was dissolved in a 10 ml glass vial using 1 ml of NaCl solution in distilled water (200 $g \cdot l^{-1}$) to which 10 μl of a methanolic solution of benzophenone (10 $mg \cdot l^{-1}$, internal standard) was added. The vial was then sealed with a septum cap (polytetrafluoroethylene or PTFE/silicone). The honey solutions were then stirred at 8 Hz and heated at 60 °C for 15 min. Volatile compounds were extracted with SPME fibre (DVB/CAR/PDMS; Supelco, Bellefonte, Pennsylvania, USA). The SPME fibre was inserted through the septum into the vial and volatile compounds were adsorbed for 30 min at 60 °C. The extracted volatiles were desorbed at 240 °C for 4 min. The method conditions were chosen based on a previous study by Kružík et al. [25].

GC-MS analysis

The volatile compounds were separated on a 30 m capillary column with the inner diameter of 0.25 mm and the stationary phase thickness of 0.25 μm (HP-5MS, Agilent Technologies). The temperature program started at 60 °C (2 min), then the temperature increased by 10 $°C \cdot min^{-1}$ to 290 °C. The total analysis time was 25 min. Helium 4.8 (purity 99.998 %) was used as the carrier gas at the flow rate of 1.4 $ml \cdot min^{-1}$. The temperature of the MS quad was set at 150 °C and the temperature of the ion source at 230 °C. The separated volatile compounds were assessed based on peak area percentages and a volatile profile was created. To ensure the quality control (QC) of the measurement, an internal standard was added to each sample and a honey control sample (rape honey 2016; R1) was added into the injection sequence after every 10^{th} sample. The injection of the control sample was done to monitor the stability of the GC-MS system. The chromatograms were evaluated using HP Chemstation software (Agilent Technologies). Reproducibility (11 %) and repeatability (6 %) for hotrienol were evaluated as a part of the method verification. Individual compounds were identified based on the comparison of the spectra with the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland) mass spectral library (NIST14) and on the assessment of Kovatch retention indeces.

Statistical analysis

To determine the differences between the honey samples, principal component analysis (PCA), linear discriminant analysis (LDA) and one-way analysis of variance (ANOVA) were conducted. The comparison of honey samples was firstly performed using PCA as an unsupervised chemometric tool. A data matrix (56 x 28) was assembled using 56 samples of honey as cases and 28 volatile compounds (relative representation in percent) as variables. Only volatiles present at least in one sample with an abundance higher than 0.1 % of total peak area were selected for the data matrix. Secondly, the supervised LDA method (with the same data matrix as for PCA) was used to investigate whether the profile of volatiles would allow discrimination of the botanical origin of honeys. The statistical analysis was performed using Statistica 12.0 (StatSoft, Tulsa, Oklahoma, USA). Analyses of volatile compound and pollen were carried out twice for each rape honey sample and the mean values were reported.

RESULTS AND DISCUSSION

In the first part of the experiment, we examined whether all 28 tested honeys met the standards to be classified as rape honey. In order to classify honey as rape honey, the relative representation of rape pollen grains in the honey needs to be at least 60 % [26]. The pollen analyses confirmed the presence of rape botanical species in all of the tested honeys, thus they were classified as rape honey (Tab. 3). The average rape pollen grain (PG) representation exceeded 80 % for all the sample groups (2014, 2015 and 2016), the total number of pollen grains ranging from 1.4×10^6 to 9.5×10^6 PG per kilogram. The relative representation corresponded to the data from a study by Persano Oddo et al. [6], in which 715 European rape honeys were analysed. The average values of the pollen grain total identified in this study amounted to approximately half of the values published in the study of Persano Oddo et al. [6]. Compared to most of the other botanical sources of nectar, rape provides a large amount of pollen grains. In addition to rape pollen grains, small proportions (< 10 %) of other types of pollen were identified, mainly apple, cherry, willow and dandelion pollen grains.

A total of 52 volatile compounds were found in the analysed rape honeys. Tab. 4 shows 28 of the most abundant compounds present in the rape honeys, which had an average representation higher than 0.1 %. The identified compounds

Tab. 3. Quantitative and qualitative pollen analysis.

Year	Number of samples	Total number of pollen grains per kilogram	Rape pollen grains [%]		
			Average	Minimum	Maximum
2014	9	3.6×10^6	87.7	80.4	96.7
2015	5	3.4×10^6	84.0	73.6	94.0
2016	14	5.0×10^6	90.2	85.3	94.5

Total number of pollen grains is expressed as average counted from 20 fields of view.

Tab. 4. The most abundant volatile compounds identified in the rape honey samples.

Number	*Tr* [min]	Compound	*RI*	Min. [%]	Max. [%]	Average [%]	Frequency of occurrence [%]
Aldehydes							
1	5.55	Benzaldehyde	964	0.7	19.0	6.7	100
2	6.87	2-Phenylacetaldehyde	1 048	0.5	4.6	2.1	100
3	9.28	Decanal	1 207	0.4	3.0	1.4	100
Alcohols							
4	3.69	Pent-2-en-1-ol	837	0.0	35.3	5.0	96
5	3.75	3-Methylpentan-1-ol	842	0.0	12.4	2.4	64
6	6.70	Phenylmethanol	1 038	0.0	7.2	2.6	89
7	7.70	β-Linalool	1 101	0.3	3.4	1.4	100
8	7.78	Hotrienol	1 107	7.2	39.3	17.2	100
9	7.95	2-Phenylethanol	1 118	1.0	10.2	5.2	100
10	8.76	Isoborneol	1 172	0.0	11.5	3.5	65
11	9.02	*p*-Cymene-8-ol	1189	0.0	6.5	0.4	25
12	10.8	4-Ethenyl-2-methoxyphenol	1 320	0.0	2.3	0.6	73
Alkanes							
13	3.19	Octane	801	0.0	4.2	1.9	93
14	4.55	Nonane	901	0.0	0.8	0.1	32
Carboxyl compounds							
15	5.23	3-Methylpentanoic acid	944	0.0	2.6	1.3	75
16	8.84	Octanoic acid	1 177	0.0	1.7	0.3	25
17	10.16	Nonanoic acid	1 270	0.4	7.0	2.3	100
Esters							
18	8.81	Ethylbenzoate	1 175	0.0	29.2	5.0	71
19	9.53	Methylnonanoate	1 225	0.0	4.6	1.9	96
20	10.04	2-Phenylethylacetate	1 262	0.0	5.4	0.7	21
Ethers							
21	7.32	Linalooloxide	1 093	0.0	7.8	3.1	96
22	8.39	Lilac aldehyde C	1 147	0.0	3.2	1.0	93
23	8.74	Lilac aldehyde D	1 171	0.0	12.0	2.4	54
Nitriles							
24	2.46	2-Methylbutanenitrile	721	0.0	12.3	3.7	38
25	2.51	3-Methylbutanenitrile	727	0.1	15.7	5.2	100
26	8.34	2-Phenylacetonitrile	1 144	0.0	3.3	0.7	61
Sulfides							
27	2.68	Dimethyldisulfide	745	0.0	2.2	0.3	57
Others							
28	11.80	β-Damascenone	1 393	0.0	3.1	0.8	64

Tr – retention time, *RI* – retention index.

were classified into 9 groups according to their chemical structure, of which alcohols represented the largest group. In all the rape honey samples, hotrienol, benzaldehyde and 2-phenylethanol were the most frequently represented compounds. These results were different from other studies, which found nonane [16], benzaldehyde [16, 19, 20, 22], hotrienol [16, 18], 3-methylbutanenitrile [16], methylnonanoate [16], phenylmethanol [17, 18, 20, 22] and ethylbenzoate [18] to be the most abundant volatile compounds in rape honey. It is evident that results from our study only partly correspond with other studies, in particular regarding hotrienol or benzaldehyde.

The basic profile of volatile compounds in rape honey was very unspecific. Therefore, 8 key compounds were selected in this study, based on available literature sources, to identify rape honey. The selected key compounds were of dimethyldisulfide [20, 21], 3-methylpentanoic acid [22], benzaldehyde [20, 22], phenylmethanol [20], 2-phenylacetaldehyde [22], 2-phenylethanol [22], 4-ethenyl-2-methoxyphenol [22] and β-damascenone [21, 22]. The content of each key compound was estimated by comparing its peak area percentage with the internal standard peak area and expressed as area ratio. To confirm uniqueness of the selected key compounds, the multifloral honey volatile profiles from our previous study [25] were re-evaluated (Fig. 1) and used for comparison. Our findings did not comply with the assumption [15] that rape honey volatile profiles are generally more featureless than profiles of other floral honeys both in terms of the number of volatile compounds and their quantities. For example, Plutowska et al. [17] identified only 10 abundant rape honey compounds and considered rape honeys to be indistinct.

Fig. 1 shows that the content of the 8 key volatile compounds slightly differed in each production year (2012, 2014, 2015 and 2016). The most atypical group of the rape honeys was the one produced in 2016. The one-way ANOVA showed statistically significant differences in the contents of 6 compounds, namely, 3-methylpentanoic acid ($p = 0.014$), 2-phenylethanol ($p < 0.001$) and 4-ethenyl-2-methoxyphenol ($p < 0.001$) were found in larger quantities in the rape honey samples from 2016. The rape honey samples from 2014 and 2015 showed a low content of benzaldehyde ($p = 0.013$), phenylmethanol ($p < 0.001$) and β-damascenone ($p < 0.001$). There were no statistically significant differences in the average content of the selected key rape honey compounds between the rape honeys (i.e. all 28 samples from 2014, 2015 and 2016) and the multifloral honeys from 2012 (Student's t-test, $p > 0.05$). Therefore, the selected key compounds cannot be considered as solely characteristic for rape honeys.

PCA was applied to create an overview of general distribution of data on profiles of volatile compounds (relative representation of 28 most abundant volatile compounds in percent). The principal components PC1 vs PC2 scatter plot (Fig. 2) shows a good visual separation of rape (2014–2016) and multifloral honeys but a rather poor clustering of rape honeys wher PC1 and PC2 explained only 39.8 % and 23.8 % of the total variance, respectively). Evaluation of PC weights (not shown) confirmed that no single volatile compound was sufficient to distinguish rape and multifloral honeys. These results highlighted the necessity to use LDA as a supervised technique.

LDA was applied to find a linear combination of the representations of the 28 volatile compounds separating the honey samples into the two previously known classes according to their botanical origin. The principle of this method is based on the determination of linear discriminatory functions that increase the variability between classes. Two significant canonical discriminant functions were identified using the LDA method. The first function correctly discriminated between the samples of rape honeys (2016 and 2014–2015; on average 89 % correctly classified) and multifloral honeys (93 % correctly classified). From the 28 samples of rape honey, only 3 were incorrectly classified as multifloral honeys. The model correctly classified 89% of the rape honeys and as such seems to be a promissing tool for deter-

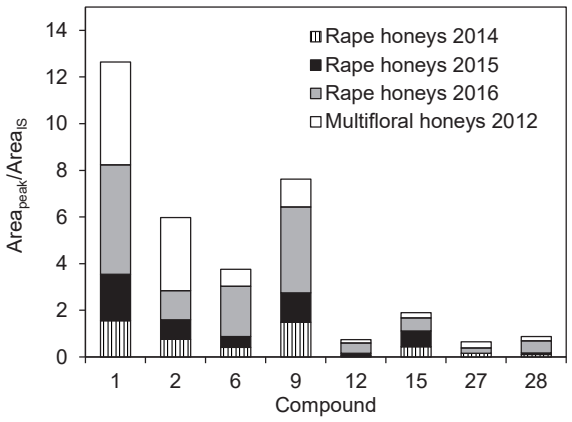

Fig. 1. Content of the key compounds in the rape and multifloral honeys.

Compounds (numbers from Tab. 4): 1 – benzaldehyde, 2 – 2-phenylacetaldehyde, 6 – phenylmethanol, 9 – 2-phenylethanol, 12 – 4-ethenyl-2-methoxyphenol, 15 – 3-methylpentanoic acid, 27 – dimethyldisulfide, 28 – β-damascenone.

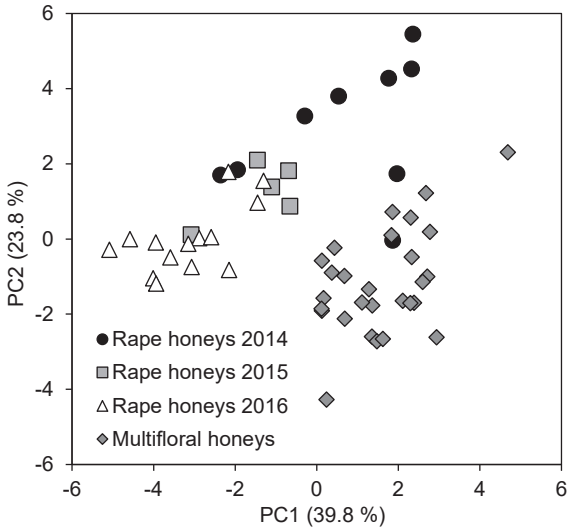

Fig. 2. Principal component scatter plot for rape and multifloral honey samples.

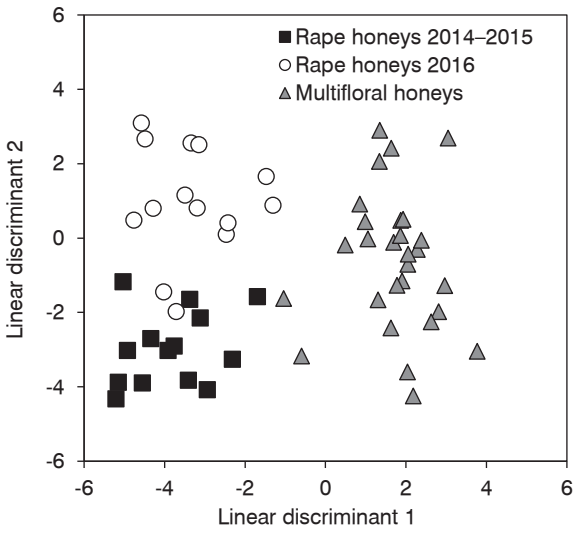

Fig. 3. Classification graph of canonical roots – distribution of the rape and multifloral honeys.

mination of the botanical origin of unifloral rape honeys.

The distribution of the honeys in the space of the first and the second discriminatory functions is shown in Fig. 3. The step-by-step method was used to select the most important variables. Based on the *p*-value and the Wilk's (λ) and Fischer criteria (*F*), the most significant discriminators were identified as (in the order of decreasing *F* value) nonanoic acid ($p < 0.001$) > 3-methylbutanenitrile ($p < 0.001$) > 2-phenylacetonitrile ($p = 0.004$) > β-damascenone ($p = 0.005$) > 3-methylpentanoic acid ($p = 0.027$). The highest *F* values corresponded to the volatile compounds that most discriminated between the objects in the classes.

The results showed that the first discriminatory function, which distinguished between the rape honeys and the multifloral honeys, was strongly associated with the representation of 3-methylbutanenitrile and nonanoic acid in the volatile profiles. The second discriminatory function, which slightly distinguished between the rape honeys from 2014–2015 and those from 2016, was strongly associated with the representation of 2-phenylacetonitrile and β-damascenone. From the 8 selected key compounds discussed above, which were analysed using an ANOVA test, β-damascenone and 3-methylpentanoic acid appeared to be the most significant compounds able to distinguish rape honeys from season 2014–2015 (β-damascenone) and 2016 (3-methylpentanoic acid) from the rest of the samples. After consulting available literary sources (Tab. 2), we concluded that characteristic compounds identified in this study, able to distinguish between the rape and the multifloral honeys (i.e. 3-methylbutanenitrile and nonanoic acid), did not correspond to characteristic compounds identified in other studies. This inconsistency may be caused by the high variability in the composition of analysed honeys and by the presence of 5–25 % of honeys of other botanical species, predominantly apple and cherry tree, willow and dandelion.

CONCLUSIONS

Main volatile compounds of rape honeys were analysed using headspace SPME-GC-MS. The obtained profiles of volatile compounds were comparatively rich. The identified compounds are commonly found in floral honeys. Hotrienol (7.2–39.3 %), benzaldehyde (0.7–19.0 %), 3-methylbutanenitrile (0.1–15.7 %), 2-phenylethanol (1.0–10.2 %) and nonanoic acid (0.4–7.0 %) were the most abundant compounds, being present in all 28 analysed samples. LDA could identify only two key compounds, 3-methylbutanenitrile and nonanoic acid, as the characteristic compounds for the rape honeys. Except for botanical origin, the occurrence and representation of individual compounds is strongly influenced by a number of factors, including storage conditions, processing technology and the botanical purity of the samples [27]. We hypothesize that the high variability in composition of honeys and the difficulty in identifying characteristic compounds was

mostly caused by the presence of 5–25 % of honeys of other botanical species (predominantly apple and cherry tree, willow and dandelion), some of which are known sources of intense aroma.

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