



Discrimination of Polish unifloral honeys using overall PTR-MS and HPLC fingerprints combined with chemometrics



Piotr Marek Kuś^{a, b, *}, Saskia van Ruth^{a, c}

^a RIKILT – Institute of Food Safety, Wageningen University and Research Centre, P.O. Box 230, 6700 AE Wageningen, The Netherlands

^b Department of Pharmacognosy, Wrocław Medical University, ul. Borowska 211a, 50-556 Wrocław, Poland

^c Food Quality and Design Group, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands

ARTICLE INFO

Article history:

Received 28 September 2014

Received in revised form

26 December 2014

Accepted 28 December 2014

Available online 7 January 2015

Keywords:

Headspace volatiles

Chromatography

Classification

Quality control

Botanical origin

ABSTRACT

A total of 62 honey samples of six floral origins (rapeseed, lime, heather, cornflower, buckwheat and black locust) were analysed by means of proton transfer reaction mass spectrometry (PTR-MS) and HPLC-DAD. The data were evaluated by principal component analysis and k-nearest neighbours classification in order to examine consistent differences in analytical fingerprints between various honeys allowing their discrimination. The study revealed, that both techniques were able to distinguish the floral origins, however the HPLC shows advantage over PTR-MS providing substantially better differentiation of all analysed honey types. Especially HPLC fingerprints recorded at 210 nm were most suitable for discrimination of botanical origin with the use of chemometric analysis. The obtained classification rates were: 100%, 93%, 100%, 83%, 100%, 100% (HPLC) and 69%, 67%, 78%, 67%, 100%, 88% (PTR-MS) for rapeseed, lime, heather, cornflower, buckwheat and black locust, respectively. Even if performance of PTR-MS in general was lower than HPLC, it might be useful for fast on-line screening of buckwheat honey.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Honey is one of the oldest natural medicine and food products that is still in use in its original form or as a functional ingredient. Numerous studies provide data on phytochemical diversity and variability regarding volatile and non-volatile compounds of the honeys deriving from different floral sources (Pyrzynska & Biesaga, 2009; Soria, Martínez-Castro, & Sanz, 2008). It exhibits a strong impact on differences in biological activity, flavours, colours and other properties of honeys (Kuś, et al., 2014; Piana et al., 2004; Tuberoso et al., 2014) that are crucial for their application, customer preferences and market value. Therefore, the most valuable are unifloral honeys providing predictable and desirable physical-chemical, sensory and biological characteristics (Bryant & Jones, 2001). The determination and confirmation of floral origin has been performed by pollen analysis based on the presence of characteristic pollen in the nectar. However, this method has many limitations (Ruoff, 2006). For example, due to specific botanical

conditions, characteristic pollen can be often underrepresented in strawberry tree, asphodel, black locust, lime and heather honey (Bryant & Jones, 2001; Jerković, Tuberoso, Kasum, & Marijanović, 2011; Tuberoso et al., 2010). Therefore, work in recent decades has focused mainly on phytochemicals present in honey, particularly on finding specific and non-specific markers that could be useful for distinguishing different floral origin (Kaškonienė & Venskutonis, 2010). Such compounds are not always available, however chemical composition of each honey is different and complex, thus application of multivariate statistical analysis may be very useful for their characterization. To date various chromatographic and spectroscopic methods employing chemometric approach have been developed to distinguish the honey botanical origin. Recently NIRS has been reported useful for identification of botanical origin of Chinese honeys (Zhao, He, & Bao, 2011) and NMR for Polish honeys (Zieliński, Deja, Jasicka-Misiak, & Kafarski, 2014). High performance liquid chromatography with diode array detector (HPLC-DAD) or mass spectrometry (HPLC-MS), have been proposed to investigate chemical profiles of the honey in the search of markers of floral origin (Pyrzynska & Biesaga, 2009; Tuberoso et al., 2011) as well as for classification of honey of different floral origin in combination with chemometric approach (Bertoncelj, Polak, Kropf, Korošec, & Golob, 2011; Zhou et al., 2014). Bertoncelj et al. (2011) focused on pre-cleaned flavonoid fraction. Fingerprints of

* Corresponding author. Department of Pharmacognosy, Wrocław Medical University, ul. Borowska 211a, 50-556 Wrocław, Poland. Tel.: +48 71 7840211; fax: +48 71 7840218.

E-mail address: kus.piotrek@gmail.com (P.M. Kuś).

the honey headspace volatiles obtained by gas chromatography – mass spectrometry (GC-MS) and combined with chemometric analysis allowed to distinguish honeys of different floral origin from Spain (Soria et al., 2008) and Greece (Aliferis, Tarantilis, Harizanis, & Alissandrakis, 2010). The MS techniques provide good quality results, however their relatively high cost makes them less attractive for routine analyses in food industry. Additionally, many methods build discrimination models based only on several selected compounds (Bertoncelj et al., 2011; Soria et al., 2008) which makes them vulnerable to fraud since few specific marker compounds can be added to falsify the honey. An approach considering utilization of whole fingerprints includes contribution of all compounds of the complex mixture and makes adulteration more difficult. Similarly human olfaction is perceived as a global fingerprint and applied in form of sensory analysis to assess quality of numerous food products (Dickinson, White, Kauer, & Walt, 1998). In recent decades the electronic nose was developed to mimic human olfaction and was useful to classify honeys in combination with Artificial Neural Network models (Ampuero, Bogdanov, & Bosset, 2004; Čačić Kenjerić, Mannino, Bennedetti, Primorac, & Čačić Kenjerić, 2009). Similar principle is provided by proton transfer reaction mass spectrometry (PTR-MS) which allows rapid on-line, non-separative dynamic headspace analysis, without complicated sample pre-treatment and with high sensitivity which makes it good tool for fingerprinting combined with unsupervised classification methods (Luykx, Peters, van Ruth, & Bouwmeester, 2008). It was successfully applied to several food matrices such as dry cured ham (Sánchez del Pulgar et al., 2011), monovarietal virgin olive oils (Ruiz-Samblás et al., 2012), speciality coffees (Özdestan et al., 2013) and for differentiation of geographical origin of palm oils (Tres, Ruiz-Samblas, van der Veer, & van Ruth, 2013). To the best of our knowledge, PTR-MS was not yet applied for classification and distinguishing between various honeys of different floral origin.

The objective of this study was to develop analytical methodology applying chemometric methods to overall fingerprints obtained by two different techniques. Specifically, this approach will be used to distinguish the floral origin of Polish honeys based on their i) overall HPLC fingerprints of the honey solutions, recorded at different wavelengths and ii) overall fingerprints of their headspace, analysed by PTR-MS.

2. Materials and methods

2.1. Honey samples

A total of 62 samples of six honey floral origins, collected in years 2010 and 2011 in Poland were used for analyses: rapeseed (*Brassica napus* L. var. *oleifera* Metzger) [13 samples, 54.0–86.6 % of specific pollen], buckwheat (*Fagopyrum esculentum* Moench) [11 samples, 40.3–56.1 % of specific pollen], heather (*Calluna vulgaris* (L.) Hull) [9 samples, 47.8–65.0 % of specific pollen], cornflower (*Centaurea cyanus* L.) [6 samples, 23.4% - 40.5 % of specific pollen], black locust (*Robinia pseudoacacia* L.) [8 samples, 31.1–36.4 % of specific pollen], and lime (*Tilia* spp.) [15 samples, 32.1–37.1 % of specific pollen]. All the samples were obtained in Poland and their floral origin was confirmed by pollen analysis. After receipt, the honeys were stored at 4 °C in dark. Qualitative and quantitative melissopalynological analyses were carried out according to the method of the International Commission of Bee Botany and International Honey Commission (Von Der Ohe, Persano Oddo, Piana, Orlot, & Martin, 2004).

2.2. Reagents

Phosphoric acid 850 g/kg, of analytical grade was purchased from Merck (Darmstadt, Germany), Acetonitrile HPLC supra

gradient grade was purchased from Biosolve BV (Valkenswaard, The Netherlands) ultrapure MiliQ water (18.2 MΩ) was obtained using MiliQ Advantage A10 System (Millipore Corporation, Billerica, MA, USA).

2.3. Proton transfer reaction-mass spectrometry (PTR-MS)

Measurements were performed using high sensitivity PTR-MS (HS-PTR-MS) system (Ionicon GmbH, Innsbruck, Austria). Each honey sample was homogenized using a spatula and approximately 3.0 g was weighed in clean 250 mL SCHOTT screw cap glass bottle, closed and equilibrated under agitation for at least 30 min in a water bath (35 °C) with shaker to saturate the air in the bottle (headspace) with investigated volatile organic compounds (VOCs). Bottles containing analysed sample were manually connected to the inlet flow of PTR-MS and the headspace air of the analysed samples was delivered via drift tube directly to the instrument inlet with an airflow rate of 56 mL/min. The temperature of both the inlet and drift tube was maintained at 60 °C to prevent loss of the volatiles along the inlet sampling line. Before each sample of honey, a blank air sample was analysed. Measurements were carried out in the mass full-scan mode and in range of 20–180 mass-to-charge ratios (m/z) and the spectra were recorded. A mass detection rate of 0.2 s mass^{-1} was applied, resulting in a cycle time slightly above 30 s.

Analyses of the samples were carried out in triplicate, and the replicated measurements were performed on different days. For each replicate, 5 full mass scans were recorded. The first and the last cycle were excluded from calculations. The remaining scans were averaged and the data were corrected (background, transmission). Out of the three averaged mass spectra from the independent replicates of each sample a mean mass spectrum was obtained for each sample. Additionally m/z 32 (O_2^+) and m/z 37 (water cluster ion) that are associated with the PTR-MS ion source were removed. The dataset containing spectra of all the investigated samples was obtained in this manner and used for further analysis and evaluation.

2.4. HPLC-DAD analysis

The analyses were performed according to Tuberoso et al. (2011) after modifications, using HPLC-DAD Agilent 1100 Series system (Agilent Technologies, Inc., Santa Clara CA) consisting of: thermostated (G1330B) autosampler (G1329A), binary pump (G1312A), degasser (G1379A), and PDA detector (G1315B). The separation was obtained with a Phenomenex Gemini C18 110A column (150 mm \times 4.60 mm, 3 μm , Phenomenex, Torrance, CA) thermostated to 30.0 °C using 0.2 mol/L phosphoric acid (solvent A) and acetonitrile (solvent B) as mobile phase at a constant flow rate of 0.9 mL/min. The gradient (v/v) was generated, keeping 90% of solvent A for 5 min, then decreasing to 65% in 10 min, to 10% in 5 min, and remaining at this concentration for 10 min, then the initial A/B ratio (90: 10, v/v) was achieved within 1 min and the system was stabilized for 10 min before the next injection. The injection volume was 10 μL . The obtained chromatograms and spectra were elaborated with an Agilent Chemstation software for LC 3D systems (Rev. B.01.03). The honey samples were homogenized in a jar and carefully diluted with ultrapure water (0.2 g/mL), filtered through \emptyset 25 mm Acrodisc[®] Syringe Filter 0.45 μm HT Tuffryn[®] (Pall Life Sciences, Port Washington, NY, USA) and injected in HPLC without any further purification. Analyses of the samples were carried out in duplicate.

2.5. Statistics

The data were treated and evaluated by Principal Component Analysis (PCA) comparing different data pre-processing techniques,

resulting in the selection of no data pre-treatment, autoscaling or mean-centring (when indicated). For determination of honey type, based on PTR-MS or HPLC fingerprint a k-nearest neighbour classification (k-NN) algorithm ($k = 3$) was used after application of appropriate data pre-treatment. The models and classifications were performed in Pirouette 4.0 software (Infometrix, Seattle, USA).

3. Results and discussion

3.1. PTR-MS fingerprinting

The most abundant mass-to-charge ratios (m/z) found in various proportions and higher or lower amounts in all the honeys were m/z 33, 45, 47, 59, 61, 73, 75 that could be tentatively attributed e.g. to methanol, acetaldehyde, ethanol, acetone, acetic acid and butan-2-one, isobutanol respectively along with m/z 43 that may be assigned to acetone or acetic acid fragments (de Gouw & Warneke, 2007) and m/z 29, presumably ethanol fragment ($C_2H_5^+$). These compounds could be probably products of slight fermentation and/or amino acids metabolism (Bouseta, Collin, & Dufour, 1992; Mehta, Kamal-Eldin, & Iwanski, 2012) generated naturally by yeast present in honey. These processes may occur before final ripening of each honey, since evaporation of excess water by the bees takes at least several days until the osmotic inhibition of microorganisms activity is reached (de la Fuente,

Sanz, Martínez-Castro, Sanz, & Ruiz-Matute, 2007). Similar compounds were previously found in honeys of various floral origin using dynamic headspace GC-MS system (Bouseta et al., 1992). Other ions found in the honeys, were e.g. m/z 69, 97, 101 that may be tentatively attributed to furan, furfural, dihydro-5-methylfuran-2(3H)-one that can form during storage or heat treatment as products of the Maillard reaction (Bouseta et al., 1992; de la Fuente et al., 2007). However, the levels of these compounds in the investigated samples were generally low except in buckwheat honey samples where they were higher. Elevated levels of such compounds in this honey type were observed previously also by other authors (Brudzynski & Miotto, 2011; Wolski, Tambor, Rybak-Chmielewska, & Kędzia, 2006), suggesting that some of these compounds may occur naturally or their formation is particularly favoured in this honey type. Buckwheat honey was characterized by the presence of m/z 97, 101 and 103 that could be tentatively attributed to furfural, valerolactone and its structural isomers, 2-/3-methylbutyric acid respectively (Wolski et al., 2006). On the other hand, the profile of black locust honey is quite poor in volatiles and not very specific. Cornflower honey is characterized by m/z 121 and 153 which can be tentatively associated with phenylacetaldehyde and 4-ketoisophorone found previously in its headspace (Kuś, Marijanović, & Jerković, 2013). Heather honey could be characterized by m/z 107, 121, 139, 153, 155 that may be assigned to benzaldehyde, phenylacetaldehyde, isophorone, 4-ketoisophorone, 2-hydroxyisophorone respectively, that were

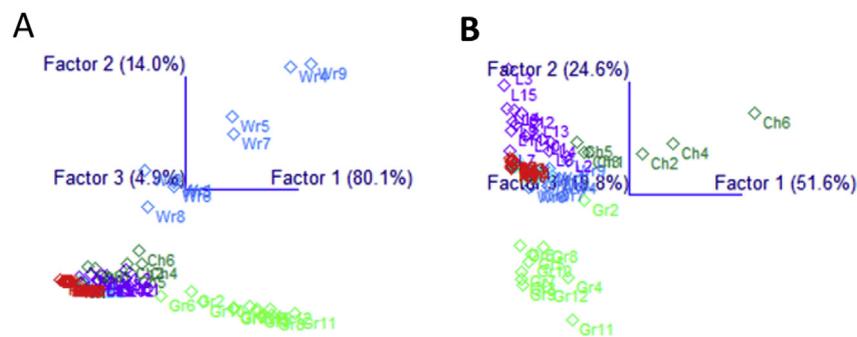


Fig. 1. Principal component analysis (PCA) scores plot based on HPLC fingerprints recorded at 210 nm and 360 nm for 62 honey samples of six botanical origins. (A) PCA scores plot on the HPLC fingerprints of 62 honey samples at 210 nm (first three factors, data without pre-processing); (B) PCA scores plot on the HPLC fingerprints of 62 honey samples at 360 nm (first three factors, mean-centred data); The variables are retention times sampled 150 times per minute and their value is absorbance measured at 210 nm (A) or 360 nm (B). R (blue) – rapeseed honey; L (violet) – lime honey, Wr (navy blue) – heather honey; Ch (dark green) – cornflower honey; Gr (light green) – buckwheat honey; A (red) – black locust honey; (regarding the references to colour, the reader is referred to the on-line version of this article).

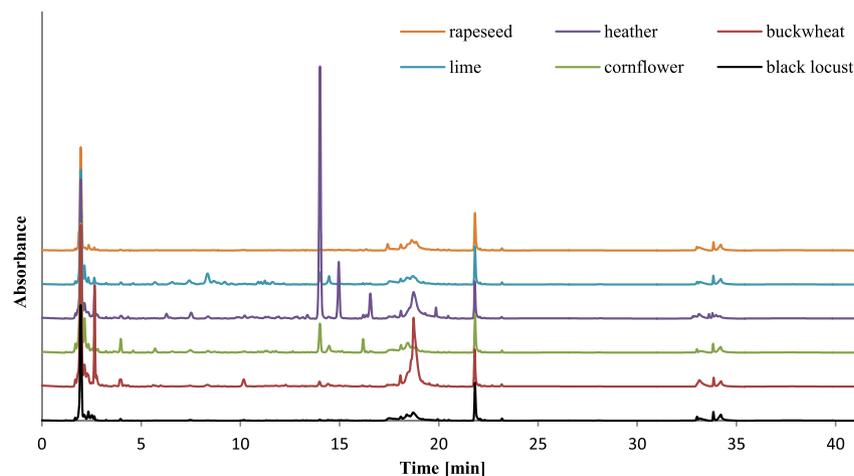


Fig. 2. Comparison of representative HPLC fingerprints of each investigated honey type, recorded at 210 nm. Fingerprints were obtained from chromatograms recorded at 210 nm, previously exported as dataset and aligned.

found in headspace of this honey type by GC-MS following HS-SPME (Wolski et al., 2006). Lime honey could be characterized by m/z 133, 135, 137, 149, 152, 153 that may be assigned to *p*-cymenene, *p*-cymene, terpinene, estragole, 1-methoxy-4-propylbenzene, 2- and 3-methoxyphenylethanol respectively, which were previously reported in this honey type (Guyot, Bouseta, Scheirman, & Collin, 1998). The profile of rapeseed honey shows high variation and is not very specific, however it could be characterized by particularly abundant m/z 33 (methanol). The comparison of PTR-MS fingerprints of different honey varieties are presented in Fig. 3. The tentative assignment of the volatile organic compounds to ions by comparison with previously

identified e.g. by GC-MS in honeys (literature data) is presented in Table 1.

3.1.1. Principal component analysis (PCA)

The PCA was conducted on the whole dataset obtained as described previously including data of 62 samples and 159 ions. The data was autoscaled prior to analysis and the PCA scores plot revealed some natural clustering, according to the floral origin. The two first factors explained 41.6% of variance between the different samples. The most distant from the other samples were those from lime, buckwheat and heather origin showing clear differences from the other samples as well as higher

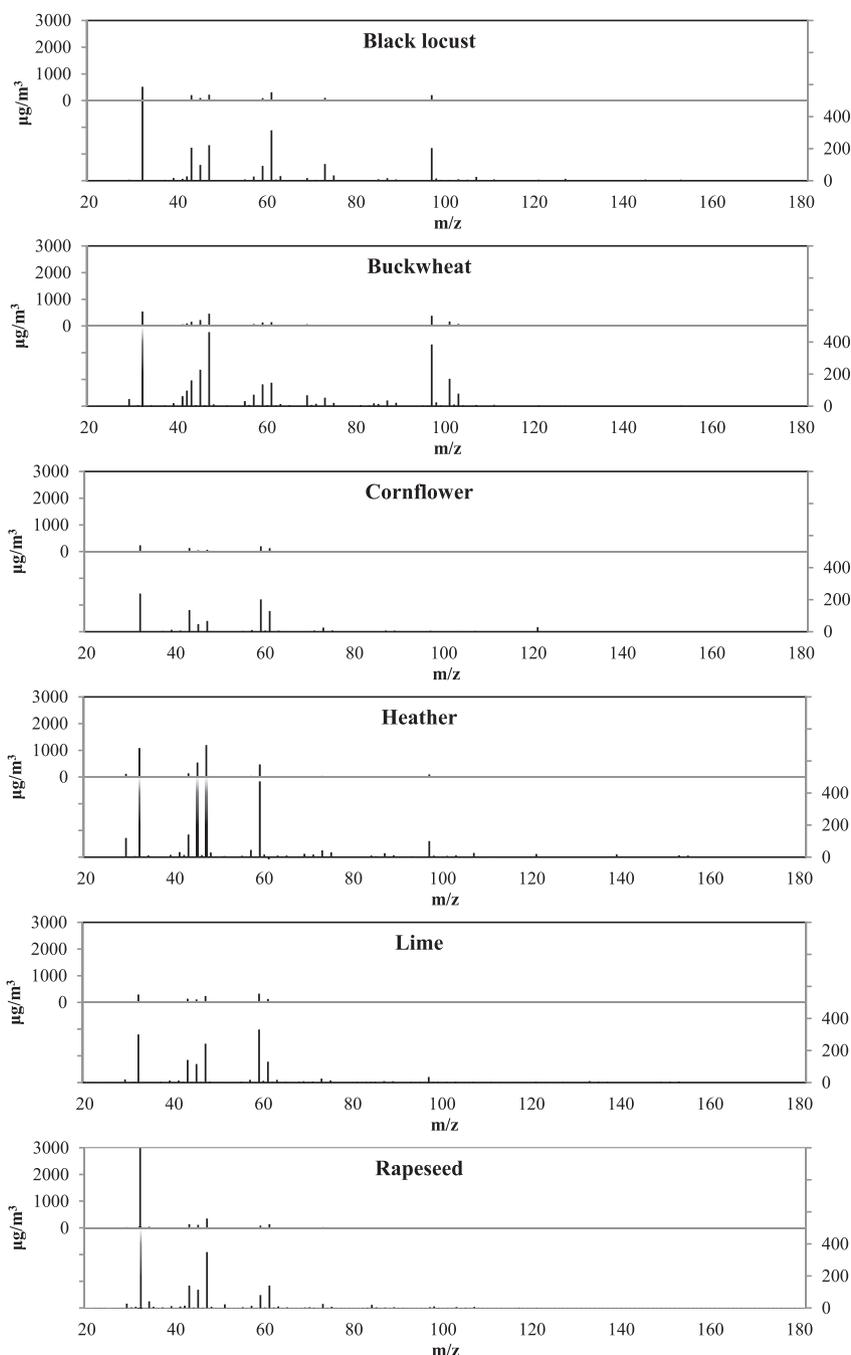


Fig. 3. Representative headspace mass spectra of honey from different botanical origin. The same spectrum for every honey is presented in two scales (bottom and top) to allow comparison between honeys and visibility of their peculiarities; m/z 's 32 and 37 related to PTR-MS ion source were excluded.

Table 1

Average amount and tentative assignment of selected ions detected in the headspace of honey from different botanical origins.

m/z	Concentration ($\mu\text{g}/\text{m}^3$)												Tentative assignment of ions to compounds
	Black locust		Buckwheat		Cornflower		Heather		Lime		Rapeseed		
	(n = 8)	$\pm\text{SD}$	(n = 11)	$\pm\text{SD}$	(n = 6)	$\pm\text{SD}$	(n = 9)	$\pm\text{SD}$	(n = 15)	$\pm\text{SD}$	(n = 13)	$\pm\text{SD}$	
29	10.6	14.7	53.4	137.2	125.9	86.2	164.0	235.6	129.8	121.9	33.8	107.8	C_2H_5^+ (ethanol fragment) ^a
33	518.1	176.3	735.0	690.4	286.1	61.3	1465.9	2694.5	387.1	164.2	4039.1	12648.8	methanol ^a
39	17.1	1.3	30.3	5.8	428.6	103.6	149.3	189.9	277.9	260.3	18.6	1.9	furan fragment ^d , isoprene (fragment) ^d
41	11.7	6.4	103.5	51.8	305.4	88.0	113.8	92.5	199.5	201.7	14.7	9.9	2- and/or 3-methylbutanal (fragment) ^e
42	26.7	62.4	167.1	110.7	3.5	2.8	28.1	15.6	6.2	5.5	24.8	60.5	acetonitrile ^{d,f} , C_3H_6^+ (propene or C_4 – C_5 hydrocarbons fragment) ^f
43	206.0	75.9	283.0	120.1	238.2	153.1	304.2	116.0	232.2	98.1	244.3	160.2	acetone (fragment) ^a , acetic acid (fragment) ^a
45	98.2	94.5	418.6	528.1	545.8	166.9	1145.4	1325.8	482.3	285.5	209.5	578.8	acetaldehyde ^a
47	224.8	177.6	1357.4	2271.5	616.2	356.6	2376.2	3681.0	819.2	533.2	704.2	1649.8	ethanol/formic acid ^{a,b}
55	8.0	1.3	70.3	26.2	189.1	133.9	63.4	69.3	129.5	144.6	10.4	3.7	protonated water trimer ^d
57	26.2	22.5	167.2	58.1	377.1	170.2	143.1	87.8	282.4	323.3	31.3	22.6	C_4H_9^+ (butanol fragment) ^d
59	92.3	56.4	328.9	182.6	623.3	103.1	1198.2	831.7	909.8	380.2	196.8	227.9	acetone ^{a,b}
61	314.4	133.1	364.5	165.1	313.3	86.4	840.0	1283.6	638.5	458.6	368.7	223.5	acetic acid ^{a,b} , propanol ^g , methyl methanoate ^g , pentane-2,3-dione (fragment) ^e
63	29.0	42.8	34.0	28.4	268.9	173.7	67.3	61.2	252.8	260.4	26.5	39.3	dimethyl sulphide ^{b,g}
65	4.3	4.1	15.8	24.9	61.9	25.5	45.9	45.1	45.6	47.3	10.1	19.0	methanol dimer ^d
69	16.6	20.4	192.2	125.3	183.3	125.9	139.2	99.6	200.7	236.1	8.6	8.5	furan ^g , isoprene ^d
73	104.3	32.8	157.6	101.9	316.5	271.6	257.7	216.9	288.3	247.1	77.6	27.7	butan-2-one ^a , tetrahydrofuran ^g
75	33.0	33.4	61.1	29.1	440.9	264.2	203.9	215.9	323.2	298.8	21.5	28.5	2-methylpropan-1-ol ^b , ethyl methanoate ^g
85	9.4	7.2	47.4	22.1	304.6	110.9	92.1	137.6	219.7	270.2	13.1	14.7	2-methylbut-2-enal ^b
87	16.0	13.6	127.4	84.4	292.4	190.3	130.8	69.1	483.0	482.7	11.9	6.0	3-methylbut-3-en-1-ol ^b , 2- and/or 3-methylbut-2-en-1-ol ^b , dihydrofuran-2(3H)-one ^b , 3-methylbutan-2-one ^g , hexane ^g
89	8.8	3.1	76.9	21.6	470.3	278.5	83.7	77.6	317.1	415.6	17.4	12.8	ethyl acetate ^{b,g} , 2- and/or 3-methylbutan-1-ol ^b , 2-methyl-propanoic acid ^b , butyric acid ^b
97	204.1	438.7	1526.4	1294.3	372.7	265.6	471.7	399.0	555.6	372.9	18.9	38.6	furfural ^{a,b} , methanol trimer ^d
101	6.0	2.0	706.0	188.1	237.9	91.8	104.0	126.1	190.1	233.7	6.0	2.2	dihydro- 3-/4-/5-methylfuran-2(3H)-one ^b , pentane-2,3-dione ^{e,g} , hexanal ^g
103	9.1	6.2	328.4	138.0	412.5	321.2	199.8	240.3	280.9	309.6	23.3	14.6	3- and/or 2-methylbutyric acid ^{b,c} , valeric acid ^b , ethyl propionate ^g
107	24.0	20.6	32.6	16.2	472.5	345.8	275.8	238.0	325.1	354.8	29.9	47.6	benzaldehyde ^{b,c} , xylene ^g
111	8.4	12.0	37.6	23.8	157.3	43.5	50.1	44.0	86.9	86.2	2.6	1.7	2,3-dimethyl-2-cyclopenten-1-one ^c , 2-acetylfuran ^b , 5-methylfurfural ^b
121	6.7	4.2	19.5	15.4	392.2	293.2	339.2	382.1	388.5	463.7	6.2	7.0	phenylacetaldehyde ^{b,c} , acetophenone ^b
133	0.8	0.8	4.6	4.6	196.7	122.8	51.7	81.8	207.5	197.3	0.7	0.5	<i>p</i> -cymene ^c , cinnamaldehyde ^b
135	1.2	1.3	5.2	3.9	233.6	200.3	26.1	43.1	116.5	85.0	0.7	0.4	<i>p</i> -cymene ^c , phenyl-2-propanone ^b
137	2.2	1.5	5.3	3.0	435.7	244.8	91.5	157.4	220.2	253.1	2.0	1.4	terpinene ^{b,c} , phenylacetic acid ^{b,c}
139	1.0	0.6	3.0	1.9	87.9	50.8	148.8	68.9	59.1	57.7	1.5	0.6	isophorone ^b
149	0.3	0.2	1.4	1.3	152.5	65.9	15.7	19.4	126.5	199.3	0.3	0.2	estragole ^c
151	0.2	0.1	0.4	0.2	23.3	18.1	4.6	7.0	27.1	35.0	0.1	0.1	1-methoxy-4-propylbenzene ^c
153	1.0	0.8	3.4	2.5	155.9	81.1	51.8	77.3	151.1	160.4	1.3	1.7	4-ketoisophorone ^{b,c} , methyl salicylate ^b
155	7.0	10.5	16.5	10.8	389.7	265.4	115.9	59.7	236.3	227.1	2.4	1.1	2-hydroxyisophorone ^b , linalool ^b
PASD	14.2												

Data are expressed as average of n samples \pm standard deviation; measurements of each sample were performed in triplicate and the pooled analytical standard deviation (PASD) is calculated; The ions were tentatively assigned to compounds, based on literature data.

^a (de Gouw & Warneke, 2007).

^b (Wolski et al., 2006).

^c (Guyot et al., 1998).

^d (Maleknia, Bell, & Adams, 2007).

^e (Lindinger et al., 2005).

^f (Dunne, Galbally, Lawson, & Patti, 2012).

^g (Bousseta et al., 1992).

concentrations of volatiles. Samples of honey of other floral origin (rapeseed, black locust and cornflower) seem to show smaller differences between each other and relatively lower concentration of volatiles.

3.1.2. k-Nearest neighbour classification results

The distinction between the floral origins was further examined by a k-NN classification model based on autoscaled PTR-MS data. For the model all 62 honey samples of 6 floral origins and 159 ions were used. Most samples of each of honey type were classified correctly giving success rate of 88%; 100%; 67%; 78%; 67%; 69%; for black locust, buckwheat, cornflower, heather, lime and rapeseed respectively. Overall success rate of classification for all the samples was 77%. Incorrect assignment of other samples in

groups of buckwheat and cornflower honey was not observed. For lime and heather classification error rates were low (1 and 3 samples respectively), but higher for black locust and rapeseed (5 samples of other origins incorrectly assigned to each of this groups). For buckwheat, heather, lime and cornflower honeys, the correct assignment is relatively high and risk of wrong assignment of other honeys to this groups is low. For rapeseed and black locust honeys, the risk of incorrect assignment was higher, demonstrating some overlap in patterns between these honeys and the others. In comparison with other chemometric approaches, the model based on headspace volatile fingerprints data (HS-SPME followed by GC-MS) in classification of Greek honey of different floral origins provided higher accuracy (98.7%) (Aliferis et al., 2010). Nevertheless, PTR-MS still provides quite fair overall

classification, especially for honey of some floral origins like buckwheat for which percentage of correct classification was 100%, while offering much shorter and cost-efficient on-line analysis. Therefore, it would be very useful for screening and confirmation of quality of this honey-type.

3.2. HPLC fingerprinting

The chromatograms obtained at 210, 254, 280, 330 and 360 nm, covering the whole spectra of a wide range of different compounds which were aligned and the whole chromatographic fingerprints (all data points of chromatogram – time/absorbance, not peak areas) were used for further statistical analysis.

3.2.1. Principal component analysis (PCA)

The datasets obtained by different wavelengths were evaluated by PCA, showing pronounced natural clustering and good separation for all of them. The most suitable for further analysis turned out to be the datasets obtained by 210 and 360 nm. In the case of the data obtained at 210 nm, no pre-processing was applied prior to the analysis and the first PCA factor explained 80.0% and factors 1–3 together even 99.0% of variance among the samples. In the case of the data obtained at 360 nm, mean-centring was applied prior to analysis, the main factor explained 51.6% and three first PCA factors explained 86.0% of variance in the dataset. At 210 nm the most outstanding were buckwheat, heather and lime honeys (Fig. 1A) At 360 nm, additionally cornflower honey showed better separation from the other floral origins of honey (Fig. 1B). After evaluation by PCA only the chromatograms at 210 nm, as the most useful for further analysis were used to build classification model. Comparison of fingerprints obtained at this wavelength is presented in Fig. 2.

3.2.2. *k*-Nearest neighbour classification results

In order to evaluate the discrimination between the honeys of different floral origins further, a *k*-NN classification model was developed using all 62 honey samples of the 6 floral origins using the overall chromatograms at 210 nm (for which the most variance was explained by two variables) without any data pre-processing. The chromatograms consisted of 150 readings per minute resulting in 6150 variables. Most samples of each honey floral origin were classified correctly giving success rate of 100%; 100%; 83%; 100%; 93%; 100%; for buckwheat, black locust, cornflower, heather, lime, rapeseed respectively. One sample of cornflower was incorrectly assigned as lime and one sample of lime honey as black locust honey. All other samples were assigned correctly (60 out of 62). The overall success rate was very high (97%). These results confirm the consistent differences in HPLC patterns between the six honeys of different floral origins. In comparison, the classification of Slovenian honeys based on LC-DAD/ESI-MS analysis of flavonoids and abscisic acid applying chemometrics exhibited 85% overall successful classification rate and for one of the honey floral origin it was correct only in 60% (Bertoncelj et al., 2011).

4. Conclusions

The developed chemometric classification models utilizing HPLC-DAD and PTR-MS fingerprints allowed differentiation of honeys of various botanical origin. The overall accuracy of classification model based on full HPLC fingerprint is very high and it is more efficient than that obtained by other authors that based classification on several identified compounds. The general accuracy of classification based on HPLC data was also significantly higher than that based on PTR-MS. Proposed method utilizing PTR-MS fingerprints provides an accurate

classification of only some honey floral origins, especially buckwheat. Therefore, models based on HPLC fingerprints may be useful as universal and accurate method of classification and PTR-MS for quick targeted on-line screening, but only of some specific honey types.

Acknowledgements

Authors thank Dr. Grishja van der Veer and Mr. Alex Koot for their help and support during the project and gratefully acknowledge financial support of the Ministry of Economic Affairs in The Netherlands.

References

- Aliferis, K. A., Tarantilis, P. A., Harizanis, P. C., & Alissandrakis, E. (2010). Botanical discrimination and classification of honey samples applying gas chromatography/mass spectrometry fingerprinting of headspace volatile compounds. *Food Chemistry*, 121, 856–862.
- Ampuero, S., Bogdanov, S., & Bosset, J.-O. (2004). Classification of unifloral honeys with an MS-based electronic nose using different sampling modes: SHS, SPME and INDEX. *European Food Research and Technology*, 218, 198–207.
- Bertoncelj, J., Polak, T., Kropf, U., Korošec, M., & Golob, T. (2011). LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. *Food Chemistry*, 127, 296–302.
- Bouseta, A., Collin, S., & Dufour, J.-P. (1992). Characteristic aroma profiles of unifloral honeys obtained with a dynamic headspace GC-MS system. *Journal of Apicultural Research*, 31, 96–109.
- Brudzynski, K., & Miotto, D. (2011). The relationship between the content of Mailard reaction-like products and bioactivity of Canadian honeys. *Food Chemistry*, 124, 869–874.
- Bryant, V. M., & Jones, G. D. (2001). The R-values of honey: pollen coefficients. *Palynology*, 25, 11–28.
- Čaćić Kenjerić, F., Mannino, S., Bennedetti, S., Primorac, L., & Čaćić Kenjerić, D. (2009). Honey botanical origin determination by electronic nose. *Journal of Apicultural Research*, 48, 99–103.
- De la Fuente, E., Sanz, M. L., Martínez-Castro, I., Sanz, J., & Ruiz-Matute, A. I. (2007). Volatile and carbohydrate composition of rare unifloral honeys from Spain. *Food Chemistry*, 105, 84–93.
- de Gouw, J., & Warneke, C. (2007). Measurements of volatile organic compounds in the earth's atmosphere using proton-transfer-reaction mass spectrometry. *Mass Spectrometry Reviews*, 26, 223–257.
- Dickinson, T. A., White, J., Kauer, J. S., & Walt, D. R. (1998). Current trends in 'artificial-nose' technology. *Trends in Biotechnology*, 16, 250–258.
- Dunne, E., Galbally, I. E., Lawson, S., & Patti, A. (2012). Interference in the PTR-MS measurement of acetonitrile at *m/z* 42 in polluted urban air—A study using switchable reagent ion PTR-MS. *International Journal of Mass Spectrometry*, 319–320, 40–47.
- Guyot, C., Bouseta, A., Scheirman, V., & Collin, S. (1998). Floral origin markers of chestnut and lime tree honeys. *Journal of Agricultural and Food Chemistry*, 46, 625–633.
- Jerković, I., Tuberose, C. I. G., Kasum, A., & Marijanović, Z. (2011). Volatile compounds of *Asphodelus microcarpus* Salzm. et Viv. honey obtained by HS-SPME and USE analyzed by GC/MS. *Chemistry & Biodiversity*, 8, 587–598.
- Kaškonienė, V., & Venskutonis, P. R. (2010). Floral markers in honey of various botanical and geographic origins: a review. *Comprehensive Reviews in Food Science and Food Safety*, 9, 620–634.
- Kuš, P. M., Congiu, F., Teper, D., Sroka, Z., Jerković, I., & Tuberose, C. I. G. (2014). Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six Polish unifloral honey types. *LWT – Food Science and Technology*, 55, 124–130.
- Kuš, P. M., Marijanović, Z., & Jerković, I. (2013). Headspace compounds from *Centaurea cyanus* L. honey: the occurrence of 3,4-dihydro-3-oxoedulan. *Chemistry of Natural Compounds*, 49, 961–964.
- Lindinger, C., Pollien, P., Ali, S., Märk, J., Blank, I., & Märk, T. (2005). Direct inter-comparison of datasets obtained by different PTR-MS: a novel approach to optimize and adapt the fragmentation pattern using a standardization procedure. In A. Hansel, & T. D. Märk (Eds.), *Proceedings of 2nd International Conference on Proton Transfer Reaction Mass Spectrometry and its Applications* (p. 89). Oberburgl, Austria: Innsbruck University Press.
- Luykx, D. M. A. M., Peters, R. J. B., van Ruth, S. M., & Bouwmeester, H. (2008). A review of analytical methods for the identification and characterization of nano delivery systems in food. *Journal of Agricultural and Food Chemistry*, 56, 8231–8247.
- Maleknia, S. D., Bell, T. L., & Adams, M. A. (2007). PTR-MS analysis of reference and plant-emitted volatile organic compounds. *International Journal of Mass Spectrometry*, 262, 203–210.
- Mehta, B. M., Kamal-Eldin, A., & Iwanski, R. Z. (2012). *Fermentation: Effects on food properties (chemical & functional properties of food components)*. Boca Raton, FL: CRC Press.

- Özdekan, Ö., van Ruth, S. M., Alewijn, M., Koot, A., Romano, A., Cappellin, L., et al. (2013). Differentiation of specialty coffees by proton transfer reaction-mass spectrometry. *Food Research International*, 53, 433–439.
- Piana, M. L., Persano Oddo, L., Bentabol, A., Bruneau, E., Bogdanov, S., & Guyot Declerck, C. (2004). Sensory analysis applied to honey: state of the art. *Apidologie*, 35, 26–37.
- Pyrzynska, K., & Biesaga, M. (2009). Analysis of phenolic acids and flavonoids in honey. *TrAC Trends in Analytical Chemistry*, 28, 893–902.
- Ruiz-Samblás, C., Tres, A., Koot, A., van Ruth, S. M., González-Casado, A., & Cuadros-Rodríguez, L. (2012). Proton transfer reaction-mass spectrometry volatile organic compound fingerprinting for monovarietal extra virgin olive oil identification. *Food Chemistry*, 134, 589–596.
- Ruoff, K. (2006). *Authentication of the botanical origin of honey*. Doctoral dissertation. ETH Zurich.
- Sánchez del Pulgar, J., Soukoulis, C., Biasioli, F., Cappellin, L., García, C., Gasperi, F., et al. (2011). Rapid characterization of dry cured ham produced following different PDOs by proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS). *Talanta*, 85, 386–393.
- Soria, A. C., Martínez-Castro, I., & Sanz, J. (2008). Some aspects of dynamic head-space analysis of volatile components in honey. *Food Research International*, 41, 838–848.
- Tres, A., Ruiz-Samblas, C., van der Veer, G., & van Ruth, S. M. (2013). Geographical provenance of palm oil by fatty acid and volatile compound fingerprinting techniques. *Food Chemistry*, 137, 142–150.
- Tuberoso, C. I. G., Bifulco, E., Caboni, P., Cottiglia, F., Cabras, P., & Floris, I. (2010). Floral markers of strawberry tree (*Arbutus unedo* L.) honey. *Journal of Agricultural and Food Chemistry*, 58, 384–389.
- Tuberoso, C. I. G., Bifulco, E., Caboni, P., Sarais, G., Cottiglia, F., & Floris, I. (2011). Lumichrome and phenyllactic acid as chemical markers of thistle (*Galactites tomentosa* Moench) honey. *Journal of Agricultural and Food Chemistry*, 59, 364–369.
- Tuberoso, C. I. G., Jerković, I., Sarais, G., Congiu, F., Marijanović, Z., & Kuš, P. M. (2014). Color evaluation of seventeen European unifloral honey types by means of spectrophotometrically determined CIE L*a*b* chromaticity coordinates. *Food Chemistry*, 145, 284–291.
- Von Der Ohe, W., Persano Oddo, L., Piana, M. L., Orlot, M., & Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie*, 35, 18–25.
- Wolski, T., Tambor, K., Rybak-Chmielewska, H., & Kędzia, B. (2006). Identification of honey volatile components by solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). *Journal of Apicultural Science*, 50, 115–126.
- Zhao, X., He, Y., & Bao, Y. (2011). Non-destructive identification of the botanical origin of Chinese honey using visible/short wave-near infrared spectroscopy. *Sensor Letters*, 9, 1055–1061.
- Zhou, J., Yao, L., Li, Y., Chen, L., Wu, L., & Zhao, J. (2014). Floral classification of honey using liquid chromatography–diode array detection–tandem mass spectrometry and chemometric analysis. *Food Chemistry*, 145, 941–949.
- Zieliński, L., Deja, S., Jasicka-Misiak, I., & Kafarski, P. (2014). Chemometrics as a tool of origin determination of Polish monofloral and multifloral honeys. *Journal of Agricultural and Food Chemistry*, 62, 2973–2981.