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Original Article

STUDY ON HONEY QUALITY EVALUATION AND DETECTION OF ADULTERATION BY ANALYSIS OF VOLATILE COMPOUNDS

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Abstract

Fast evaluation of honey quality is a topical and significant problem of the food industry, bee keepers and consumers. In this work, 22 samples of commercially available honey aromas (with methyl and ethyl esters of phenylacetic acid predominated), 13 samples of authentic honey collected directly from bee keepers (characterised by high content of benzaldehyde, 2-phenylethanol, hotrienol and 2-phenylacetaldehyde) and 63 honeys purchased from an outdoor market were evaluated based on volatiles profiles determined through solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC/MS) and then suspicious samples were identified. The results were statistically processed and compared with results of a sensory analysis. Six honeys, which differed significantly in volatiles profiles (outliers detected by Factor Analysis), selected volatile substance representation (furan-2-carbaldehyde, 1,4-dimethylpyrazole, benzaldehyde, 2-phenylacetaldehyde) and honey aroma intensity and pleasantness were subjected to targeted analyses (i.e. determination of 5-(hydroxymethyl)-2-furaldehyde, diastase activity, unauthorized additive presence). Four of these suspicious samples were found to have high content of 5-(hydroxymethyl)-2-furaldehyde (more than 40 mg/kg), three honeys had low values for diastase activity (less than 8) and three samples positive for triacetin addition. The fact that all these samples revealed a breach of least one of the selected quality parameters defined by the Codex Alimentarius standard proved the proposed methodology to be a useful tool for fast quality evaluation of honey.

Keywords: adulteration, honey, quality, sensory assessment, SPME-GC/MS, volatile compound.

INTRODUCTION

Honey is characterised by its aroma, sweet taste and potential biomedical activity. Due to continuous expansion of the world honey market, the importance of apiculture as an industry has also grown. Composition and quality criteria of honey are defined by the Codex Alimentarius standard (CODEX Stan 12, 2001) and the EU Honey Directive (Council Directive, 2001) which state that honey should not have any ingredients added; no particular constituent can be removed from it; it does not have any objectionable matter, flavour, aroma or taint absorbed from foreign matter during processing and storage; and it should not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired.

Honey is commonly adulterated through the addition of sweeteners (e.g. sugar syrups and molasses inverted by acids or enzymes from corn, sugar cane, sugar beet and natural syrups such as maple), different botanical and geographical origins, heat treatment or improper storage conditions or the filtration or addition of colorants (Bogdanov & Martin, 2002; Potraviny na pranýři, 2013). The quality of honey is determined by its sensorial, physicochemical and microbiological characteristics. A combination of

sensory and analytical criteria is considered to be the best way to evaluate the quality of honey and determine better conditions of storage to preserve its quality worldwide. Colour, aroma and taste are key factors in determining quality of honey. They vary according to geographical and seasonal conditions and to floral source type. Sensory evaluation enables us to distinguish between botanical origins of honey and to identify and quantify defects, such as fermentation, impurities, off-odours and flavours (Piana et al., 2004). Flavours range from delectably mild in a lighter-coloured honey to distinctively bold in a darker one. Flavour is directly influenced by different floral sources of nectar or honeydew origin, environmental factors, bee-keeping practices, processing and storage conditions.

Volatile substances also affect the quality of honey. Volatiles including aldehydes, ketones, esters, alcohols, hydrocarbons, and sulfur compounds come from plants or nectar, transformation of plant compounds by or directly generated by honeybees, heating or treatment during honey processing and storage, and microbial or environmental contamination (Jerković & Marijanović, 2009). Therefore, it is possible to identify which volatile components are responsible for a unique flavour of a particular honey and use them to estimate the botanical source. For example, methyl anthranilate is typical of honey from citrus varieties and linden ether is typical of linden honey (Molan, 1998; Belitz et al., 2009). However, some limitations of using single unique compounds as markers of floral sources should be considered if there are doubts about their uniqueness, because not all botanic species have been analysed; if a large natural variance among these markers is reported by different authors; or if a "pure" monofloral honey is not usually available, even if one species significantly dominates (Molan, 1998).

Improper manufacturing practice and adulteration also affect volatiles. Storage excessively long or at a temperature above 30°C and an intentionally added sweetener cause the concentration of most volatile substances to decline and the concentration of sugar degradation products (e.g. maltol, 5-(hydroxymethyl)-2-furaldehyde and furan-2-carbaldehyde) to increase (Castro-Vázquez et al., 2012; Agila & Barringer, 2013). The combination of volatiles fingerprints and the chemometric methods seems to be more potential than the use of single markers in majority of the above mentioned cases.

Market surveys show that enriching a sensorially unsatisfactory honey (or even honey adulterated by sugar syrup) with an artificial honey aroma is one example of consumer deception. Characteristic artificial aroma-forming compounds are most often esters of phenylacetic acid (methyl phenylacetate and ethyl phenylacetate) and 2-phenylethanol (Rowe, 2005). Other fragrances contained in artificial honey aromas include allyl phenylacetate, pentyl phenylacetate and benzyl phenylacetate. Sometimes bee wax extract or natural essential oil from honey can be added as well (Belitz et al., 2009).

The aim of this work was to evaluate the quality of honey of different categories based on the determination of volatiles profiles through SPME-GC/MS and to identify suspicious samples. According to specific fingerprints and sensory analysis, we differentiated between standard quality samples (e.g. those which fulfil legislative requirements) and samples adulterated by unsuitable storage, prolonged heating, dilution with exogenous substances or artificial aroma additives.

MATERIAL AND METHODS

Honey and artificial honey aroma samples

Honey samples (n = 76; Honey 01–76) were analysed. The samples were separated into two sets: 1) authentic honeys (n = 13; honey of known origin and history derived from verified Czech local beekeepers, harvested in 2011 and 2012); 2) commercially available honeys (n = 63; purchased at the market, unknown quality and unknown harvest year, originating from the Czech Republic and countries outside the European Union, as was declared on the labels). Categories of the honey samples were as follows: 1) authentic honeys: blossom honeys (n = 6), honeydew honeys (n = 5) and blend honeys

(n = 2); 2) commercially available honeys: blossom honeys (n = 32), honeydew honeys (n = 12) and blend honeys (n = 19). The samples were kept at approx. 20°C until analysed.

Simultaneously, 22 samples of commercially available artificial honey aromas (Aroma 01–22) from various manufacturers were analysed. The artificial aroma samples differed in aroma solvents/carriers (e.g. ethanol with propylene glycol, ethanol with water, and propylene glycol with water) and their recommended dosage. All samples were stored in a refrigerator (at approx. 5°C) until they were analysed.

Reagents

Sodium chloride (p.a.) and sodium hydroxide (p.a.) were purchased from Lach-Ner (Neratovice, Czech Republic). Methanol (p.a.) and a 5-(hydroxymethyl)-2-furaldehyde (5-HMF) standard (99%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). 1,2,3-propantriyl triacetate (triacetin) (min. 99%) and ethanol (min. 96%) standards were purchased from P-Lab (Prague, Czech Republic) and Penta (Prague, Czech Republic), respectively. Phadebas honey diastase tablets were purchased from Magle Life Science (Lund, Sweden); sodium acetate trihydrate (p.a.) from Lachema (Neratovice, Czech Republic) and acetic acid (100%) from Merck (Darmstadt, Germany).

Volatile compounds analysis by SPME-GC/MS

Sample preparation

A sample of honey (1 g) was weighed into a 10-mL vial with polytetrafluoroethylene (PTFE)/silicone septa, and then 1 mL of NaCl solution (200 g/L of distilled water) was added (Alissandrakis et al., 2007). A sample of artificial honey aroma was prepared in the following way: 1.7 mL of a honey aroma solution (20 µL/L in NaCl solution mentioned above) was put into a vial. Volatile compounds were tested three times for each sample and mean values were reported.

Isolation of volatile compounds by solid phase microextraction (SPME)

A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre was used to extract headspace volatiles from the samples. First, a prepared sample was stirred (8.3 Hz) and conditioned at 60°C for 15 min. Then a SPME needle was introduced through the septum and the fibre was exposed in the vial headspace for 30 min; a temperature of 60°C was maintained during the headspace sampling. Volatile compounds adsorbed on the SPME fibre were immediately thermally desorbed in the injection port of GC for 4 min at 240°C. The conditions were modified from those by Alissandrakis et al. (2007) and selected based on a achieved high yield of volatile substances, good repeatability and the possibility of results comparison with the literature (Cuevas-Glory et al., 2007).

Gas chromatography/mass spectrometry (GC/MS) instrumentation and conditions

The samples were analysed by GC-MS (7890A/5975C, Agilent Technologies, Santa Clara, California, USA) on a HP-5MS column (Agilent Technologies). Chromatographic conditions for GC-MS were a HP-5MS column (30 m×250 µm×0.25 µm) and a temperature programme, initially held at 60°C for 2 min and then ramped at 10°C/min to 290°C. The detector temperature: 230°C MS source and 150°C MS Quad; helium (4.8; purity 99.998%) was used as carrier gas at a flow rate of 1.4 mL/min. The conditions were modified from those by Alissandrakis et al. (2007). Profiles of volatile compounds were evaluated based on their relative representation. With regard to that approach of evaluation, the method was verified only in terms of reproducibility for ten major volatile compounds; reproducibility expressed as relative standard deviation (RSD) was less than 8% for all of them. Identification of the individual components was based on comparisons with NIST 11 Mass Spectral Library and Kovats Retention Indices.

Sensory analysis

Sensory evaluation of the honey samples was performed by a panel of ten highly trained assessors from the Department of Food Preservation (University of Chemistry and Technology,

Prague, Czech Republic) according to procedure described by Piana et al. (2004). The assessors were selected, trained and monitored according to ISO standard 8586-1 (1993). The performance was in agreement with international standard ISO 6658 (2005).
The panellists evaluated intensity (numeric category scale: intensity scaling; 5 = strong, 0 = weak), pleasantness of honey aroma (numeric category scale: hedonic scaling; 5 = like extremely, 0 = dislike extremely) and intensity of artificial honey aroma (numeric category scale: intensity scaling; 5 = strong, 0 = weak). Total score was calculated as a sum of the individual panelists' results. Relative standard deviation of their results was on average 25%. Aroma was evaluated immediately after the samples were prepared.

Physicochemical analysis
The physicochemical standard quality parameters determined for selected honey samples with abnormal volatiles and sensory profiles were 5-HMF content, diastase activity, ethanol and triacetin content. 5-HMF content was determined using the method of Bogdanov et al. (1997), with these changes: 5 g of a sample weighed into a 100-mL volumetric flask and the volume adjusted with a mobile phase. Diastase activity, expressed as a diastase number, was assessed spectrophotometrically using Phadebas honey diastase tablets (Magle AB, Lund, Sweden) precisely following the Phadebas Honey Diastase Test (Magle AB) Instructions for use (Phadebas®, 2010).
Ethanol content was analysed with SPME-GC/FID (gas chromatography with flame ionization detector; 6890N, Agilent Technologies); the conditions were modified from those by Grégrová et al. (2012). SPME conditions were the same as the conditions for the honey volatiles isolation mentioned above. The initial temperature for GC/FID conditions was held at 40°C for 5 min, then ramped at 10°C/min to 260°C and held at 260°C for 6 min. The detector temperature was 300°C, and nitrogen (4.0; purity 99.990%) was used as the carrier gas at a flow rate of 1.7 mL/min. Quantification was performed by an external calibration curve, and the concentration ranged from 1.0 mg/kg to 2 000 mg/kg.
Triacetin content was determined by SPME-GC/MS with the same method as the one for the honey volatiles analysis. SPME-GC/MS was followed by SPME-GC/FID quantification and performed in the same way as during the ethanol analysis mentioned above using an external calibration curve. The concentration ranged from 0.5 mg/kg to 10.0 mg/kg. All physicochemical parameters were tested three times for each sample and mean values are reported.

Statistical analysis
Factor analysis was used to explain similarities between individual honey samples. Statistical analyses were performed using the STATISTICA 10.0 (StatSoft, USA) statistics programme.

RESULTS

Determination of volatile substances
In total 48 volatile compounds were identified in honey samples (Tab. 1) in accordance with the literature (Pérez et al., 2002; Wolski et al., 2006). These were those whose peaks with relative representation were higher than 0.05% or whose representation of the volatile substances was highly variable. Most abundant in the authentic samples were benzaldehyde, 2-phenylethanol, hotrienol and 2-phenylacetaldehyde which, according to literature, contribute significantly to either the general honey aroma or the unique scent of certain botanical species (Wardencki et al., 2009; Pino, 2012; Seisonen et al., 2015). A representation of 2-phenylacetaldehyde content was comparable with its content in analysed European honeys (average representation 5.7%; Alissandrakis et al., 2007). Wolski et al. (2006) reported analyses of a set of honeys originating from Poland with relative representations of selected volatile compounds for benzaldehyde 8.1%, linalool oxide 0.5%, furan-2-carbaldehyde 4.0% and 2-phenylethanol 2.7%. The honey samples' content differed mostly in furan-2-carbaldehyde, 1,4-dimethylpyrazole, benzaldehyde and 2-phenylacetal-

Table 1

Volatile substances identified in investigated authentic honey samples (n=13), representation expressed as a percentage of total chromatogram area

Peak no.	RT[a]	Compound[b]	Characteristic fragment ions, m/z	KI[c]	Min[d]	Max[d]	Average[d]	Detection frequency[e]
1	1.36	Dimethyl sulfide	62, 61, 47	584	ND	5.0	1.7	92
2	1.69	3-Methylbutanal	58, 44, 43	610	ND	2.4	0.5	54
3	2.06	2-Methyl-butanenitrile	55, 54, 29	638	ND	1.4	0.5	54
4	2.13	3-Methyl-butanenitrile	43, 41, 27	643	1.0	8.8	4.6	100
5	2.27	Dimethyl disulfide	94, 79, 45	653	ND	2.5	0.3	23
6	2.43	(3E)-3-(2-propenylidene)-1-cyclobutene	92, 91, 65	666	0.1	3.0	1.1	100
7	2.75	Octane	57, 43, 41	691	0.3	5.4	2.2	100
8	3.18	Furan-2-carbaldehyde	96, 95, 39	822	0.5	5.1	1.5	100
9	3.25	1,4-Dimethyl-pyrazole	96, 95, 42	827	ND	29.4	5.6	77
10	4.29	Acetylfuran	110, 95, 39	902	ND	0.4	0.1	31
11	4.67	α-Pinene	93, 91, 77	925	ND	4.9	1.7	69
12	5.09	Benzaldehyde	106, 105, 77	951	6.1	32.3	10.8	100
13	5.74	Octanal	84, 56, 43	991	0.1	2.7	0.8	100
14	5.87	Unknown	152, 109, 41	999	ND	4.6	0.7	46
15	6.12	β-Cymene	134, 119, 91	1014	ND	2.0	0.2	15
16	6.18	D-Limonene	93, 68, 67	1018	ND	14.7	1.5	62
17	6.25	Benzyl alcohol	108, 107, 79	1022	0.5	7.8	4.2	100
18	6.43	2-Phenylacetaldehyde	120, 91, 65	1033	0.7	40.3	5.5	100
19	6.67	γ-Terpinene	136, 93, 91	1048	ND	1.1	0.3	46
20	6.89	Linalool oxide	11, 94, 59	1062	0.8	10.6	3.0	100
21	7.16	p-Cymenene	132, 117, 115	1078	0.6	8.0	2.8	100
22	7.30	β-Linalool	121, 93, 71	1086	ND	14.3	4.3	92
23	7.37	Hotrienol	82, 71, 67	1090	2.7	18.9	6.1	100
24	7.54	2-Phenylethanol	122, 92, 91	1101	1.6	24.8	6.6	100
25	7.68	Methyl octanoate	127, 87, 74	1110	0.8	2.6	1.6	100
26	7.95	Benzyl nitrile	117, 116, 90	1127	ND	10.4	3.0	92
27	8.01	Lilac aldehyde isomer	111, 93, 55	1132	ND	4.4	1.4	54
28	8.12	Lilac aldehyde isomer	111, 93, 55	1139	0.3	13.4	4.0	100
29	8.35	Lilac aldehyde isomer	111, 93, 55	1153	ND	11.5	3.2	92
30	8.38	Isoborneol	110, 95, 41	1155	ND	7.5	2.9	69
31	8.46	(S)-cis-Verbenol	109, 94, 79	1160	ND	2.4	0.4	46
32	8.53	Methyl phenylacetate	150, 91, 65	1165	0.2	3.3	1.2	100
33	8.74	Terpinen-4-ol	111, 93, 71	1178	ND	8.3	1.5	92
34	8.86	Safranal	121, 107, 91	1186	ND	8.5	1.2	69
35	8.92	Decanal	70, 57, 43	1189	ND	2.9	1.0	92
36	8.97	Unknown	148, 119, 91	1193	ND	4.9	0.5	38
37	9.20	Eucarvone	150, 107, 91	1208	0.2	5.7	3.0	100
38	9.32	Unknown	126, 111, 55	1217	ND	1.2	0.2	38
39	9.45	1-(2-butoxy-1-methyle-thoxy)-2-propanol	103, 59, 57	1225	ND	2.5	1.0	92
40	9.52	Ethyl phenylacetate	164, 91, 65	1230	ND	5.2	1.8	92
41	9.65	(E)-Chrysanthenylacetate	134, 119, 91	1239	ND	1.2	0.2	31
42	9.76	Nonanoic acid	73, 60, 57	1247	0.4	2.1	1.4	100
43	10.15	Unknown	121, 107, 79	1273	ND	1.5	0.2	23
44	10.31	Isothymol	150, 135, 91	1284	ND	1.8	0.2	31
45	10.51	3,5-Diethylphenol	150, 135, 121	1298	0.1	2.7	1.3	100
46	11.09	Eugenol	164, 149, 131	1340	ND	2.9	0.7	85
47	11.49	β-Damascenone	190, 121, 69	1368	ND	4.6	1.3	85
48	12.55	Unknown	123, 83, 55	1451	ND	0.8	0.3	69

[a] Retention time (min); [b] Compounds identified using NIST 11 Mass Spectral Library; [c] Kovats retention index calculated on a HP-5MS column; [d] % of total chromatogram area; [e] occurrence findings in % of 13 authentic honey samples; ND – not detected

dehyde. However, other volatile compounds with a low threshold of perception, which are usually present in traces in honeys, were noted to contribute to the overall sensory perception of honey.

Esters of phenylacetic acid were determined as dominant components of 16 out of 22 samples of artificial honey aromas. Specifically, methyl and ethyl esters of this acid were found; other compounds occurred in minor amounts in the artificial aromas (Tab. 2). Volatiles profiles of the honey aromas were always less intense than those for the honey samples. The majority of the samples (14 out of 22) contained seven or less volatile components and vice versa three artificial honey aromas contained from 13 to 16 components. Unfortunately, most of the substances identified in the artificial aromas were also found in the samples of authentic honey (Tab. 1), which has negative implications for the possibility of using volatile profiles to detect added honey aromas. Therefore, there was a problem to identify specific compounds which could serve as reliable markers of aroma additives. Honey aroma addition can be detected only for extremely adulterated honey samples or in the cases of excessive added aroma (predominating representation of the main volatile compounds in honey aromas – Tab. 2, or aroma solvents/carriers, e.g. ethanol and triacetin). Furthermore, volatiles profiles of commercial artificial honey aromas originating from different manufacturers were quantitatively different from each other in which the total area of volatiles varied more than 50 times.

Sensory analysis

An important aspect of aroma research is exploration of relationships between sensory and instrumental data. For most of honey samples s it was possible to conclude that the more volatile compounds they contained, the better they were assessed in the sensory evaluation. Only the samples with a high content of furan-2-carbaldehyde, methyl phenylacetate and certain samples containing triacetin proved to be an exception to this rule. The results of the sensory evaluation of honey aroma intensity and intensity of some artificial honey aroma and the pleasantness evaluation of honey aroma of the samples are summarised in Fig. 1. Only five samples were classified as having significant (slight-moderate) intensity of artificial honey aroma, rated as unnatural and candy-like; these samples also showed moderate intensity of overall honey aroma but low pleasantness.

DISCUSSION

The results of the sensory evaluation of 76 samples of honey were correlated with one another and with the total volatiles content

Table 2

Representation of the main volatile compounds in honey aromas

	Representation (%)		
Compound	Min	Max	Median
2-Phenylethanol	0.1	3.2	0.6
Methyl phenylacetate	0.2	98.3	36.9
Ethyl phenylacetate	0.1	96.4	65.1
Nonanoic acid	ND	0.4	0.1
Anethole	ND	8.4	1.6
Phenylallyl alcohol	ND	5.3	1.3
Sum of other minor compounds	0.9	17.5	3.6

representation expressed as a percentage of total chromatogram area; ND – not detected

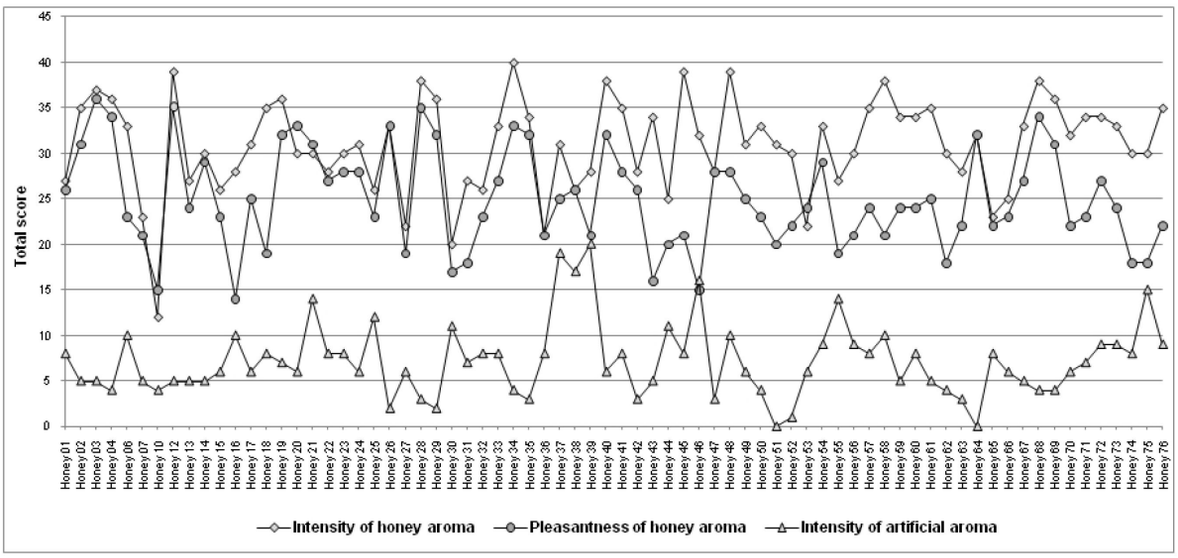

Fig. 1. Sensory analysis of the honey samples.

(total area of 48 identified compounds). Generally this approach is used for the identification of monofloral honey authenticity, but the atypical sensory flavour profile can for example also serve as an indicator of thyme honey adulteration by thyme essential oil (Mannaş & Altuğ, 2007). A statistically significant correlation (α = 0.05) was demonstrated only between honey aroma intensity and pleasantness of honey aroma (Pearson correlation coefficient r = 0.5; critical value for correlation coefficient 0.2; p < 0.001), and pleasantness of honey aroma and intensity of artificial aroma (r = –0.3; critical value for correlation coefficient 0.2; p = 0.003).

The volatile substance profiles (% relative representation of 48 identified compounds) of all honey samples were processed by a factor analysis (FA), which allowed the evaluation of internal data structures and revealed good separation between honeys of different botanical origin in the study by Papotti et al. (2012). Using a Cattell scree test plot (Scree Plot), the first two main factors were deemed significant. The first and the second factor described 68.2% and 12.1% of the data variability (variance), respectively; the first two factors described 80.3% of the data variability. The factor weight figure for the individual compounds using a Varimax was used to explain the correlations between the factors and the original characters. In the first factor, the largest weights were described for 1-(2-butoxy-1-methylethoxy)-2-propanol (retention time t_R = 9.45 min), terpinen-4-ol (t_R = 8.74 min), isoborneol (t_R = 8.38 min) and ethyl phenylacetate (t_R = 9.52 min), while in the second factor the above-mentioned characters had the smallest weights. In the second factor, 3-methyl-butanenitrile (t_R = 2.13 min), lilac aldehyde (t_R = 8.12 min), benzaldehyde (t_R = 5.09 min), and benzyl nitrile (t_R = 7.95 min) had the largest weight.

The factor scores of the individual objects in a scatter diagram after the Varimax rotation are shown in Fig. 2. This diagram shows the position of the objects (H = honey) in the factor space. Except for slightly distant objects H61, H6, H21, H36, H63, H18, H2, H70, and H71, the objects were placed in a single cluster. Objects H62, H19, and H74 were considered as completely outlying and excluded from the main cluster regardless of their category (blossom, honeydew or blend honeys).

Honey 62 (H62) expressed primarily by the value of the first factor was outlying due to its high content of ethyl phenylacetate (6 times higher than the average value) and isoborneol (5 times higher than the average value). Ethyl phenylacetate is a typical compound occurring in artificial honey aromas (Tab. 2) but also in dandelion honey (Piasenzotto et al., 2003), and isoborneol is a significant volatile compound present in eucalyptus honey (Castro-Vázquez et al., 2012). Honey 61 and Honey 6 (H61 and H6) were characterized by contents of the same compounds (3 times higher than the average

Table 3

Characteristic indicators present (yes/no) in suspect samples

Sample	Volatile compounds atypical profile	High furan-2-carbaldehyde content	Triacetin presence	Outlier samples according to volatiles profiles[a]	Low intensity of honey aroma[b]	Low pleasantness of honey aroma[b]	High intensity of artificial aroma[b]
Honey 21	No	No	Yes	Yes	No	No	Yes
Honey 27	Yes	Yes	No	No	Yes	No	No
Honey 31	Yes	Yes	Yes	No	No	Yes	No
Honey 36	Yes	No	No	Yes	Yes	No	No
Honey 39	Yes	Yes	No	No	No	No	Yes
Honey 62	Yes	No	Yes	Yes	No	Yes	No

[a] determined by statistical evaluation
[b] using sensory analysis

Table 4

Standard quality characteristics of suspect samples

Sample	Country of origin	Honey category	5-HMF (mg/kg)	Diastase activity (DN)	Ethanol (mg/kg)	Triacetin (mg/kg)
Honey 21	CZ	blossom linden	19.7	10.1	5.3	<LOQ
Honey 27	EU, non-EU	baker´s	404.1	<0.9	193.3	ND
Honey 31	EU, non-EU	blossom	247.1	1.7	25.4	1.2
Honey 36	EU, non-EU	blossom linden	50.3	1.7	12.5	ND
Honey 39	EU, non-EU	blossom acacia	52.3	10.5	4.5	ND
Honey 62	CZ	blend	5.1	15.6	1 364.7	1.2

DN – diastase activity is expressed by diastase number; LOQ – limit of quantification; ND – not detected

value).

Conversely significantly outlying Honey 19 and Honey 74 samples (H19 and H74) were mostly specified by the second factor value. Honey 19 (H19) differed from the other samples in benzyl nitrile content (7 times higher value than for the other samples). A similar trend was found for sample H74 whose its benzyl nitrile content was found to be approximately five times higher than the average value.

Honey 21 and 36 (H21 a H36) were suspected of aroma addition. Honey 21 had an atypical volatiles profile with triacetin present and it was later sensorially evaluated as a sample with high intensity of artificial aroma. Honey 36 had a completely atypical volatiles profile with a relatively high content of methyl phenylacetate (5.8%) and p-cymenene (21.3%), which has been found to be not typical for any described honeys (Serra Bonvehí & Ventura Coll, 2003; Wolski et al., 2006; Alissandrakis et al., 2007). Conversely it had low benzaldehyde content.

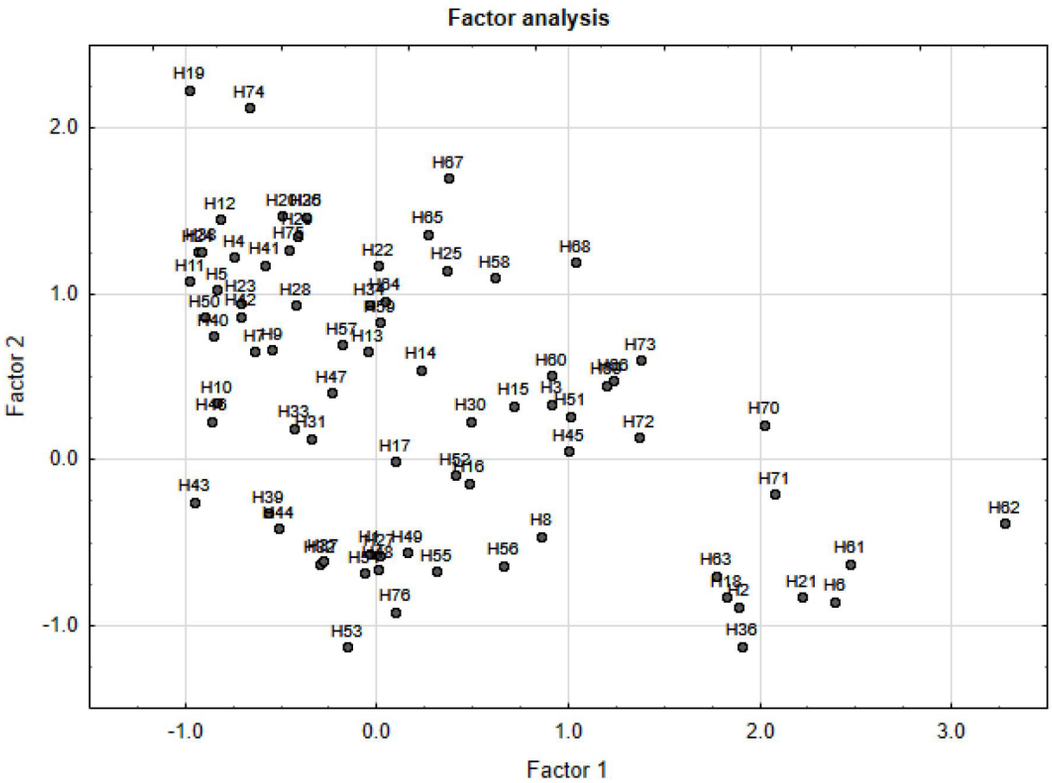

Fig. 2. Variability of the honeys evaluated by FA with a Varimax rotation (scatter diagram of factor scores for the first two factors; H = honey; honey samples specified by all 48 volatile compounds).

Based on selected volatiles and their representation (general profile, absence of triacetin) in the authentic honey samples and the sensory analysis results (Tab. 3), six honey samples were identified to not meet three or more of the characteristic quality indicators, therefore, suspected of dilution, heating, improper storage or aroma addition. To prove this statement these honey samples were subjected to targeted analyses of the selected quality parameters (Tab. 4). In four of the samples, an increased content of 5-HMF (max. limit of 5-HMF is 40 mg/kg for honey and 80 mg/kg for tropical honey) was found (Council Directive, 2001), which pointed to heating or improper long-term storage of the samples. Diastase activity was also analysed as an indicator of heating and/or improper storage and expressed as diastase number ranged from <0.9 to 1.7 in three (i.e. 50%) of these samples (limit min. 8, with exceptions not relevant for the analysed samples) (Council Directive, 2001). Higher ethanol content indicated fermentation or an unauthorized additive, and its contents ranged from 4.5 mg/kg to 1 364.7 mg/kg. Triacetin, triester of glycerol and acetic acid, commonly used food additives as solvents in aromas, were identified in three of the six honey samples, indicating unauthorized added aroma addition.

By the measurement of volatiles profiles by SPME-GC/MS and the sensory analysis, we were able to identify suspicious samples and prove the proposed methodology to be a useful tool for fast quality evaluation of honey. This was confirmed through the standard quality characteristics of 5-HMF content, diastase activity, flavour solvents/carriers content, when all outliers were found not to comply with one or more of the selected quality requirements. Unfortunately, since most substances identified in the artificial aromas were also found in the authentic honeys, the possibility to use volatiles profiles is limited to extremely adulterated honey samples or for the detection of excessive aroma addition.

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