Physicochemical parameters and botanical origin of Czech honeys

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Summary

In this study, 7 physicochemical parameters of quality and botanical origin of 37 honeys harvested in 2003 in Czech Republic were analysed and evaluated. The parameters were as follows: electrical conductivity 9.8–127.3 $mS.m^{-1}$; water contents 15.0–20.0 %; water activity 0.485–0.607; pH value 3.64–4.95; hydroxymethylfurfural contents 0.00–15.51 $mg.kg^{-1}$; invertase activity 6.8–238.1 $U.kg^{-1}$; diastase activity 9.8–40.9 degrees on the Schade scale. Microscopic analysis of pollen grains revealed that the main part of the honeys originated from rape, lime and acacia. Dependence of the electrical conductivity on the botanical origin was observed, when rape and acacia honeys displayed the lowest electrical conductivity. The highest electrical conductivity of all blossom honeys was found in lime tree honeys. The four monitored physicochemical parameters correlated with electrical conductivity of the honey with a statistical significance (invertase activity – $p < 0.0001$; pH – $p < 0.0001$; diastase activity – $p < 0.001$; water activity – $p < 0.05$). No significant dependence was observed between the electrical conductivity and water contents or hydroxymethylfurfural contents.

Keywords

honey; quality; physicochemical parameters; botanical origin

Honey is an easily digestible, energy-rich food that contains a lot of nutritionally valuable components. Honey types (blossom and honeydew) differ significantly as to the sensory as well as chemical composition, that is reflected by its physical and chemical parameters. At the same time, many of them provide information on how the honey was handled and on possible procedures that can significantly reduce its dietary value. Several of the physicochemical parameters of quality are covered by legislative limits on the national level (Decree No.76/2003 Coll. [1], as amended in later regulations) that matches international legislation (Council Directive 2001/110/EC [2]). Some of the physicochemical parameters of honey are significantly influenced by its botanical origin.

A prevailing part of the honeys originating in the Czech Republic have another significant feature apart from a high nutritional value, namely, the antibacterial activity. Thanks to the capacity to inhibit the growth of various bacterial strains including pathogenic ones, honey can be involved in the therapy of various gastrointestinal infections but also skin injuries, bedsores or burn injuries. Antibacterial activity is not identical in all honeys. From the tested Czech honeys, the highest antibacterial activity was displayed by honeydew ones [3]. An important prerequisite for the antibacterial activity of honey is its high quality. Some practices of handling honey, e.g. heating, reduce its quality in terms of the reduction of its antibacterial. Many honeys produced by Czech honey bee keepers are of a high quality, which is evidenced by physicochemical parameters, hydroxymethylfurfural (HMF) contents, diastase and invertase enzyme activities, water contents etc. We monitored these in a series of studies in the past [3-7].

The goals of this study were to identify the origin of honeys on the honey market in the Czech Republic, to compare their physicochemical parameters as quality or safety markers, and to investigate possible relations of these parameters with the botanical origin of honeys.

MATERIAL AND METHODS

Material

A total of 37 honeys obtained from Czech beekeepers from the harvest of 2003 were analysed. The majority of the samples originated in the South Moravia region (23 honeys) but there

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were also some samples from the North Moravia (9 honeys) and East (3 honeys) and Central Bohemia (2 honeys; Table 1). Honey samples were accompanied with a record containing the date of extraction, storage conditions, location, possible treatment status of the bees and other information. The volume of the collected samples ranged from 900 g to 1 000 g. The origin of the samples declared by the honey bee keepers is presented in Table 1. Some honey bee keepers omitted the declaration of the botanical origin of the honey, presenting only the date of the harvest when the honey was collected. Based on this information and on the knowledge on the blossoming periods of individual nectar sources, it was possible to estimate the honey origin. Analyses were carried out within 5 months after the extraction. Honey was stored in well-sealed glass containers away from sunlight at temperatures of 20 ± 5 °C between the extraction and the analysis.

Methods

Six out of the seven monitored physicochemical parameters were established based on the methods described in Harmonised methods of the European Honey Commission [8]:

Water contents in honey were determined using the refractometric method on the Abbé refractometer (AR 2, A-Krüss Optronic, Hamburg, Germany).

Tab. 1. Origins of the analysed honey samples as declared by the honey bee keepers.

Sample No.	Origin of the honeys	Region
1	meadow	North Moravia
2	spring	North Moravia
3	acacia	North Moravia
4	acacia	South Moravia
5	acacia	South Moravia
6	acacia, rape, lime	Central Bohemia
7	acacia	South Moravia
8	raspberry	South Moravia
9	lime, rape	South Moravia
10	from the harvest until June 23, 2003	East Bohemia
11	fruit trees, acacia	South Moravia
12	phacelia, acacia, lime	South Moravia
13	floral	Central Bohemia
14	sunflower	South Moravia
15	from the harvest between June 13 and August 4, 2003	South Moravia
16	acacia, fruit trees, dandelion	South Moravia
17	fruit trees, rape, acacia	South Moravia
18	sunflower	South Moravia
19	sunflower	South Moravia
20	lime, phacelia, acacia	North Moravia
21	lime, raspberry	South Moravia
22	sunflower	South Moravia
23	clover, fruit trees, sunflower	South Moravia
24	lime	North Moravia
25	from the harvest of July 2003	North Moravia
26	lime	South Moravia
27	sunflower	South Moravia
28	from the harvest until June 7, 2003	North Moravia
29	lime	South Moravia
30	from the harvest until June 7, 2003	North Moravia
31	lime, fruit trees, meadow	North Moravia
32	from the harvest between June 3 and July 29, 2003	South Moravia
33	acacia, lime	South Moravia
34	forest	East Bohemia
35	honeydew	South Moravia
36	from the harvest of June 2003	East Bohemia
37	from the harvest between June 25 and August 6, 2003	South Moravia

Electrical conductivity was determined using the conductometric method on an inoLab Cond Level 2 conductometer (WTW, Weilheim, Germany).

Hydroxymethylfurfural contents in honey were determined using the HPLC method on a liquid chromatograph Alliance 2695 with a PDA detector 2996 (Waters, Milford, Massachusetts, USA) and Zorbax Eclipse XDB-C18 column, 4.6 × 150 mm, 5 μm (Agilent, Santa Clara, California, USA). Gradient elution was applied with water - methanol (90 : 10) mobile phase used for first 6 min and then the ratio gradually changed down to 50 : 50 until 11 min when all sample components were eluted. HMF was used as external standard.

Diastase activity in honey was determined based on the Schade method using a single-ray UV-VIS spectrophotometer Lambda 11 (Perkin Elmer, Norwalk, Connecticut, USA).

Invertase activity in honey was determined using the same instrument.

The pH value of honey was established using a combination of the ROSS electrode and an Orion pH-meter (model 250 A; Orion Research, Beverly, Massachusetts, USA).

Water activity was determined using an a_W-meter (Thermoconstanter TH 200, Novasina, Pfäffikon, Switzerland).

Botanical origin of honeys was determined by diluting 10 g of honey in 20 ml of distilled water and then applying centrifugation 4 times. The centrifugal sediment was transferred onto a clean slide. After drying (at the temperature of 35 °C), the sediment was covered with a glycerol-gelatin drop and a cover slide around which lacquer was applied (ex. Celox). Two samples were always prepared. Using a microscope (objective 20–45×, ocular 10×), pollen grains from various spots of the sample were monitored and were classified to plant species or species group. Presence of microflora (fungi, algae) was monitored as well.

Statistical evaluation based on the correlations between electrical conductivity and other physicochemical parameters was carried out using Spearman rank correlation coefficients in the statistical package Unistat 5.1. (Unistat, London, United Kingdom).

RESULTS AND DISCUSSION

With regard to the practice of honey production, it is almost impossible to obtain a monofloral honey in Czech Republic. For this reason, determination of the botanical origin of honey based on the predominance of pollen grains presence was applied. In Tab. 2, the dominant pollen grains and other honey sediment particles as well as the declared botanical origin of the analysed honeys are presented. It is clear from this table that in 2003 in the areas of the Czech Republic from which the honeys came (South and North Moravia, East and Central Bohemia), the main part of the harvest was formed from rape (*Brassica*), lime (*Tilia*) and acacia (*Robinia*). To a lesser extent, was formed from sunflower (*Helianthus*) and fruit trees (*Pomum*) and, in some honeys, there was a significant portion of phacelia (*Phacelia*), dandelion (*Taraxacum*), raspberry (*Rubus*) and clover (*Trifolium*). Woods debris which is a designation for typical microscopic image of honeydew honey containing fungal hyphae, algal cells, dust particles and various trichomes, was found only in three samples, which indicates a high predominance of blossom honey within the 2003 harvest.

When comparing the origin of the analysed honey samples in Tables 1 and 2, one can state that Czech honey bee keepers are able to follow the botanical origin of their honeys because the botanical origins declared by them (Table 1) and those determined in the laboratory (Table 2) match in a majority of honeys (in some cases, at least one nectar source matches).

Electrical conductivity and botanical origin of honeys

In Tab. 2, the analysed honey samples are sorted according to the declared botanical origin and in these groups, the samples are sorted in an ascending order according to their electrical conductivity levels. This means that the lowest electrical conductivity is to be found in honeys formed mostly from rape or acacia. As the fruit tree, phacelia, dandelion or sunflower pollen grain counts increase, the electrical conductivity of the honey gradually grows. It is clear from Tab. 2 that the highest electrical conductivity of all blossom honeys is found in honeys containing mostly lime tree pollen. Honeys that, judging by their electrical conductivity, belonged to the honeydew group, were also confirmed to be honeydew by identifying the woods debris content. An exception is honey with the highest electrical conductivity that should thus be honeydew but woods debris was not identified in it and, moreover, it contained pollen grains of plants that are contained in honeys with a much lower electrical conductivity. This sample confirms the conclusions made by Přidal and Vorlová [5] who reported that when sorting honeys by their origin to blossom and honeydew, it is necessary to pay attention, apart from electrical conductivity, to optical rotation and pollen analysis. These authors

Tab. 2. Characteristics of the analysed honeys.

Sample No.	Conductivity [mS.m-1]	HMF [mg.kg-1]	Water content [%]	Water activity	pH	Invertase activity [U.kg-1]	Diastase activity [degrees on Schade scale]	Dominant pollen grains and other particles	Declared botanical origin
1	9.8	4.66	16.4	0.528	4.09	6.8	10.1	rape	nectar
2	10.3	2.97	16.8	0.592	3.96	19.4	12.0	rape	nectar
15	24.0	1.09	16.6	0.538	3.77	151.4	32.4	rape, clover	nectar
4	12.6	0.73	18.6	0.607	4.12	48.6	9.8	acacia	nectar
5	13.2	0.00	16.2	0.553	3.82	74.3	15.7	acacia	nectar
18	30.5	2.25	16.9	0.504	3.82	110.3	21.7	sunflower	nectar
23	36.9	3.43	15.2	0.552	3.78	125.5	22.0	sunflower, rape	nectar
22	32.5	0.00	18.3	0.563	3.87	169.7	31.2	lime, sunflower	nectar
25	38.5	3.04	19.0	0.572	4.00	180.1	36.9	lime, rape	nectar
26	40.2	1.90	16.4	0.556	4.15	151.7	30.1	lime, rape	nectar
33	73.0	0.00	15.5	0.558	4.77	104.4	25.8	lime, acacia	nectar
34	82.3	0.80	15.3	0.570	4.30	163.8	29.7	woods debris,fruit trees,rape	honeydew
35	119.0	2.75	17.6	0.564	4.20	238.1	40.9	woods debris,sunflower,raspberry	honeydew
36	120.6	1.05	17.3	0.587	4.85	130.6	28.6	rape,raspberry,woods debris	honeydew
3	11.9	4.88	15.5	0.554	3.79	9.8	13.0	rape, acacia	polyfloral
6	13.4	1.13	15.8	0.540	3.91	109.0	20.3	rape, lime	polyfloral
7	16.4	4.04	15.8	0.512	3.97	73.3	18.5	acacia, phacelia	polyfloral
8	16.5	1.97	19.7	0.551	3.82	87.6	25.3	rape, raspberry	polyfloral
9	18.0	2.61	15.5	0.520	3.80	134.9	23.7	lime, rape, fruit trees	polyfloral
10	18.4	15.51	18.0	0.549	3.90	10.5	12.7	rape, fruit trees	polyfloral
11	19.3	2.59	20.0	0.542	3.97	120.1	20.4	fruit trees, acacia, rape	polyfloral
12	20.7	2.79	18.0	0.485	3.64	113.4	37.5	phacelia, acacia	polyfloral
13	23.2	2.77	19.2	0.569	3.77	158.7	31.9	rape, lime, phacelia	polyfloral
14	23.8	0.00	16.1	0.547	3.75	47.7	27.3	sunflower, phacelia, acacia	polyfloral
16	24.9	3.46	15.4	0.544	4.24	94.6	22.2	fruit trees, acacia, dandelion	polyfloral
17	28.9	1.15	16.7	0.556	3.96	132.3	27.3	fruit trees, acacia, dandelion	polyfloral
19	30.9	1.94	17.7	0.511	3.92	105.4	24.2	sunflower, rape, lime	polyfloral
20	31.0	3.18	15.5	0.558	3.95	139.9	32.8	various	polyfloral
21	32.4	1.87	16.8	0.560	4.29	135.6	25.5	rape, lime, raspberry	polyfloral
24	37.2	0.00	17.4	0.604	4.03	152.7	29.9	various	polyfloral
27	40.3	3.05	15.0	0.552	3.94	141.3	33.2	sunflower, rape	polyfloral
28	47.8	1.72	16.4	0.549	4.26	162.1	25.3	fruit trees, acacia, dandelion	polyfloral
29	53.0	1.46	19.0	0.595	4.47	83.5	21.5	lime, various	polyfloral
30	61.6	0.96	15.8	0.565	4.78	101.9	19.5	fruit trees, dandelion, lime	polyfloral
31	65.7	1.20	15.5	0.589	4.61	112.6	21.7	fruit trees, lime, dandelion	polyfloral
32	69.6	2.72	16.5	0.534	4.16	223.6	39.2	lime, rape	polyfloral
37	127.3	4.25	18.1	0.555	4.95	165.1	25.8	acacia, rape, fruit trees	polyfloral
Spearman rank correlation coefficients towards conductivity:		–0.2255	–0.0804	0.3600	0.6407	0.6501	0.5581		
Statistical significance		statistically insignificant	statistically insignificant	$p < 0.05$	$p < 0.0001$	$p < 0.0001$	$p < 0.001$		

reported that when they classified honeys according to electrical conductivity only, some of the analysed samples would be included in a wrong group.

The lowest electrical conductivity in honeys originating mostly from rape or acacia correspond with the results published by DEVILLERS et al. [9] where rape (20.3 ± 4.4 mS.m^{-1}) and acacia honeys (19.5 ± 4.0 mS.m^{-1}) had the lowest electrical conductivity of all analysed French honey types. In this paper, the sunflower honey group had an electrical conductivity by ten units higher on average (30.6 ± 5.7 mS.m^{-1}) as compared to rape and acacia honeys. This also corresponds with our results. For acacia honeys, GOLOB and PLESTENJAK [10] reported a similar electrical conductivity of 23.5 ± 3.5 mS.m^{-1} , which was the lowest electrical conductivity of all Slovene honeys analysed in the study. Regarding other results of this study, electrical conductivity of lime tree honeys from 70.1 mS.m^{-1} to 91.5 mS.m^{-1} corresponds with our results with respect to the fact that the analysed honeys with a predominance of lime pollen grains occupied the highest electrical conductivity levels of all blossom honeys.

Enzyme activity, pH value and electrical conductivity

It is clear from Tab. 2 that invertase and diastase enzyme activities and pH value correlate at a statistical significance (invertase activity and pH very highly at $p < 0.0001$, diastase activity at $p < 0.001$) with electrical conductivity of honey. Values determined are presented in Fig. 1–3. All three of these physicochemical parameters increase with the increasing electrical conductivity honey. This is evident from the Spearman rank correlation coefficient in Tab. 2, which implies that the lowest values of pH and both enzyme activities are displayed by honeys that contain a majority of rape and acacia pollen grains. The values of these three physicochemical parameters gradually increase along with the increasing fruit tree, phacelia, dandelion or sunflower pollen grain counts, with lime tree and woods debris at the tail. These results correspond with data presented in literature stating that honeydew honeys have higher pH values than blossom honeys and invertase and diastase activities depend on the botanical origin of honey [9-14].

Diastase activity

Our results on diastase activity interrelation with the botanical origin of honey correspond with data presented in the paper by DEVILLERS et al. [9] where various French honeys were analysed. The authors found the diastase activities in acacia honeys lower than in other honey types. Diastase activities of rape honeys was comparable to those of sunflower honeys and higher than of acacia honeys. This is different from our results, which indicate that honeys with a prevalence of rape pollen grains belong to those with a rather low diastase activity that is comparable with diastase activities in honeys with acacia pollen grain predominance. PERSANO ODDO et al. [15] analysed 499 Italian honeys of various botanical origins, out of which honeys containing the blossom nectar of the

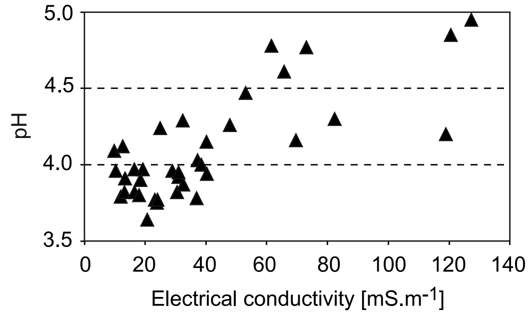

Fig. 1. The pH and electrical conductivity dependence.

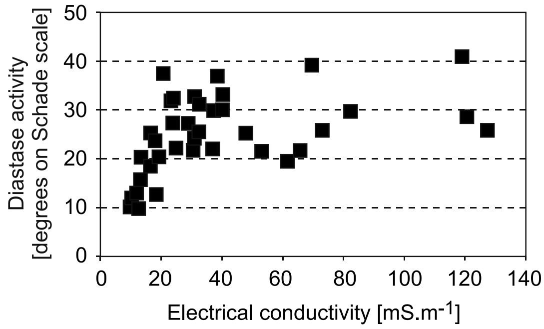

Fig. 2. The diastase activity and electrical conductivity dependence.

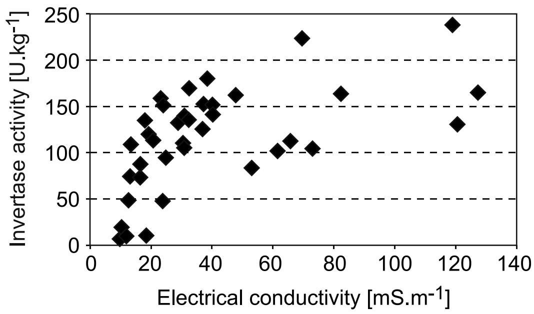

Fig. 3. The invertase activity and electrical conductivity dependence.

Robinia family can be compared with our results. These honeys had diastase activities in the range of 3.9–14.9 degrees on Schade scale that ranks among the lowest levels of all analysed honeys. These results are similar to our honeys containing mostly acacia pollen grains (9.8–18.5 degrees on Schade scale). In the lime tree honey group (the *Tilia* family), the authors determined diastase activities in the range of 12.0–27.2 degrees on Schade scale, which is by ten units lower than in our honeys with lime tree pollen grains predominance (21.5–39.2 degrees on Schade scale). Sunflower honeys (the *Helianthus* family) displayed diastase activities in the range of 12.0–23.0 degrees on Schade scale while diastase activities in our honeys with sunflower pollen grain predominance were by ten units higher on average (21.7–33.2 degrees on Schade scale). In honeydew honeys, the authors found diastase activities in the range of 15.0–49.3 degrees on Schade scale which harbours also the diastase activity of both our honeys containing a predominance of woods debris (29.7 and 40.9 degrees on Schade scale).

Results presented here on diastase activity are in a similar range as those of honeys originating in various parts of the world, with a certain influence of the botanical origin being remarkable. The correlation of diastase activity with botanical origin of honeys shows that the diastase activity does not sufficiently determine the degree of honey adulteration. It is always necessary to establish the HMF content and preferably also the botanical origin of honey.

Invertase activity

Our results on invertase activity interrelation with the botanical origin of honeys correspond with data of Horn and Hammes [16] who analysed 2 honeys of German origin, out of which one was blossom type with rape predominance displaying invertase activity at 116.4 $U.kg^{-1}$ and the other was a honeydew honey of coniferous trees displaying invertase activity at 172.7 $U.kg^{-1}$. Persano Oddo et al. [15] determined in Italian honeys invertase activities in the range of 0.4–30.6 degrees on Hadorn scale, which corresponds to approximately 2.9–224.6 $U.kg^{-1}$. Our results can be compared with honeys formed from the blossom nectar of the blossoms of the *Robinia* family plants. These honeys displayed invertase activities in the range of 2.9–56.5 $U.kg^{-1}$ and belonged to the lowest values of all analysed honeys, similarly to our honeys containing a majority of acacia pollen grains (48.6–74.3 $U.kg^{-1}$). In the lime tree honey group, they established invertase activities in the range of 49.2–145.3 $U.kg^{-1}$, which are values almost twice as low as in our honeys with lime tree pollen grain majority (83.5–223.6 $U.kg^{-1}$). Sunflower honeys displayed invertase activities in the range of 66.1–119.6 $U.kg^{-1}$ while invertase activities in our honeys with sunflower pollen grain predominance were in a wider range (47.7–141.3 $U.kg^{-1}$). In honeydew honeys, they established invertase activity at a range of 131.4–224.6 $U.kg^{-1}$ where the invertase activity of one of our honeys is situated. It contained a predominance of woods debris (163.8 $U.kg^{-1}$). The other honey of ours with a predominance of woods debris displayed a slightly higher invertase activity (238.1 $U.kg^{-1}$).

Other quality parameters and electrical conductivity

We found the water activity to correlate with electrical conductivity of honey (Tab. 2) at a statistical significance ($p < 0.05$). This is a very interesting result which we did not expect.

Other quality parameters (water contents and HMF contents) did not statistically significantly correlate with electrical conductivity. Statistically significant correlation with electrical conductivity was found neither with botanical origin of honeys (Tab. 2). This is in concordance with literature data because water contents depend on the degree of honey maturation and the HMF contents depend on the degree of rawness and thermal damage to honey [12].

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