Honey and its physical parameters

Med a jeho fyzikální parametry

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ABSTRACT: The aim of this study was to find how close relations exist between the results of microscopic analysis, optical rotation and electrical conductivity in 55 honey samples from the Czech Republic. The obtained results were to be interpreted in relation to the classification of honey samples according to their origin (honey groups: blossom, honeydew and compound honey). The relations were positive, very close ($r > 0.80$) and very highly significant ($P < 0.001$). Several analysed samples would have been inserted into honey groups wrongly if only conductivity had been measured. This fact and high correlation coefficients evidence that exact classification of honey must be carried out not only by measuring the conductivity but also on the basis of optical rotation and microscopic analysis – namely in transition intervals of conductivity between the particular honey groups.

Keywords: honey; quality evaluation; classification; specific rotation; pollen analysis; electrical conductivity

ABSTRAKT: Cílem práce bylo zjistit, zda existují závislosti mezi výsledkem mikroskopické analýzy medu a optickou rotací, elektrickou vodivostí a dalšími fyzikálními parametry u 55 vzorků medu z České republiky. Získané výsledky pak interpretovat pro použití při klasifikaci skupin medů (květový, medovicový a smíšený). Všechny uvedené závislosti mezi jmenovanými parametry byly kladné, velmi těsné ($r > 0,80$) a velmi vysoce průkazné ($P < 0,001$). Pokud by byla u analyzovaných vzorků používána ke klasifikaci medu jen konduktivita, některé z analyzovaných vzorků by bývaly byly zařazeny nesprávně do medných skupin. Tato skutečnost a těsné závislosti mezi sledovanými parametry dokazují, že přesnější zařazení medu není možné jen podle jeho vodivosti, ale současně s ohledem na jeho optickou rotaci a mikroskopickou analýzu (zejména v přechodných pásmech vodivosti mezi jednotlivými skupinami medu).

Klíčová slova: med; hodnocení jakosti; klasifikace; specifická rotace; pylová analýza; elektrická vodivost

Both European Honey Directive (1974) and Codex Alimentarius Standard for Honey (1993, 1998) specify criteria for honey quality and its classification. Both documents are revised now. Czech national criteria and other regulations for honey and other foods are laid down by Decree (1997, 2000).

Physical attributes belong to the main criteria of honey classification. Their measuring is comparatively simple and they have a good information value. The best-known and one of the most important honey characteristics is electrical conductivity. Optical rotation is a parameter that is discussed in relation to determination of botanical origin and adulteration of honey (Piazza *et al.*, 1991; Bogdanov *et al.*, 1997, 1999; Schuster *et al.*, 1998; Al-Khalifa and Al-Arify, 1999; Sanchez *et al.*, 2001). In some countries the rotation is applied to differentiation

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of honey groups – blossom, honeydew and compound honeys but the limit values have not been harmonised so far (Bogdanov *et al.*, 1997).

In Decree No. 334/97 the limit values for conductivity are determined as follows: blossom honey max. 55 mS/m, compound honeys 50–105 mS/m and honeydew honey 90–130 mS/m. Therefore, it is necessary to consider also other attributes for the determination of honey groups especially within transition intervals (i.e. 50–55 a 90–105 mS/m).

Microscopic analysis is another analytical method for the identification of botanical origin. Namely quantitative and also qualitative content of honeydew particles and pollen grains is studied for the identification of honey group and the blossom origin, respectively. On this account, the microscopic analysis is able to detect the botanical origin much more exactly than other analytical methods. However, it is difficult to correctly interpret results of melissopalynology and it needs a lot of experiences (Demianovicz, 1961; Kropáčová, 1969).

The aim of this study was to find how exactly it is possible to classify honey in relation to its botanical origin if pollen analysis and optical rotation are used. Therefore, it was needful to find how close relations exist between the results of microscopic analysis (i.e. pollen analysis), and the optical rotation, electrical conductivity and further physical parameters in 55 honey samples from the Czech Republic.

MATERIAL AND METHODS

Material

Samples of honey that came from the Czech market and different suppliers (n = 14) and samples directly from beekeepers, taken in the same year (2000), were used as the material. All of the honey samples were obtained by extraction. The samples were stored with authentic labels in eclipse at a laboratory temperature (22 ± 2°C) until the time of analysis.

Methods

Determination of moisture. Refractive index was determined by refractometer and equivalent content of water was found in the Table. Abbé refractometer AR 2 (fy A-Krüss Optronic, Germany) was used for such determination. Every sample was analysed in three parallel determinations. The determination of water content in honey was used for sample preparation to determine electrical conductivity of honey and to calculate specific rotation (Bogdanov *et al.*, 1997).

Determination of water activity (a_w). Water activity was determined by Pawkit AquaLab (fy Decagon, USA) instrument. Every sample was analysed in three parallel determinations.

Determination of electrical conductivity. Electrical conductivity of honey was determined from a honey solution containing 20% of honey dry matter in 100 ml distilled water (Bogdanov *et al.*, 1997). The measurement was carried out by help of thermostatic conductive cell and conductometer LF 315 (WTW GmbH, Germany). Every sample was analysed in three parallel determinations.

Determination of specific rotation. The angular rotation of a clear filtered aqueous solution was measured. The measurement was done on circular polarimeter 1000 (A-Krüss Optronic GmbH, Hamburg, Germany). Specific rotation was calculated from angular rotation, ray circuit length and grams of taken dry matter (Bogdanov *et al.*, 1997). Every sample was analysed in three parallel determinations.

Determination of botanical origin – pollen analysis (PA). Honey origin was verified by qualitative and quantitative microscopic pollen analyses (melissopalynology). The honey samples were divided according to their origin into several groups and subgroups (blossom origin) as follows:

a) blossom honey – 1. monofloral, 2. multifloral and 3. multifloral with predominance of some plant(s)
b) honeydew honey
c) compound honey (blend of honeydew and blossom honey)

The honeys belonging to a1) group originate mainly from nectar of only one plant species and proportions of the other nectars are fractional. All monofloral honeys had usual physical attributes and the result of their microscopic analysis was typical of the given monofloral origin (e.g. *Robinia* honeys did not crystallise). These honeys are not so called experimentally monofloral honeys gained from technically isolated growths as it was carried out e.g. by Demianowicz (1961). The mixture of nectars from different plant species is typical of honey samples belonging to a2) group. The honeys of a3) group also originate from the mixture of different nectars but one, two or three sources of nectar at maximum are obviously predominant. These honeys did not have any usual physical attributes and the result of their microscopic analysis was not typical of any monofloral honey of the found predominant nectars. The methodology was consistent with the international methodology including their supplements and adaptations proposed by Louveaux *et al.* (1970, 1978).

Results were evaluated using the Microsoft EXCEL 2000 software. Methods: analysis of variance with multiplication comparison (confidence intervals for $P < 0.05$), regression analysis, correlation analysis and descriptive statistical characterisation.

RESULTS AND DISCUSSION

Table 1 shows the summarisation of results for some analysed honey samples. The following parameters were measured in each sample: moisture, water activity, electrical conductivity, specific optical rotation and microscopic analysis. In Table 2, the results of analysis for individual honey groups (honeys divided according to the result of microscopic analysis) are summarised and characterised by descriptive statistical data.

According to our former experience the conductivity of monofloral *Robinia* honeys is not higher than 12 mS/m. It was also found in samples No. 1–3. Samples No. 4–5 with conductivity above 12 mS/m did not crystallise either and their appearance was consistent with usual *Robinia* honey but the result of pollen analysis detected a greater portion of rape nectar (higher quantity of *Brassica* pollen grains). This fact can cause difficulties during consecutive honey technology. One of these two samples also had a higher number of pollen grains per 1 g of honey (above 2000), which is not typical of pure monofloral *Robinia* honeys.

Samples of honeys No. 29, 30 and 49 had very low conductivity with respect to the result of pollen analysis – compound honeys. Otherwise, these samples contained only a few honeydew particles but with reference to comparatively high rotation these samples were classified as a compound honey. In spite of the higher conductivity in sample No. 13 (similar to samples No. 29 and 30), sample No. 13 was a blossom honey because it was free of honeydew particles. Sample No. 49 was a typical example when honey bees were foraging equally on two sources; in this case on *Phacelia* and honeydew. *Phacelia* is a plant flowering usually during the period of honeydew appearance. Both sources are very attractive for foragers and according to our experience increasingly more honeys with higher portion of *Phacelia* nectar usually contain a higher or a lower amount honeydew. This sample contained a higher number of honeydew particles and, therefore, the sample was a compound honey in spite of low conductivity (under standard value 50 mS/m).

Sample No. 55 was another unusual honey – by honeydew honey this once. Conductivity of this sample was rather low and lay in the transition interval between compound and honeydew honeys. However, the sample contained many hon-

Table 1. Results of analysis of individual samples ordered according to honey group

Samples	Result of pollen analysis (honey group – blossom origin)	Moisture (g/100 g)	a_w	Conductivity (mS/m)	Rotation $(\alpha)^{20}_D$
1	monofloral – *Robinia*[C]	15.4	0.50	10.3	–13.9
2	monofloral – *Robinia*	15.3	0.50	10.4	–16.1
3	monofloral – *Robinia*[C]	16.7	0.51	11.3	–15.0
4	monofloral – *Robinia* (*Brassica*)	16.0	0.48	12.3	–16.9
5	monofloral – *Robinia* (*Brassica*)[C]	16.4	0.49	13.8	–16.0
13	Multifloral	15.6	0.47	43.4	–13.5
29	compound honey	14.9	0.49	45.8	–9.9
30	compound honey	15.4	0.48	41.0	–8.5
43	compound honey	16.2	0.48	109.8	–2.1
49	compound honey[C]	15.6	0.49	43.8	–7.7
55	honeydew honey	16.4	0.49	96.6	6.6

[C] sample of commercial honey

Table 2. Descriptive statistical characterisation of the individual physical parameters according to honey groups

	Moisture (g/100 g)	a_w	Conductivity (mS/m)	Rotation $(\alpha)^{20}$
Monofloral – *Robinia* (n = 5)				
Mean	16.0	0.49	11.6	–15.6
Minimum value	15.3	0.48	10.3	–16.9
Maximum value	16.7	0.51	13.8	–13.9
Standard deviation	0.6	0.01	1.5	1.1
Standard error	0.3	0.00	0.7	0.5
Coefficient of variation (%)	3.8	1.80	12.7	–7.3
Monofloral – others (n = 7)				
Mean	17.1	0.51	20.6	–15.2
Minimum value	15.1	0.47	13.3	–22.2
Maximum value	19.5	0.54	28.4	–12.0
Standard deviation	1.5	0.03	5.7	3.3
Standard error	0.6	0.01	2.2	1.2
Coefficient of variation (%)	9.0	5.20	27.9	–21.6
Multifloral (n = 16)				
Mean	16.5	0.52	26.0	–13.1
Minimum value	14.8	0.46	14.0	–18.9
Maximum value	18.5	0.59	43.9	–9.6
Standard deviation	1.1	0.04	9.2	2.5
Standard error	0.3	0.01	2.3	0.6
Coefficient of variation (%)	6.7	8.00	35.4	–19.3
Compound honey (n = 21)				
Mean	15.9	0.50	68.0	–4.2
Minimum value	14.0	0.41	41.0	–9.9
Maximum value	18.6	0.59	109.8	3.8
Standard deviation	1.1	0.04	18.5	4.0
Standard error	0.2	0.01	4.0	0.9
Coefficient of variation (%)	6.9	8.60	27.1	–97.4
Honeydew honey (n = 6)				
Mean	15.3	0.49	107.5	10.5
Minimum value	13.8	0.48	96.6	–1.0
Maximum value	16.5	0.50	111.6	20.4
Standard deviation	1.0	0.01	5.8	7.8
Standard error	0.4	0.00	2.4	3.2
Coefficient of variation (%)	6.8	1.80	5.4	74.5

eydew particles and a very low number of pollen grains (only 894 pollen grains per 1 g of honey). Furthermore, a major part of these pollen grains was represented by anemophile pollen or pollen from plants without nectar production as it is typical of honeydew honeys. On the other hand, sample No. 43 would have been classified as a honeydew honey if it had been classified only on the basis of conductivity. However, very low content of honeydew particles and very high content of pollen

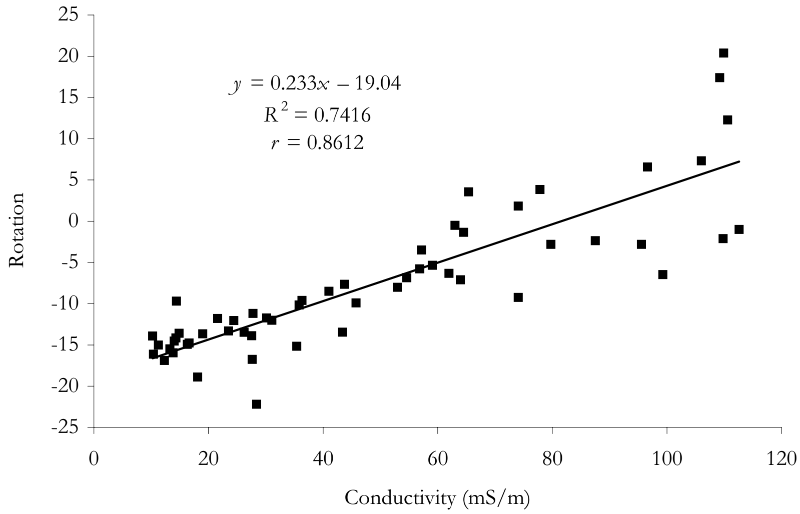

Figure 1. Regression line for conductivity and rotation

was found in this sample (10 049 PG/g – typical of honey with great portion of nectar source at least). Therefore sample No. 43 is a compound honey and this result corresponds with negative rotation.

Several closer relations were found between the physical attributes of analysed samples (Table 3). These are the relations between electrical conductivity, optical rotation and microscopic analysis that are the most important for honey classification and sorting into the individual honey groups. All mentioned relations are positive, very close ($r > 0.80$) and very highly significant. To calculate correlation coefficients between the individual honey groups (also blossom origin of honey) and other physical attributes, individual samples had to be numbered as follows: *Robinia* – 1, *Robinia* (*Brassica*) – 1.5, monofloral – 2, multifloral – 3, compound – 4 and honeydew honey – 5.

Regression line for rotation and conductivity is represented in Figure 1. Arithmetic means for conductivity and rotation of individual honey groups and blossom origin are shown in Figure 2.

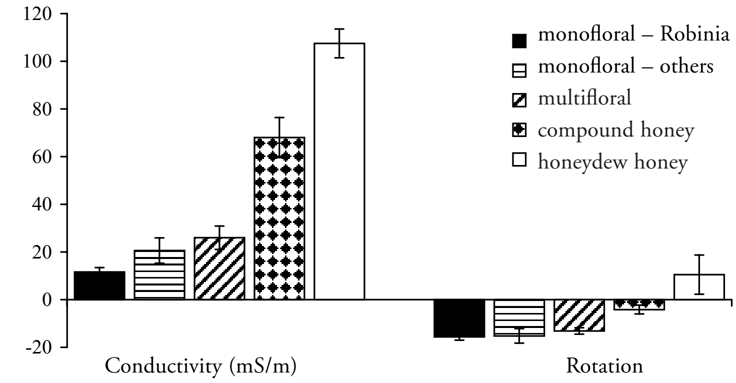

Figure 2. Average values of conductivity and rotation according to honey groups

Table 3. Coefficients of correlation between the physical parameters of honey

Parameter	PA	Moisture	a_w	Conductivity	Rotation
PA	1				
Moisture	–0.29*	1			
a_w	-0.09^{ns}	0.59***	1		
Conductivity	0.86***	0.23^{ns}	-0.08^{ns}	1	
Rotation	0.80***	–0.35**	-0.08^{ns}	0.86***	1

* $P < 0.05$;** $P < 0.01$;***$P < 0.001$; ns = no significance

Differences are very highly significant ($P < 0.001$). Significance of differences between individual groups is demonstrated by abscissas representing confidence intervals of reliability for $P < 0.05$. Only one difference in conductivity – between monofloral-others and multifloral honeys – is not significant, which was expected. In the case of rotation, only differences among blossom honeys (i.e. monofloral – *Robinia*, monofloral – others and multifloral honeys) are not significant; differences among blossom, compound and honeydew honeys are significant.

This fact and high correlation coefficients evidence that exact classification of honey must be carried out not only by measuring the conductivity but also in relation to optical rotation and microscopic analysis – namely in transition intervals of conductivity between the individual honey groups.

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