Authentication of honey by multivariate analysis of its physico–chemical parameters

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Summary

The aim of this study was the characterization of honeys by selected analytical methods proposed by International Honey Commission (IHC) and comparison of these parameters with those specified by regulations of Slovak Republic and EU. HPLC assays of sugar profiles and 5-hydroxymethylfurfural contents were modified by using Polymer IEX H form and Lichrospher 100 NH_2 columns (Watrex, Berlin, Germany) and by the modification of mobile phase composition. Adulteration of honey by almost 20 % addition of high-fructose corn syrups was detected in commercial honey by stable carbon isotope ratio $^{13}C/^{12}C$ analysis. In order to characterize and classify various honeys and distinguish honeys from starch syrups, principal component analysis and cluster analysis were used for statistical evaluation of physico-chemical parameters of honeys. The sum or ratio of glucose, fructose and water were found to be more specific indicators of the honey quality than any individual parameter. Low electrical conductivity values of 0.252 $mS \cdot cm^{-1}$ and 0.211 $mS \cdot cm^{-1}$ determined in two samples of forest honey purchased from the local market underlined the significance of previous Slovakian regulation for this type of honey in the interval from 0.55 $mS \cdot cm^{-1}$ to 1.00 $mS \cdot cm^{-1}$ in consequence of the assumed adulteration by adding colorants.

Keywords

honey; authentication; principal component analysis; cluster analysis

Honey is the most important primary product of beekeeping both from quantitative and economic points of view [1]. The properties and composition of honey can vary widely depending on the region, season, bee variety, plant source of nectar and storage time in the honeycomb, mode of harvesting and post-harvest storage [2].

Honey is a complex of various compounds of plant and honeybee origin such as sugars, proteins, enzymes, amino acids, vitamins, hormones, flavonoides, inorganic acids and trace elements. Physico-chemical parameters of honey are specified by a European Council Directive [3] which has been transposed into Slovakian legislation in 2004 [4].

Sugars account for 95–99% of honey dry matter. Majority of these (85–95%) are simple sugars, namely, fructose and glucose. Generally, fructose is more abundant than glucose. Small quantities of other sugars such as disaccharides (sucrose, maltose, isomaltose), trisaccharides and oligosaccharides are also present, and though quantitatively of minor importance, they provide information about botanical origin of the honey [1] and can indicate adulteration of honey as well.

The the most important minor constituents of honey are organic acids, among which gluconic acid, a by-product of enzymatic digestion of glucose, predominates. Organic acids are responsible for the acidity of honey and contribute largely to its characteristic taste [1].

Of minerals present in traces, potassium is the most abundant. Dark honeys, particularly honeydew honeys, are the richest in minerals [1, 5]. The mineral content influences the colour and the taste of honeys: the higher the quantity of minerals and the darker colour, the stronger taste [6]. Approximate relationship was found between the mineral profile of honeys and their botanical origin [7].

Proteins, enzymes, amino acids or water-soluble vitamins are thought to result from pollen contents and from honeybee secretions in honey [1].

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The average protein content of honeys is about 0.7% [8]. Since apalbumin 1, the most abundant royal jelly (RJ) protein, is a regular component of honeys, it is a suitable marker of adulteration of honey [9]. The proteins originating from plants (pollen and nectar) [10–12] and the amino acids profile may be useful for determination of botanical origin of honey [13–15] as well as for differentiation of floral and honeydew honeys [16].

The enzymes originating from salivary secretions of worker honeybees play an important role in processing the nectar to honey. The main enzymes are β-D-fructofuranoside-fructohydrolase (invertase), diastase (amylase), glucose oxidase [1], α-glucosidase and β-glucosidase [9]. Their activity is lost during heating or prolonged storage of honey [17] and for this reason, these enzymes are not suitable authentication markers.

Honey adulteration is a reprehensible practice consisting of incorporating sugar syrups into the genuine product. More importantly for apiculture practice, honey adulteration is also caused by incorporation of sugars into honeys via feeding of bees [18, 19]. The sale of honey under a fraudulent origin name is the third way of honey adulteration [18]. Floral honey is made by honeybees from the nectar of blossoms, while honeydew honey is prepared from secretions of living parts of plants or excretions of plant-sucking insects. Differentiation between floral and honeydew honey is a response to consumer demands [20], e.g. honeydew honey is preferred in by consumers in Slovakia. Physicochemical parameters such as acidity, ash content, colour, electrical conductivity and optical rotation have been considered useful for the differentiation of the two types of honey [20, 21]. Extensive compositional analysis is required to prove the authenticity of honey [22].

Honey is rich in flavonoids and other phenolic compounds, which act as natural antioxidants and are becoming increasingly popular because of their potential role in human health. These compounds can also be used as indicators of floral and geographical origin of honey [23–38]. Flavanone hesperetin is characteristic in particular for citrus honey [39]. Eucalyptus honey has a typical flavonoid profile comprising myricetin, tricetin, quercetin, luteolin and kaempferol. Quercetin and kaemperol are flavonoids common for monofloral honeys. Kaempferol was found to be the main flavonol in rosemary honey. Quercetin was detected as one of main flavonoids in sunflower honey, suggesting its possible use as a marker of this floral origin [26–28, 32].

Quality control methods, in conjunction with multivariate statistical analysis, have been found to be able to classify honeys according to their geographical origin, botanical origin, adulteration and chemical characteristics [6, 12, 15, 16, 18, 19, 22, 40–50]. In order to understand and improve quality of honey, the industry needs new tools that can measure a wide variety of properties as well as data analysis methods that can handle large and complex data. Multivariate analysis involves the use of mathematical and statistical methods. Multivariate analysis helps to look at the honey sample in its entirety and not just in its single component so that we can untangle all the complicated interactions among individual constituents and understand their combined effects on the whole object. Nowadays, the application of statistical methods, such as principal component analysis (PCA) or cluster analysis (CA), provides the possibility to analyse and classify a food sample as a whole [20, 51–52]. Therefore, it is not always necessary to determine all constituents of the sample to know whether it falls within a defined range or group. Instead, trends or correlation among individual quality characteristics can be used [41, 52].

The aim of this work was to test the suitability of PCA and CA for distinguishing honeys from starch syrups using physico-chemical parameters pH, acidity, conductivity, contents of water, proline, fructose, glucose, sucrose, maltose and 5-hydroxymethylfurfural (HMF).

MATERIALS AND METHODS

Chemicals and materials

Honeys, invert and starch syrups

Samples of 33 honeys were subjected to physico-chemical analysis (Tab. 1). Series A and B represented samples of honeys purchased from markets in Slovakia during 2004 and 2005, respectively. Most of these samples were Slovakian products except for samples AB and BB, which originated in Argentina, samples AF, BH and BI from European Union (EU) countries, and samples BF and BG which were honeys containing admixtures of honeys from tropical countries. Most samples were processed in Slovakia except for the sample AB packed in Hungary, and samples BF and BG packed in Czech Republic. The samples included multifloral, unifloral (robinia, linden, sunflower, rape), forest and honeydew honeys. Series C represented samples of honeys obtained directly from beekeepers in Slovakia. As for their botanical origin (robinia, linden, sunflower, rape, cherry and chestnut), it reflected the flower season during the nectar flow. Samples were stored

Tab. 1. Characteristics of honeys, invert and starch syrups.

Supplier source	Series	Sample	Type	Cuntry of origin
Market honeys	Series A (2004)	AA	Multifloral	SR
		AB	Multifloral	A
		AC	Multifloral	SR
		AD	Forest	SR
		AE	Robinia	SR
		AF	Linden	EU
		AG	Forest	SR
		AH	Linden	SR
		AI	Robinia	SR
		AJ	Honeydew	SR
		AK	Sunflower	SR
	Series B (2005)	BA	Multifloral	SR
		BB	Multifloral	A
		BC	Multifloral	SR
		BD	Forest	SR
		BE	Multifloral	SR
		BF	Forest	TC
		BG	Multifloral	TC
		BH	Rape	EU
		BI	Multifloral	EU
Beekeepers' honeys	Series C	CA	Linden	SR
		CB	Robinia	SR
		CC	Sunflower	SR
		CD	Cherry	SR
		CE	Robinia	SR
		CF	Linden-chestnut	SR
		CG	Multifloral	SR
		CH	Sunflower	SR
		CI	Rape	SR
		CJ	Robinia	SR
		CK	Linden	SR
Artificial honeys	Series D	DA	Dandelion	–
		DB	Inverted sucrose (Metko)	P
Starch syrups	Series SS	SS1	Starch hydrolysed	SR
		SS2		SR
		SS3		SR

SR – Slovakia, A – Argentina, EU – blend of honeys from EU countries, TC – honey mixtures containing also honeys from tropical countries, P – Poland.

protected from light in screw-capped glass flasks at a laboratory temperature. For comparison, artificial dandelion honey (sample DA) was prepared as follows: 250 pieces of dandelion (*Taraxacum officinale*) flower heads were boiled with 3 slices of lemon in 800 ml of water for 20 min, strained and, after addition of 1.5 kg of sugar, boiled for about 40 min to reach a proper solidification density. Artificial honey (inverted syrup, sample DB) consisting of inverted sucrose was obtained from the market. As model adulterated honeys, commercial starch hydrolysates SS1 (R.B.S., Martin, Slovakia), SS2 and SS3 (both Amylum Slovakia, Boleráz, Slovakia) were used.

Determination of physico-chemical parameters of honeys

Samples of honeys were analysed by standard methods of the Slovak Technical Standard (STN) No. 57 0190 [53] and by harmonized methods of the European Honey Commission [54]. The water content was determined by measuring refractive index (*RI*) using an Abbé analogue refractometer (A. Krüss Optronic, Hamburg, Germany) at 20 °C. The water content was derived from *RI* according to the STN mentioned above. Acidity was determined by a titration method using 0.1 M NaOH solution [53, 54]. In order to determine pH, 10 g of honey was dissolved in 75 ml of CO_2-free distilled water and pH of the solution was measured by a pH-meter InoLab Level2 (WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) [54]. The proline contents weres determined after the reaction with acidic ninhydrin solution by measuring A_{520} in a UV-VIS spectrophotometer UV-1601 (Shimadzu, Tokyo, Japan) [54]. The botanical origin of honey, in particular the nectar or honeydew source, was determined by measuring the electrical conductivity at 20 °C using a conductometer OK-102/1 (Radelkis, Budapest, Hungary) [53, 54].

Sugar profile determination by HPLC-RI

For separation of sucrose, glucose, fructose and maltose, two chromatographic conditions (I and II) were applied using a DeltaChrom equipment (Watrex, Berlin, Germany) consisting of a HPLC pump DeltaChrom SDS 030, a thermostated column DeltaChrom CTC 100 and a RI detector WellChrom K-2301 (Knauer, Berlin, Germany). In condition I, an IEX H-form column (250 × 8 mm, 5 μm; Watrex) at 25 °C and 9 mM H_2SO_4 solution as a mobile phase at a flow rate of 1 ml·min^{-1} was used. In condition II, a Lichrospher 100 NH_2 column (250 × 4 mm, 5 μm; Watrex) at laboratory temperature and acetonitrile - water mixture (8 : 2, v/v) as a mobile phase at a flow rate of 1 ml·min^{-1} was used. Before injection, diluted samples of honey were filtered through a ProFill Nylon 0.45 μm membrane (La-Pha-Pack, Langerwehe, Germany). Results of analysis were processed using the Clarity Version 2.4.1.65 software (DataApex, Prague, Czech Republic).

HMF content determination by HPLC-UV

Content of HMF was determined by HPLC-UV according to an IHC method [54] with some

modifications in the mobile phase composition, using absorbance wavelengths repored in the previous study [55]. A liquid chromatography equipment from Laboratorní přístroje (Praha, Czech Republic) consisted of a pump HPP 5001, an UV detector LCD 2040 and a line recorder TZ 4620. A Nucleosil C18-RP column (250 × 4 mm, 5 μm; Watrex) with a guard column with the same stationary phase were used. A mixture of acetonitrile and water (1 : 9, v/v) was used as a mobile phase at a flow rate of 1 ml·min^{-1}. Before injection, diluted samples of honey were clarified with the Carrez I (15 g $K_4Fe(CN)_6.3H_2O$ in 100 ml of deionized water) and Carrez II solution (30 g $Zn(CH_3COO)_2.2H_2O$ in 100 ml of deionized water), and filtered through a 0.45 μm nylon membrane. The HMF content was determined from the absorbance value at 280 nm using a calibration curve [55].

Stable carbon isotope ratio determination

Stable carbon isotope ratio determination was done by the methods AOAC 978.17 [56] and AOAC 991.41 [57] after complete combustion to carbon dioxide using an Elemental Analyzer FLASH EA 1112 equipped with IRMS DELTA^{Plus} XP (both from Thermo Finnigan, Bremen, Germany). PDB (*Belemintella Americana)*, a fossil limestone from National Institute of Standards and Technology (Gaithersburg, Maryland, USA), was used as a reference. The ISODAT NT software (Thermo Finnigan) was used. The honey adulteration (in %) by addition of starch syrups was calculated according to the formula

$$Adulteration = \frac{\left(\delta\,^{13}C_P - \delta\,^{13}C_H\right)}{\left(\delta\,^{13}C_P - \delta\,^{13}C_{CS}\right)} \times 100 \qquad (1)$$

where $\delta^{13}C_H$ represents carbon isotopic ratio of honey, $\delta^{13}C_P$ carbon isotopic ratio of the protein fraction of honey, and $\delta^{13}C_{CS}$ carbon isotopic ratio of the sweetener. $\delta^{13}C$ (in ‰) takes into account the PDB reference value according to the formula

$$\delta\,^{13}C = \left[\left(\frac{^{13}C}{^{12}C_{sample}}\right) \div \left(\frac{^{13}C}{^{12}C_{standard}}\right) - 1\right] \times 1000 \qquad (2)$$

Proteins were precipitated using 10% sodium tungstate and 0.67 N H_2SO_4 according to AOAC 991.41 [57]. Reference adulterated honeys at a level of 10, 20, 30, 50 and 75%, respectively, were prepared by adding high-fructose corn syrup (HFCS). Analysis of honeys with added starch hydrolysates was done by a method based on the Fiehe reaction [53].

Multivariate statistical analysis

Results of physico-chemical analysis of honeys were processed by PCA and CA using SG WIN Staticgraphic for Windows Version 3.1 (Statpoint, Herndon, Virginia, USA).

RESULTS AND DISCUSSION

Physico-chemical analysis of honeys

Results of physical-chemical analysis of genuine and artificial honeys, as well as invert and starch syrups are presented in Tab. 2. In the samples, the following parameters were determined: pH, acidity, electrical conductivity, and contents of water, proline and HMF, for which reference values were set by the regulation in Slovak Republic (water content, acidity, conductivity,and HMF content) [4] or recommended by IHC (proline content) [54, 58].

Adulteration of honey by the addition of water is unusual due to a risk of undesirable fermentation [4, 59]. In the CI sample of rape honey, a rather high water content (21.40%) was detected, which led to an assumption that this honey was harvested unripe in an attempt to avoid the beginning of the crystallization process [60] and/or that this honey was blended with monofloral or other type of honey. Water contents of all samples of starch syrups were above 20%. The water contents of DA and DB samples of artificial honey were within the limit.

The tolerable limit of honey acidity is 50 mmol·kg^{-1} or mekv·kg^{-1} (milliekivalent per kg) [4]. The acidity of samples ranged from 6.18 mmol·kg^{-1} to 34.74 mmol·kg^{-1}, indicating a good microbiological quality. A higher acidity was found for the AJ sample of honeydew honey, which was in agreement with previously published data [20]. Correlation analysis of pH and acidity values yielded a coefficient of –0.03643, which indicated that there was no significant dependence between these parameters. The reason may be a different buffering capacity of the contained organic acids and their salts [2].

One of the forms of adulteration of honey is deliberately false labelling of nectar honey as forest or honeydew honey because of their higher price [20]. Blossom honey (from nectar) has electrical conductivity typically below 0.8 mS·cm^{-1}, while honeydew honey exceeds that value because of higher minerals and organic acids contents. This parameter is limited by national as well as international regulations [4]. Electrical conductivity is a parameter suitable also for characterization of

Tab. 2. Selected parameters of honeys.

Honeys samples	Water [%]	Proline [mg·kg^{-1}]	HMF [mg·kg^{-1}]	pH	Acidity [mmol·kg^{-1}]	Conductivity [mS·cm^{-1}]
AA	18.96	169	68.2	4.1	7.10	0.132
AB	19.12	339	32.9	3.6	19.46	0.244
AC	15.92	520	13.6	3.9	16.71	0.388
AD	15.92	591	11.7	4.3	20.84	0.656
AE	16.16	246	11.5	3.9	9.39	0.173
AF	17.32	79	46.8	4.5	6.18	0.321
AG	15.64	356	59.8	3.9	12.82	0.252
AH	16.93	461	9.9	4.1	18.86	0.441
AI	16.32	218	14.0	3.8	10.96	0.146
AJ	15.50	821	4.4	4.3	34.74	0.987
AK	18.84	320	55.8	3.7	14.80	0.281
BA	17.40	223	13.0	3.5	18.99	0.175
BB	17.20	422	25.8	4.0	17.82	0.171
BC	17.61	712	91.3	3.8	30.21	0.386
BD	16.70	243	60.6	3.8	13.87	0.211
BE	16.90	290	12.5	3.7	14.01	0.182
BF	17.88	104	nd	4.2	24.57	0.547
BG	19.04	314	39.6	4.1	14.44	0.357
BH	17.00	224	11.2	3.8	12.50	0.179
BI	16.90	458	5.2	3.5	11.34	0.335
CA	16.44	344	2.0	3.3	18.99	0.339
CB	14.80	252	7.7	3.3	10.13	0.255
CC	16.72	511	2.6	3.7	25.79	0.591
CD	18.00	403	2.4	4.1	18.62	1.010
CE	15.96	556	2.6	3.7	12.24	0.444
CF	16.72	725	3.3	3.7	30.41	0.928
CG	16.28	609	nd	4.3	15.43	1.187
CH	16.44	596	7.7	3.5	32.45	0.372
CI	21.40	215	nd	3.6	13.98	0.163
CJ	16.72	280	nd	3.7	13.97	0.176
CK	17.40	505	nd	4.3	14.99	0.569
DA	14.80	nd	192.0	3.7	32.88	0.043
DB	19.40	nd	926.2	3.4	19.27	0.147
SS1	21.20	nd	49.0	3.3	5.88	0.048
SS2	21.44	nd	38.8	4.1	6.94	0.054
SS3	27.60	nd	24.2	4.0	5.00	0.051

nd – not detected.

botanical origin of honey. A lower conductivity is typical for robinia honey [21, 42, 61], while chestnut honey and honeydew honey show higher values [4]. Results of this study are in agreement with this knowledge, except for two samples of forest honey, AG (0.252 mS·cm^{-1}) and BD (0.211 mS·cm^{-1}), which had exceptionally low conductivities typical for blossom honey. It is interesting that both of these forest honey samples were purchased from the same manufacturer at a local market in two subsequent years (2004 and 2005). For this reason it can be assumed that the AG and BD samples were deliberately mislabelled by the manufacturer as forest honey while, in fact, they represented blossom honey. It should be quoted that there is no regulation for forest honey since 2004 in Slovak Republic as well as in EU. Before 2004, the limit for electrical conductivity of forest honey in Slovak Republic was 0.55–1.00 mS·cm^{-1} [62].

The minimum content of proline in honey has been set to 180 mg·kg^{-1} by IHC [54]. Three samples of honey, namely, AA, AF and BF did not fulfil this criterion and therefore were considered adulterated by the addition of sugar syrups. It should be mentioned that in sample BD, in which the addition of starch hydrolysates was detected by the analysis of stable carbon isotope ratio, a lower proline content was not observed. Proline content

used to be considered as a good indicator of the origin of honey, but recent studies demonstrated that it is considerably variable and not very useful for this purpose [20, 63].

Content of HMF is a widely accepted indicator of freshness and quality of honey. According to the regulation in Slovak Republic, maximum HMF content for honey from non-tropical countries is 40 $mg \cdot kg^{-1}$, and that for honey from tropical countries is 80 $mg \cdot kg^{-1}$ [4]. Six samples of honeys exceeded the limit, including samples AA, AG, BC and BD, which were obtained from the same producer. A high HMF content was also found in two samples of artificial honey (DA and DB), which was apparently caused by acidic hydrolysis of sucrose and favourable reaction conditions for HMF formation.

Sugar profiles of honeys and starch hydrolysates was determined by HPLC-RI in two different conditions, I and II (Tab. 3). Using condition I, co-elution of sucrose and maltose occurred. Using condition II, appropriate separation of sucrose, fructose, glucose and maltose was achieved. The limit of detection was found to be 0.05% for sucrose, 0.03% for fructose, 0.03% for glucose and 0.04% for maltose. The limit of quantification was

Tab. 3. Sugar profiles of honeys determined by HPLC-RI in conditions I and II.

Sample	HPLC-RI						
	Conditon I			Conditon II			
	Fructose [%]	Glucose [%]	Sucrose [%]	Fructose [%]	Glucose [%]	Sucrose [%]	Maltose [%]
AA	37.41	33.75	5.61	38.58	34.34	nd	2.26
AB	37.59	34.02	6.61	42.36	35.13	nd	2.98
AC	37.79	30.11	9.43	40.85	30.23	nd	3.56
AD	38.20	30.94	9.10	41.46	30.96	nd	3.55
AE	41.97	26.80	10.49	41.78	28.66	nd	3.63
AF	43.54	27.14	8.17	41.21	31.18	1.11	2.28
AG	38.27	32.94	8.40	38.03	33.42	nd	3.04
AH	39.64	30.48	8.76	43.22	29.05	nd	3.34
AI	41.37	26.75	8.91	44.26	26.76	0.47	3.58
AJ	34.90	27.92	7.86	35.97	28.30	nd	3.53
AK	36.47	36.50	5.50	38.83	35.64	0.42	1.62
BA	38.00	26.69	3.15	42.00	33.13	0.72	1.95
BB	38.35	26.59	3.27	42.04	32.61	0.61	3.05
BC	43.46	26.33	1.41	37.85	33.22	nd	2.66
BD	34.12	25.80	10.77	38.84	31.67	2.72	6.15
BE	35.11	27.92	4.23	35.01	39.95	nd	2.62
BF	32.93	25.56	8.07	37.04	31.20	nd	4.75
BG	33.73	27.69	6.25	38.39	34.40	1.29	2.82
BH	37.54	35.42	6.87	40.28	36.50	nd	2.86
BI	37.76	32.83	5.84	40.25	32.55	nd	3.29
CA	43.29	28.73	10.32	–	–	–	–
CB	46.23	26.81	10.86	–	–	–	–
CC	41.46	40.29	3.96	–	–	–	–
CD	41.11	31.97	9.85	–	–	–	–
CE	38.40	28.06	8.62	–	–	–	–
CF	43.28	35.81	4.98	–	–	–	–
CG	43.80	33.00	8.05	–	–	–	–
CH	40.34	34.94	5.14	–	–	–	–
CI	40.55	36.25	5.89	39.48	37.23	nd	1.37
CJ	41.32	29.17	8.85	42.97	28.84	nd	4.00
CK	39.14	30.23	9.06	39.54	28.04	nd	4.00
DA	31.70	26.09	25.23	27.15	29.92	24.75	0.29
DB	37.23	37.98	6.21	37.30	38.05	5.61	0.33
SS1	29.53	22.11	13.86	30.48	25.90	nd	12.15
SS2	19.45	26.40	13.42	24.44	38.57	nd	14.01
SS3	35.65	21.96	6.62	36.41	27.08	nd	6.28

nd = not detected.

found to be 0.18% for sucrose, 0.11% for fructose, 0.11% for glucose and 0.15% for maltose. The DA and DB samples of artificial honeys were characteristic by higher sucrose contents (above 5%). It was interesting to find that sample BD, for which adulteration by the addition of HFCS was proven later by stable carbon isotope ratio analysis, had a higher content of maltose. Since the contents of this disaccharide were significantly higher also in starch hydrolysates, we are of the opinion that the maltose content could be regarded as an authentication parameter for honey, in agreement with ABDEL-AAL et al. [64].

Detection of starch hydrolysates, in particular HFCS, in honey samples BA to BG, was achieved using stable carbon isotope ratio analysis. The $\delta^{13}C$ values of honeys and starch hydrolysates were in the range from –26.34‰ to –22.37‰, and from –11.45‰ to –10.95‰, respectively. The results of analysis and calculated percentage values of added C4 sugars are presented in Tab. 4. A nearly 20% adulteration by HFCS was detected in sample BD. In other samples, the addition of HFCS was not detected. The eligibility of this method for the detection of HFCS was tested on laboratory adulterated honey BA with 10, 20, 30, 50 and 75% of HFCS. A linear dependence was observed between $\delta^{13}C$ and the percentage of HFCS with a correlation coefficient of 0.9968 (Fig. 1). According to the literature data, an addition of HFCS to 7% corresponding to an increase in $\delta^{13}C$ by about 1% would be needed for a sample to be considered suspicious [65].

Tab. 4. Percentage of added C4 sugars in honeys determined by stable carbon isotope ratio analysis.

Samples	$\delta^{13}C_H$ [‰]	$\delta^{13}C_P$ [‰]	Added C4 sugars [‰]
BA	–25.10	–25.18	0.5
BB	–24.86	–25.15	1.9
BC	–24.40	–24.79	2.6
BD	–22.37	–25.39	19.3
BE	–26.34	–25.83	3.2
BF	–25.27	–25.05	1.4
BG	–25.39	–25.27	0.8

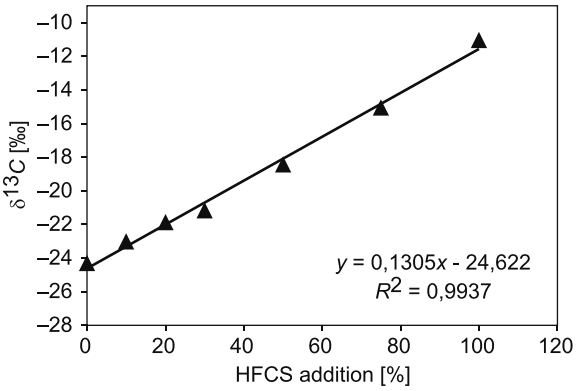

Fig. 1. Linear dependence of $\delta^{13}C$ (‰) of honey from percentage of the added HFCS.

Multivariate statistical evaluation of results

The results of physical-chemical analysis of various kinds of honey are widely processed by PCA and CA [7, 13, 19, 20, 43, 45-48, 52, 66-75]. PCA serves for (i) reduction in the number of variables while losing only a small amount of information and (ii) presentation of data in just two dimensions corresponding to individual variable and type of honey. Our PCA results are presented in Tab. 5, Fig. 2 and Fig. 3. PCA disclosed three principal

Tab. 5. Saturation of variables for the first three principal components as determined by PCA.

	Component 1	Component 2	Component 3	Component 4
Acidity	0.241	-0.054	0.301	-0.262
Conductivity	0.263	0.246	-0.134	-0.344
Fructose	0.304	0.191	-0.132	0.443
Fructose + glucose	0.357	-0.120	-0.289	0.230
Glucose	0.285	-0.386	-0.328	-0.092
HMF	-0.012	-0.462	0.079	0.114
Maltose	-0.352	0.068	-0.064	-0.256
pH	-0.011	0.305	-0.078	-0.421
Proline	0.312	0.263	-0.092	-0.238
Fructose : glucose ratio	-0.011	0.467	0.159	0.485
Glucose : water ratio	0.385	-0.213	-0.045	-0.075
Refractive index	0.326	0.088	0.361	-0.041
Sucrose	-0.023	-0.268	0.588	-0.011
Water	-0.307	-0.112	-0.394	0.029

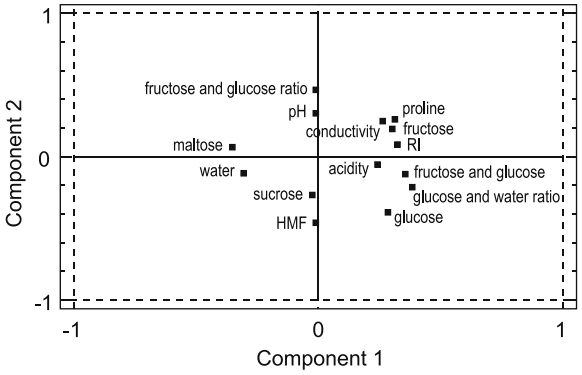

Fig. 2. Saturation of variables in axes of first two principal components.

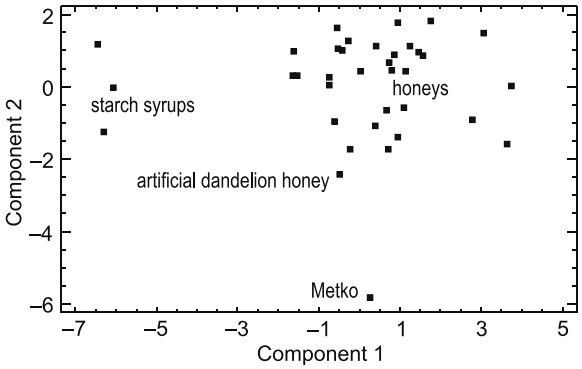

Fig. 3. Score of samples of honey and starch syrups in axes of first two principal components.

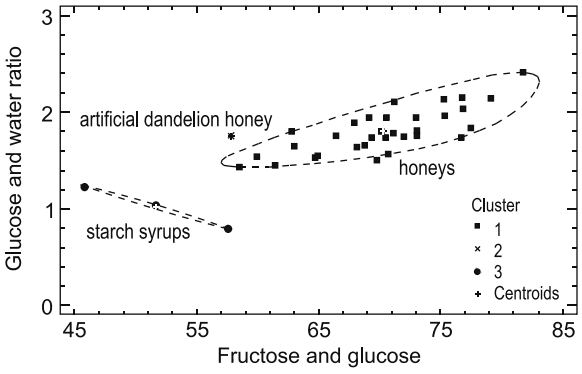

Fig. 4. Clusters of samples of honey and starch syrups in axes of two selected variables.

components that accounted for 80.7% of variability in the original data. These components were the following most important variables for distinguishing honey from artificial honey and starch syrup: the sum of fructose and glucose, the glucose : water ratio and the fructose : glucose ratio.

CA belongs to the methods that study similarity of multidimensional objects and group objects into clusters. This method is usually applied to the objects that have natural tendency to group [74, 75]. The purpose of applying CA to the results of this study was to group honey samples into clusters. In Fig. 4, a two-dimensional plot of clustered samples versus two characteristic variables (the sum of fructose and glucose contents, and the glucose : water ratio) is presented. This approach separated honey samples into a cluster of honeys, a cluster of starch syrups, and a sample of artificial dandelion honey.

CONCLUSIONS

The multivariate statistical analysis applied to various parameters of honeys, namely, pH, acidity, conductivity, content of water, sucrose, fructose, glucose, maltose, HMF and proline, proved suitable for distinguishing honey from starch syrup. It was found that the sum of glucose and fructose contents, the fructose : glucose ratio and the glucose : water ratio are better indicators of honey quality than any individual parameter. Furthermore, a standard value of electrical conductivity turned out to be an essential parameter of forest honey.

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