Research article

Classification of acacia, rape and multifloral Hungarian honey types

Emese Dominkó [a,*], Zsolt István Németh [b], Tamás Rétfalvi [a]

[a] *Institute of Environmental Protection and Nature Conservation, Faculty of Forestry, University of Sopron, 9400, Sopron, Hungary*
[b] *IngvestingTeam Ltd., 9400, Sopron, Hungary*

A B S T R A C T

The composition of honey is mostly determined by the species-specific characteristics of flowering plants, which is reflected in the significant deviations in composition of honey varieties. The high-quality acacia honey is assessed based on both physical-chemical parameters and melissopaly-nology. The appearance of rape pollen in acacia honey makes the acacia honey be sorted into the multifloral honey category. Over carrying out melissopalynology, the 149 samples of various honeys (acacia, rape and multifloral) have also been analysed by using physical-chemical and elemental analysis. Multivariate data analysis revealed that multifloral honey is much closer to acacia honey than to rape honey, as it can be observed from the examined unique parameters. By the PCA (Principal Component Analysis) analysis based on united set of physico-chemical and melissopalynology results the acacia and rape honey samples are entirely separated for each other, while multifloral honey samples are very close to acacia honey group and partially overlap with it. On ignoring the pollen analysis and based on the rest of the results, the multifloral honey category is almost indistinguishable from the declared and verified acacia honey category.

1. Introduction

Honey is a very precious bee product containing vitamins, microelements, minerals, proteins, enzymes, and carbohydrates [1,2]. For thousands of years, honey has been one of the most valuable food [3,4]. Due to its antibacterial effect [5], it is also utilized in pharmaceutical fields [6].

The macroscopic composition of honey is mostly determined by the species-specific characteristics of flowering plants, which is reflected in the significant differences in composition of honey varieties [7,8]. In order to uncover the deviations among various honeys, beyond the melissopalynological analysis [9], several physicochemical methods are also generally applied [1,8,10]. In research of Wang et al. [11] (2014) the analysis of phenolic metabolites was applied to determine adulteration of acacia honey, due to the rape honey added to it. The variation of the elemental components is also influenced by the variability of the botanical and soil properties of the growing area [12–15], that is why it is necessary to specify and identify the geographical origin of different types of honey products, as well.

In the Carpathian Basin, rape honey and acacia honey have outstanding features. These two honey varieties differ characteristically from each other. Acacia honey is able to held its liquid state, due to the high ratio of fructose to glucose (acacia honey F/G = 1.5; rape honey F/G = 1.0) [16]. Considering the prestige of acacia honey, it is highly sought-after and one of the most well-known type in

---

European Union [15]. Acacia honey has a high content of nectar nevertheless hardly contains pollen [17]. Therefore, as a quality indicator, a European protocol fixes the admissible pollen amount in acacia honey, furthermore, acacia honey has a high enough premissible sucrose content ($Suc_{max} \leq 10g/100g$ [18]; Council Directive, 2001/110/EC), because of the high quantity of sucrose in acacia nectar.

In the past decades, the flowering periods of rape and acacia have been changing and may get closer to each other [19,20].

This is a significant problem for bee brood because the pollen-rich rape flowers better attract bees even during acacia flowering and thus, they will prefer the rape flowers during their pollen accumulation. Rape plantations next to acacia forests could contaminate acacia honey with rape pollen. As a result, the extracted acacia honey may not meet the requirements of regulations due to the presence of rape pollen. Such honey must be labelled and can only be sold as multifloral honey product on the market. The non-crystalling and stably liquid-stated honey batch, which have been collected after the acacia blossoms but contaminated with rape pollen, can only be sorted into multifloral category, despite the fact that it is most similar to acacia honey in terms of its other characteristics.

In evaluating the results of honey tests, multivariate data analysis (chemometrics) become more and more to be followed [21]. The utilization of PCA (principal component analysis) has already proven to be effective in the classification of different types of honey [3, 13].

Having been collected after flowering of rape and/or acacia, some Hungarian honey samples of the 2021th year were classified by their pollen contents referring to Council Directive, 2001/110/EC into three honey types: acacia, rape and multifloral honey categories.

Based on the means of the organic and elemental components, as well as that of the physicochemical properties, these three kinds of honey are also compared to each other in order to highlight the potential honey features that support the melissopalynological distinction between the acacia and rape types. Moreover, the similarity or the comparability of the multifloral type to the other two has been revealed and shown by the application of PCA decomposition. Our statistical assessment strategy uncovered five variables of the examined ones that are able to make the two authentic honey types distinguishable.

2. Material and methods

2.1. Honey samples

A total of 149 rape and acacia honey samples were collected and posted from 67 various Hungarian apiaries in the period from June to August of the year 2021.

Having applied the Directive 2001/110/EC [18] applying of melissopalynological analysis, 44 samples were classified as rape honey, 68 samples were classified as acacia honey, and 37 samples were classified as a multifloral honey types however, considering their origins, they can be related to the flowering of acacia.

Based on our own sensory impression, the physical characteristics of multifloral honey samples like colour, scent, flavour, and liquidity resembled that of acacia honey. Additionally, the amount of acacia pollen in these samples was acceptable according to the Directive 2001/110/EC [18] but the percentage of rape pollen exceeded the threshold for acacia honey certification.

The honey samples (about 200–250 g weight) were collected in plastic container and were stored in a dark place at a low temperature of 4 °C during the analysis period in autumn of the year 2021. The low temperature and dark place were needed to slow down the Maillard-reaction. Over time at room temperature the fructose and glucose content, the value of diastase activity, the pH, colour and the electrical conductivity decrease, but the acidity and HMF content increase [22].

2.2. Melissopalynological analysis: the applied method was reported by rex Sawyer (2010) [23]

Ten g of honey sample was measured from the homogenized sample into a 50 ml centrifuge tube and 20 ml of distilled water was added to it. The honey solution was centrifuged (Hettich Zentrifugen EBA 21 Germany) for 10 min (RCF (Relative Centrifugal Force) = 1000) because of the sedimentation of pollen content. Having been rested for 3–5 min, the supernatant was poured off. This sample preparation action was repeated again adding 10 ml of distilled water. The remaining solution in the centrifuge tube (20–30 ml, depending on the honey sample) was applied with a micropipette to a glass slide prepared with lacquer felt, evenly distributed by drawing 20 × 20 mm cover plates around it. The sample applied to the slide was dried at 40 °C (Stuart hotplate stirrer SB162-3, UK). Afterward, the sample was stained with basic fuchsin dissolved in 20 % ethanol. The sample prepared in this way must be evaluated within five days. We also prepared a permanent sample from the pollen sample for later analysis. During this process, heated liquid glycerin gelatin was applied to the surface of the dried pollen preparation on the slide [23,24].

2.3. Determination of physico-chemical parameters of honey

Moisture content and degree Brix were determined by applying refractometric method (ATC-3 Portable refractometer, China). The measurements were performed in triplicate, and the data were adjusted to the standard temperature of 20 °C [16,25,26].

pH [27] and acidity measurements were carried out by the Hungarian standard of MSZ 6943/3–80 [28]. In case of pH determination 10 g sample was diluted in 25 ml distilled water at 20 °C and a pH meter (CyberScan 510 PC pH meter with Hanna Instruments HI 1131 electrode, Germany) was applied. For determination of acidity 100 ml of 10 w%-honey solution was titrated with 0.1 M NaOH solution using bromothymol indicator.

Electrical conductivity measurement (Radelkis OK-114; with Radelkis OK-0907P electrode, Hungary) was performed by using a 20

w%-honey solution at a temperature of 20 °C [5,27,29].

Diastase activity was defined by the Hungarian protocol MSZ-6943/6–81 [30]. A 20 % w/v solution was prepared from a homogenized honey sample. To neutralize the solution based on the pre-calculated acidity, Na_2CO_3 was added. The dilution series were repeated in triplicate, and the diastase number was determined based on the colour reaction [26].

HMF (hydroxymethyl-furfural) content was determined by Hungarian protocol MSZ 6943/5–1989 [31]. 5g of honey sample was 10-fold diluted, after filtration samples were measured photometrically (Shimadzu UV-2600 Spectrophotometer (Shimadzu Co, Japan), 284–336 nm) [25,27,32].

Chromatographic separation was used in order to determine the concentrations of the fructose, glucose and sucrose. 2.5 g of honey sample was diluted in 50 ml 50:50 acetonitrile-distilled water solution. The analytical system consisted of Shimadzu LC-20 chromatograph (Shimadzu Co, Japan), applied column was Waters XBridge BEH amide, 250 mm × 4.6 mm, 5 μm, 40 °C stationary phase and the mobile phase was a mixture of acetonitrile:distilled water:ammonium with a ratio of 75:25:0.1. The injected volume was 10 μl. Qualitative analysis was done by RID detector [27,33].

2.4. Microelement analysis

Determination of elemental composition was performed by ICP-OS method. Approximately 0.4 g of homogenized samples were weighed to three decimal places and placed in Teflon-coated (iPrep) digestion vessels. 10 mL of HNO_3 (65 % w/w, 1.41 g/cm^3, CARLO ERBA Reagents S.A.S.) was added, followed by a waiting period of 30 min. Subsequently, the samples were digested in a Mars 6 iWave microwave digester (MARS 6240/50, CEM, Matthews, NC, USA) at 205 °C for 15 min, with a 25-min heating time. To the destructived samples, 2 mL of H_2O_2 (30 % w/w, 1.11 g/cm^3, Scharlab S.L.) was added. The contents of the Teflon vessels were transferred to 50 mL measuring cylinders using ultrapure deionized water and then filtered through ashless filter paper (MN 640 m).

The analysis of the prepared samples was carried out using an inductively coupled plasma optical emission spectrometer (iCAP 6300 Duo ICP-OES, Thermo Fisher Scientific Inc, Waltham, MA, USA). The calibration curve was obtained using Merck multi-element standard solution (Certipur® ICP multi-element standard solution IV 1000 mg/L) [14,34].

2.5. Statistical analysis

Our evaluation strategy involves (a) normality investigation of the variable (indicator) distributions of the honey samples and determining the outliers, (b) the confidence intervals of the variables with normality, (c) comparisons of the means of the various honey types by Student t-test, (d) correlation analysis according to honey types, (e) PCA (principal component analysis), (f) determination of Wilks lambda values between honey groups in the coordinate system of the PCs. To the execution of the evaluation strategy, the software background was composed of Windows Excel, Chemometrics-Add-In, StatsKingdom online [35] and Scilab FACT

Table 1
Results of the physicochemical parameters of the analysed honey samples and literatural data [1–3,11,15,26,27,29,37,38].

		Water content	Brix	Acidity	pH	Electrical Conductivity	Sucrose	Fructose	Glucose	F/G ratio	HMF	Diastase activity
		(%)	(%)	(NaOH ml/100g)		(mS cm^{-1})	(%)	(%)	(%)		(mg kg^{-1})	Goethe unitsg^{-1}
Acacia honey	mean	**15.24**	**83.28**	**0.91**	**3.77**	**0.14**	**1.99**	**40.84**	**26.48**	**1.55**	**3.60**	**8.15**
	min-max	**13.5 - 17.5**	**80.6 - 85.0**	**0.6 - 1.35**	**3.54 - 3.99**	**0.1 - 0.19**	**0.07 - 0.87**	**38.66 - 46.7**	**19.9 - 31.87**	**1.29 - 1.71**	**0.04 - 7.05**	**2.5 - 10.9**
Literature data	*[27]*	18.04			3.76	0.14	0.45	42.60	29.70		2.12	18.40
	[26]	16.91				0.16	0.55				4.34	11.82
	[11]	16.40			3.98	0.14	1.37	39.50	30.10		1.86	24.80
	[29]	16.40				0.60	0.30	37.00	31.80	1.20	1.70	24.40
	[2]	15.96			4.31	0.12	0.45	37.19	25.93	1.42	18.97	
Rape honey	mean	**16.07**	**82.37**	**1.16**	**3.74**	**0.19**	**0.30**	**38.26**	**38.84**	**0.99**	**2.19**	**10.70**
	min - max	**13.5 - 18.0**	**80.5 - 84.5**	**0.85 - 1.5**	**3.56 - 3.97**	**0.13 - 0.28**	**0.0 - 0.69**	**33.09 - 41.64**	**33.4 - 43.33**	**0.83 - 1.09**	**0 - 5.0**	**5.0 - 17.9**
Literature data	*[37]*	17.30			4.10	0.20		37.60	37.30	1.01		14.00
	[11]	19.00			4.05	0.17	3.85	40.20	30.30		2.95	11.10
	[29]	17.00				0.24	0.10	37.00	36.50	1.00	2.50	16.00
	[2]	17.31			4.11	0.15	ND	35.23	36.09	0.97	9.48	
		B	**K**	**Mg**	**Mn**	**Na**	**Zn**	**Al**	**Cu**	**Ca**	**Fe**	**Ni**	**Pb**
		(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Acacia honey	mean	**4.72**	**225.42**	**6.82**	**0.24**	**26.15**	**1.50**	**4.08**	**0.13**	**20.13**	**5.07**	**0.17**	**0.03**
	min-max	**1.87 - 8.11**	**28.39 - 354.2**	**0.63 - 14.63**	**0.08 - 0.48**	**0.01 - 91.39**	**0.01 - 6.15**	**0.01 - 13.04**	**0.01 - 0.55**	**0.01 - 66.38**	**0.01 - 17.04**	**0.01 - 0.7**	**0.01-0.08**
Literature data	*[27]*	3.05	185.00	5.09		3.44	1.14				0.27		
	[1]	4.89	164.00	6.57	0.13	5.23	1.82	0.48	0.15	20.90	0.68		
	[15]	5.30	327.90	10.40	3.30	23.50	2.60	1.60		28.10			0.50
	[26]		325.54	22.01	0.17	95.85	7.12	1.55	0.33	111.24	1.23	0.45	0.05
	[3]	2.99	226.56	5.24	0.12	5.99	0.15		<0,1	12.39	<0,05		
Rape honey	mean	**9.41**	**242.81**	**18.68**	**0.04**	**21.20**	**0.59**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
	min-max	**4.58 - 17.79**	**23.54 - 436.2**	**10.69 - 31.27**	**0 - 0.14**	**0.01 - 76.84**	**0.01 - 2.31**						
Literature data	*[1]*	10.00	209.00	18.30	0.14	11.20	2.39						
	[15]	11.50	399.40	19.20	0.90	22.80	2.30						
	[38]		310.59	23.39	0.49		0.53						

Comparing the measured values with the literature data by using the Grubbs test, we found outlier values are signed by grey background.

Comparing the measured values with the literature data by using the Grubbs test, we found outlier values are signed by grey background.

1.4.2 chemometrics extension.

3. Results and discussion

3.1. Physicochemical and elementary analysis of honey samples

Among the investigated honey samples, the results of monofloral samples were compared with the literature data. The comparison was done by using the Grubbs test [36] which can give information about the conformity of the literature data to ours.

The summarized results of honey samples (149; see the data in the Supplementary), which include that of the 11 most important and commonly used physico-chemical variables (or indicators) together with the relevant literature data, are presented in the table (Table 1). The average values of all variables befit to the current EU limits [18].

In the case of acacia honey, with respect to physico-chemical properties, water content, sucrose, fructose, glucose, and F/G ratio match to the literature data. pH, conductivity, and HMF content showed minimal differences. The lower diastase activity measured in acacia honey may be attributed to the abundant nectar production, resulting in fewer enzymes being added to the nectar in the honey stomach. Our acacia honey data aligns with the authentic acacia honey ranges reported in the literature [27].

For rape honey, unlike acacia honey, differences were observed in sucrose, glucose, and HMF content, but all other test results match the reported literature averages. Furthermore, it is important to highlight that no significant differences were observed when our results were compared with that of any publication.

There were no literature data found for the refractometer-determined Brix% (total sugar content) and the acid degree values.

Different varieties of honey contain very deviating concentrations of minerals, which are influenced by botanical origin, geographical source, and the composition of soil, water, and rocks [34,39]. In the elemental analysis, 12 element contents of the honey samples were measured. For acacia honeys, every measured data was comparable to the literature values. In contrast, for several rape honeys, we obtained the results under the detection limit.

On comparing the measured values of acacia honey (B, K, Mg, Mn, Na, Zn, Al, Cu, Ca, Fe, Ni, Pb) with the literature data by using the Grubbs test, we found outlier values (signed by grey background) for Mn, Zn, and Ca content in one instance each.

For rape honey, among the meaningful elemental results (B, K, Mg, Mn, Na, Zn), we observed outlier values in the Mn content when compared to our own data.

The elemental composition can also influence the colour of honey; in general, the lighter the honey, the lower its mineral content [40].

3.2. Data analysis of samples

t-test (normally distribution)

For the application of *t*-test to the comparisons of the means of honey variables, 28 of 44 rape honey samples and 29 of 68 acacia honey samples were picked out because of eliminating the lacking data. Those honey samples were only involved into the further

Table 2
Results of two-pair *t*-test analysis of honey types.

	B	K	Mg	Water content	Brix	Acidity	pH	Electrical Conductivity	Sucrose	Fructose	Glucose	F/G ratio	Diastase activity	HMF
	(mg kg⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)	(%)	(%)	(NaOH ml/100g)		(mS cm⁻¹)	(%)	(%)	(%)		Goethe units g⁻¹	(mg kg⁻¹)
Acacia honey	4.518 a	218.499 a	6.896 a	14.913 a	83.495 a	0.876 a	3.781 a	0.138 a	0.582 a	41.178 a	26.482 a	1.548 a	8.189 a	2.846 a
Acacia n	67	62	62	42	43	37	45	44	52	59	61	59	44	44
Acacia SD	1.82	42.87	3.43	0.95	1.04	0.08	0.10	0.02	0.69	2.60	2.64	0.10	1.47	2.20
Rape honey	7.709 b	226.271 b	17.482 b	16.065 b	82.374 b	1.141 b	3.738 a	0.185 b	0.133 b	38.681 b	38.841 b	1.001 b	10.695 b	1.592 b
Rape n	36	42	44	42	44	37	36	33	29	35	38	33	40	38
Rape SD	1.93	52.26	5.68	1.11	1.16	0.16	0.13	0.02	0.09	1.52	2.20	0.05	3.84	1.32
Acacia honey	4.518 a	218.499 a	6.896 a	14.913 a	83.495 a	0.876 a	3.781 a	0.138 a	0.582 a	41.178 a	26.482 a	1.548 a	8.189 a	2.846 a
Acacia n	67	62	62	42	43	37	45	44	52	59	61	59	44	44
Acacia SD	1.818	42.872	3.431	0.946	1.043	0.079	0.103	0.019	0.692	2.597	2.639	0.097	1.473	2.201
Multifloral honey	4.545 a	248.496 a	5.284 a	14.761 a	83.729 a	0.995 a	3.762 a	0.141 a	0.441 a	39.599 a	26.111 a	1.501 a	9.795 b	3.543 a
Multifloral n	36	34	36	22	21	22	22	22	29	30	30	31	22	23
Multifloral SD	1.73	46.94	3.36	1.11	1.07	0.20	0.08	0.02	0.22	2.57	1.62	0.12	2.24	1.43
Rape honey	7.709 a	226.271 a	17.482 a	16.065 a	82.374 a	1.141 a	3.738 a	0.185 a	0.133 a	38.681 a	38.841 a	1.001 a	10.695 a	1.592 a
Rape n	36	42	44	42	44	37	36	33	29	35	38	33	40	38
Rape SD	1.93	52.26	5.68	1.11	1.16	0.16	0.13	0.02	0.09	1.52	2.20	0.05	3.84	1.32
Multifloral honey	4.545 b	248.496 a	5.284 b	14.761 b	83.729 b	0.995 b	3.762 b	0.141 b	0.441 b	39.599 b	26.111 b	1.501 b	9.795 a	3.543 b
Multifloral n	36	34	36	22	21	22	22	22	29	30	30	31	22	23
Multifloral SD	1.73	46.94	3.36	1.11	1.07	0.20	0.08	0.02	0.22	2.57	1.62	0.12	2.24	1.43

Various letter in same column represents significant difference at P ≤ 0.05 level. The Shapiro-Wilk normality investigation don't exclude the normal character of the variable distributions are indicated by grey background.

Various letter in same column represents significant difference at P ≤ 0.05 level. The Shapiro-Wilk normality investigation don't exclude the normal character of the variable distributions are indicated by grey background.

evaluations that all have measured values of the all investigated variables. It is necessary to highlight that missing some honey samples out of all ones did not produce significant variations in the variable means in statistically sense.

To reveal the statistical differences of the variable means among the honey types two-pair *t*-test was executed.

The *t*-test requires the normality of data, thus after their outlier analysis, we checked the distributions of the variables of the datasets ([35] StatsKingdom: www.statskingdom.com). Addressed as investigated honey properties, the variables deviating from normal distribution are marked in the table of Supplementary file (H0 or H1 hyphotesis; see in Supplementary). Although, the values of probability variable t can be calculated for the three combination pairs of all the data in Table 2, in the non-normality cases, the results of the *t*-test are biased and thus statistically conclusions cannot be drawn out of them.

The Shapiro-Wilk normality investigation don't exclude the normal character of the variable distributions are indicated by grey background (Table 2).

At both acacia and rape honey samples, two of the 14 measured variables (K and pH) do not show significant difference, while at 12 of the others the means of the variables can significantly be differed from each other. Since the data of diastase activity, acidity and HMF show non-normality in the kind of their distributions, thus calculation of the probability variable t onto them can lead to biased comparisons.

The honey variables with normal distribution character (B, K, Mg, Water content, pH, Electrical Conductivity, Fructose, Glucose, F/G ratio) provide various values of the variable t (see in Supplementary data). Since the formula of variable t involves in the difference of the average values of compared variables, this is why, the distinguishing capability of the variables for acacia and rape honeys, can be established from the decreasing order of the variable t values. This order was obtained as next: F/G ratio > Glucose > Mg > Electrical Conductivity > B > Fructose > Water content. The bigger is the value of the variable Student t, the bigger is the probability of the significant difference between the compared means.

For acacia and multifloral honey samples, among the 14 parameters studied, 8 do not show significant deviations, but the others are significantly differentiable from each other.

When comparing rape to multifloral honey samples, 4 of the 14 studied variables do not show significant differences, but the rest 10 variables show significantly differences from each other.

Based on the two-sample *t*-test, the multifloral honey group has been established to show fewer significant deviations in the variables compared to acacia honey samples than in its comparison to rape honey samples.

No significant differences were found in pH values in any pairwise comparisons, while the F/G ratio, Mg, and acid degree showed significant differences in all comparisons between pairs.

The distribution investigation revealed the non-normality of HMF content and diastase activity. Both variables in honey samples are affected by the honey pre-treatment processes [41].

The non-normal distribution of sucrose content in acacia honey can be explained by the fact that acacia's main flowering period is short (7–10 days), so local weather conditions can significantly affect the efficiency of honey collection. Acacia flowers typically provide abundant and complex sugars in their nectar, and under favourable weather conditions, bees can collect a significant amount of nectar. However, influenced by environmental condition, the enzymes required for the breakdown of complex sugars may not have a sufficient activity that can lead to only partial fermentation of sugar molecules, what can also explain the non-normal distribution of honey samples' acidity.

The standard deviations of the paired t-tests for acacia and rape honey varieties reveal that the most significant difference between the two types of honey is in terms of carbohydrates (glucose, $t_{AB} = 24.115$, and $t_{crit}(AB) = 1.9847232$; the F/G ratio, $t_{AB} = 29.793$, and $t_{crit}(AB) = 1.9866745$). There are also notable differences in the standard deviations of acidity ($t_{AB} = 9.0844$, $t_{crit}(AB) = 1.9934636$) and conductivity ($t_{AB} = 9.7641$, $t_{crit}(AB) = 1.9921022$).

Table 3
Correlation matrices of Acacia and Rape honey samples.

	B	K	Mg	Water content (%)	Brix (%)	Acidity	pH	El. Cond. ($mS\ cm^{-1}$)	Sucrose (%)	Fructose (%)	Glucose (%)	F/G ratio	Diastase activity	HMF	Acacia pollen	Rape pollen
B		-0.28	0.118	0.155	-0.178	-0.1	-0.309	0.207	-0.135	0.284	0.361	-0.195	-0.465	-0.21	0.12	-0.069
K	-0.268		0.182	0.074	-0.09	0.649	0.021	0.457	-0.208	-0.002	0.074	-0.078	0.461	0.384	-0.21	0.139
Mg	-0.077	0.596		-0.012	-0.027	0.353	-0.225	0.309	0.229	0.298	0.263	-0.12	0.092	0.024	0.089	-0.092
Water content (%)	-0.063	-0.15	-0.15		-0.992	0.304	-0.124	0.168	-0.373	-0.174	0.233	-0.41	-0.018	-0.362	0.087	-0.327
Brix (%)	0.064	0.098	0.074	-0.971		-0.343	0.165	-0.226	0.362	0.132	-0.267	0.423	0.009	0.354	-0.089	0.305
Acidity	-0.327	0.114	0.033	0.175	0.008		-0.244	0.631	-0.128	0.169	0.309	0.36	0.101	-0.047		-0.212
pH	-0.123	0.419	0.281	-0.355	0.285	-0.267		-0.793	-0.017	-0.393	-0.544	0.393	-0.009	0.175	-0.06	0.068
El. Cond. ($mS\ cm^{-1}$)	-0.413	0.566	0.282	-0.159	0.195	0.113	0.128		-0.094	0.305	0.514	-0.418	0.182	0.048	-0.073	-0.056
Sucrose (%)	-0.181	0.004	-0.321	0.146	-0.068	-0.029	0.132	0.137		0.192	0.164	-0.072	-0.327	-0.139	0.322	-0.07
Fructose (%)	-0.13	0.315	0.416	-0.019	0.008	-0.378	0.089	0.273	-0.144		0.561	0.053	-0.311	0.184	0.189	-0.145
Glucose (%)	0.119	-0.229	0.204	0.249	-0.277	0.166	-0.333	-0.004	-0.288	0.033		-0.792	-0.213	-0.338	0.115	-0.449
F/G ratio	-0.133	0.361	0.102	-0.164	0.185	-0.381	0.277	0.155	0.138	0.645	-0.728		0	0.576	-0.016	0.466
Diastase activity	0.236	0.413	0.472	-0.37	0.429	0.083	-0.008	0.253	-0.192	0.248	0.029	0.154		0.196	-0.108	0.157
HMF	-0.395	0.142	-0.138	-0.127	0.142	-0.084	-0.165	0.235	0.167	0.282	-0.359	0.462	-0.191		-0.199	0.452
Acacia pollen	-0.144	0.214	-0.003	0.084	0.037	-0.092	-0.134	0.38	-0.024	0.284	-0.082	0.26	0.41	0.15		-0.105
Rape pollen	0.286	-0.353	-0.193	-0.251	0.18	-0.099	-0.184	-0.393	-0.127	-0.125	0.316	-0.296	-0.023	0.042	-0.151	

Upper triangle: Pearson R correlations for Acacia honey samples. Bevington criterion (0.05; 29) = 0.3675.
Lower triangle: Pearson R correlations for Rape honey samples. Bevington criterion (0.05; 28) = 0.374.

Upper triangle: Pearson R correlations for Acacia honey samples. Bevington criterion (0.05; 29) = 0.3675.
Lower triangle: Pearson R correlations for Rape honey samples. Bevington criterion (0.05; 28) = 0.374.

Similar values are observed for the paired *t*-test of rape and multifloral honeys, where the most significant difference also appears in terms of carbohydrates (glucose, $t_{AB} = 26.532$, t_{crit} (AB) = 1.9965644; the F/G ratio, $t_{AB} = 21.853$, t_{crit} (AB) = 1.9989715; for sucrose, $t_{AB} = 7.014$, tcrit (AB) = 2.0032407).

3.2.1. Correlation analysis

The honey is a biological product made by bees from various flower pollens. The parallel biochemical reactions catalysed by bee enzymes system lead to the terminal composition the particular honey. With respect to a particular flower pollen, year after year or accumulation district to district and for changing environmental condition, some small variations in honey composition can be expected and experienced that do not change the function of the bee enzymes. Therefore, correlation relationships can be assumed among the components and properties of particular honey types. To check this hypothesis the Pearson R correlation matrices for the data of acacia and rape honey samples were calculated. To the correlation analysis of the honey samples without lack of data, 24 of acacia honey samples and 28 of rape honey samples were picked out (see them in Supplementary). The next honey variables (indicators) were taken into the input matrices of correlation analysis: B, K, Mg, Water content, Brix, Acidity, pH, Electrical Conductivity, Sucrose, Fructose, Glucose, F/G ratio, Diastase activity, HMF content, Acacia pollen and Rape pollen. The united correlation matrix of acacia and rape honey samples can be seen in Table 3. The upper triangle of the table contains the Pearson R values among the acacia honey variable pairs, while the data in the lower triangle belong to that of rape honey variables.

Among the 120 combination variable pairs, there are 22 and 18 variable pairs of acacia and rape honeys that have significant values (in grey background in Table 3) with respect to their Bevington criteria [42]. It is worth noting the correlation patterns in the upper and the lower triangles can not be reflected to each other. The correlation patterns are different.

3.2.2. PCA analysis and discrimination

The correlation analysis has uncovered there are some correlations among the variables in data sets of honey samples. The existence of these correlations supplies a starting base for the application of PCA. Khansaritoreh et al. (2021) reported the substantial overlap of our measured variables in the PCA space effectively illustrates the capability to differentiate between authentic and adulterated Iranian honey types [43].

The same acacia and rape samples were utilized in the PCA decomposition that were picked out to the correlation analysis. In addition, 20 multifloral honey samples were even chosen. The variables of their input data matrices were also the same the applied ones at the correlation analysis. Because of decreasing the condition numbers of the matrices, scaling data-pretreatment was used on the tree data matrices. The scalling factors of the variables were the next: 1(B), 10(K), 1(Mg), 1(Water content), 1(Brix), 0.1(Acidity), 0.1(pH), 0.01(Electrical Conductivity), 0.1(Sucrose), 1(Fructose), 1(Glucose), 0.1(F/G ratio), 1(Diastase activity), 1(HMF), 1(Acacia pollen) and 10(Rape pollen). With the application of these scaling factors, the values of standard deviations of the scaled variables spread within the same order in magnitude (see in Supplementary). The condition numbers of three input matrices, which were calculated as the rate of the maximum and minimum of singular values, decreased from 8178, 6268 and 19850 to 153, 101 and 345.

There are two main viewpoints in the PCA decomposition of the honey data. The first was to obtain information about the separation of acacia and rape samples in the PCs space and afterward about where the multifloral samples projected into beforehand stretched PCs space appear (case A; see Fig. 1A). Similar findings were published by Wang et al. (2014). The adulteration of acacia honey with rape honey at different levels (5–50 %, w/w) has been established to end up in higher chlorogenic acid content and much
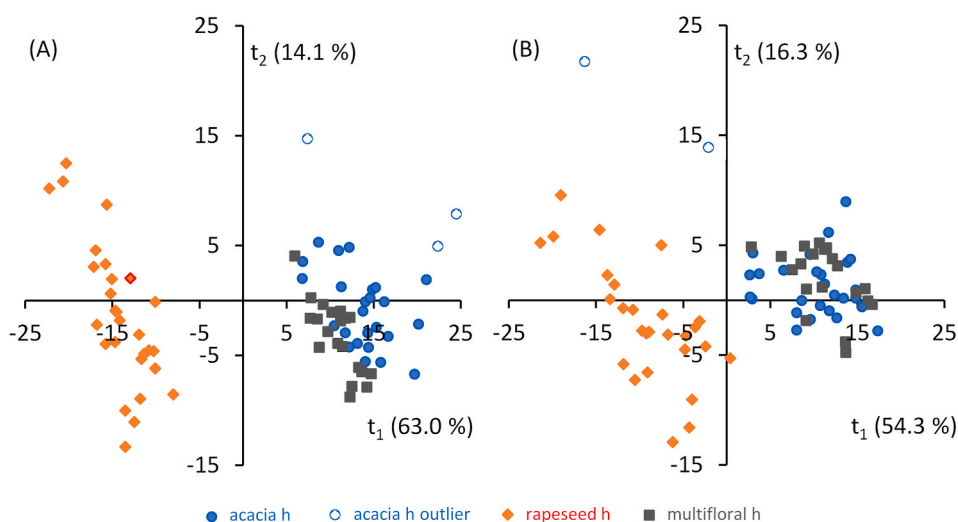

Fig. 1. The grouping of honey samples in the score plots of the first two PCs, coordinate system of t_2 versus t_1. (t_1 and t_2 are the column vectors of sore matrix T in PCA decomposition; the explained variances of t_1 and t_2 are in brackets (Fig. 1A: The input matrices include the data of the variables 'A pollen' and' R pollen'. Fig. 1B: The PCA decomposition of the measured honey variables without the variables 'A pollen' and 'R pollen'.).

lower ellagic acid content. These findings suggested chlorogenic and ellagic acids as potential markers for differentiating between acacia and rape honey types, respectively.

The second curiosity was to experience the effect of the elimination of the variables 'A pollen' and 'R pollen' out of the PCA input matrices on the samples grouping (case B; see Fig. 1B). The application of pollen data on classification of acacia honey types caused incorrect results; corroborated by the observations of the article Zieliński et al. (2014) [44].

The PC number was predicted by Kaiser's rule [45]. This estimated the significant PCs of 5. However, the multifloral samples projected into PCs coordinate system later has relevant portion of their variance along the axis of the PC 6. Thus, to investigate the separations of acacia, rape and multifloral samples in PCs space, the first 6 of PCs was chosen as the basis of discrimination.

The score plots of PCA decompositions are depicted in Fig. 1. Fig. 1A pertains to the PCA involving all the variables. Fig. 1B shows honey samples in the first two PCs after having eliminated the data of the variables 'A pollen' and 'R pollen' out of the input matrices.

As can be seen in both Fig. 1A and B, the acacia and rape honey samples are entirely separated from each other. The multifloral honey samples in Fig. 1A appear with partial overlapping at the edge of the acacia honey group. The multifloral honey samples in Fig. 1B are undistinguishable from that of acacia honey.

To quantify the discrimination extents among the three group pairs, the values of Wilks lambda were calculated for the PC spaces with various two PC numbers, as well as for the input matrices with and without the data of flower pollens. The Wilks lambda expresses the ratio of the variance within the groups to the total variance of the groups. Its value is in the range from zero to one. When it is equal to zero then the two groups are totally separated and if its value is one then the two groups are overlapped and undistinguishable from each other.

The Wilks lambda results are summarized in Table 4.

The Wilks lambda values are close enough to zero in the comparisons of both acacia-rape and rape-multifloral group pairs. The rape honey samples are separated from both acacia and multifloral samples. The Wilks lambdas for acacia and multifloral groups have high values which reflects and/or suggests overlapping group positions in the PCs coordinate systems.

4. Conclusion

In this study after having analysed the physical-chemical parameters and pollen analysis of 149 early spring honey samples from Hungary following rape and/or acacia flowering, these samples were classified into acacia, rape, and multifloral honey categories according to the Directive 2001/110/EC standard. The paired *t*-test comparison of 14 chemical variables (indicators) for rape-acacia and multifloral honeys revealed significant differences between rape and acacia honey, except for two parameters (K content and pH). Moreover, significant differences were experienced between multifloral and acacia honey in case of 6 variables and between multifloral and rape honey in 11 parameters. The most significant difference between acacia and rape honey was found in sugar composition (glucose content and F/G ratio). PCA analysis uncovered 6 principal components, in the PCs space of which acacia and rape honey samples were clearly distinct from each other, while the group of multifloral honey samples partially overlapped with that of acacia honey samples. Excluding pollen analysis results from the PCA analysis, acacia and multifloral honey groups could not clearly be differentiated. To sum up our main conclusion, the acacia variety honey classification based on the current EU Directive is to be considered too strict, and distinguishing between rape and acacia honey is advisable to also be extended to the other characteristic indictors, for example, Student t-values related to fructose/glucose ratio, glucose content, conductivity, acidity, and magnesium content.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Emese Dominkó: Writing – original draft, Investigation. **Zsolt István Németh:** Writing – review & editing, Validation, Supervision, Software. **Tamás Rétfalvi:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

Table 4

Wilks lambda values for discrimination of the three various honey groups.

PCs		PCA with pollen data		PCA without pollen data	
		t1: t2	t1: t6	t1: t2	t1: t6
Acacia Honey	Rape Honey	0.169	0.313	0.32	0.471
Acacia Honey	Multifloral Honey	0.855	0.818	0.989	0.873
Rape Honey	Multifloral Honey	0.196	0.361	0.298	0.463

wilks lambdas of honey groups in the coordinate systems of PCs.

influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30498.

References

[1] N. Czipa, B. Kovács, Electrical conductivity of Hungarian honeys, J. Food Physics 27 (2014) 13–20. http://www.foodphysics.net/journal/2014/paper_2.pdf.

[2] D. Pauliuc, F. Dranca, S. Ropcius, M. Oroian, Advanced Characterization of monofloral honeys from Romania, Agriculture 12 (2022) 526, https://doi.org/10.3390/agriculture12040526.

[3] A. Bodó, L. Radványi, T. Kőszegi, R. Csepregi, D.U. Nagy, A. Farkas, M. Kocsis, Quality evaluation of light and dark-colored Hungarian honeys, focusing on botanical origin, antioxidant capacity and mineral content, Molecules 26 (2021) 2825, https://doi.org/10.3390/molecules26092825.

[4] A. Chirsanova, T. Capcanari, A. Boistean, Palynological, physico-chemical and biologically active substances profile in some types of honey in the Republic os Moldova, J. Eng. Sci. XXVIII (3) (2021) 175–186, https://doi.org/10.52326/jes.utm.2021.28(3).14.

[5] S. Bogdanov, Nature and origin of the antibacterial substances in honey, LWT–Food Sci. Technol. 30 (1997) 748–753, https://doi.org/10.1006/fstl.1997.0259.

[6] M. Madejczyk, D. Barałkiewicz, Characterisation of honey from different areas of Poland by their physico-chemical parameters and trace elements, Anal. Chim. Acta 617 (2008) 11–17, https://doi.org/10.1016/j.aca.2008.01.038.

[7] M. Kücük, S. Kolayh, S. Karaoglu, E. Ulusoy, C. Baltaci, F. Candan, Biological activities and chemical composition of three honeys of different types from Anatolia, Food Chem. 100 (2007) 526–534, https://doi.org/10.1016/j.foodchem.2005.10.010.

[8] Zs Bodor, Z. Kovacs, Cs Benedek, G. Hitka, H. Behling, Origin Identification of Hungarian honey using melissopalynology, physicochemical analysis, and near infrared spectroscopy, Molecules 26 (2021) 7274, https://doi.org/10.3390/molecules26237274.

[9] B. Herrero, R.M. Valencia-Barrera, R. San Martín, V. Pando, Characterization of honeys by melissopalynology and statistical analysis, Article in Canadian Journal of Plant Science (2002), https://doi.org/10.4141/P00-187. January 2002.

[10] A. Bodó, L. Radványi, T. Kőszegi, R. Csepregi, D.U. Nagy, A. Farkas, M. Kocsis, Melissopalynology, antioxidant activity and multielement analysis of two types of early spring honeys from Hungary, Food Biosci. 35 (2020) 100587, https://doi.org/10.1016/j.fbio.2020.100587.

[11] J. Wang, X. Xue, X. Du, Ni Cheng, Lanzhen Chen, J. Zhao, Wei Cao, Identification of Acacia honey adulteration with honey using liquid chromatography-electrochemical detection and chemometrics, FOOD ANAL METHOD 7 (2014) 2003–2012, https://doi.org/10.1007/s12161-014-9833-7.

[12] R. Fernández-Torres, J.L. Pérez-Bernal, M.Á. Bello-López, M. Callejón-Mochón, J.C. Jiménez-Sánchez, A. Guiraúm-Pérez, Mineral content and botanical origin of Spanish honeys, Talanta 65 (2005) 686–691, https://doi.org/10.1016/j.talanta.2004.07.030.

[13] G. Di Bella, V. Lo Turco, A.G. Potortí, G.D. Bua, M.R. Fede, G. Dugo, Geographical discrimination of Italian honey by multi-element analysis with a chemometric approach, J. Food Compos. Anal. 44 (2015) 25–35, https://doi.org/10.1016/j.jfca.2015.05.003.

[14] N. Bilandžić, M. Sedaka, M. Đokić, A. Gross Bošković, T. Florijančić, I. Bošković, M. Kovačić, Z. Puškadija, M. Hruškar, Element content in ten Croatian honey types from different geographical regions during three seasons, J. Food Compos. Anal. 84 (2019) 103305, https://doi.org/10.1016/j.jfca.2019.103305.

[15] Zs Sajtos, P. Herman, S. Harangi, E. Baranyai, Elemental analysis of Hungarian honey samples and bee products by MP-AES method, Microchem. J. 149 (2019) 103968, https://doi.org/10.1016/j.microc.2019.103968.

[16] M. Kędzierska-Matysek, M. Florek, A. Wolanciuk, P. Skałecki, A. Litwińczuk, Characterisation of viscosity, colour, 5-hydroxymethylfurfural content and diastase activity in raw rape honey (Brassica napus) at different temperatures, J FOOD SCI TECH 53 (4) (2016) 2092–2098, https://doi.org/10.1007/s13197-016-2194-z.

[17] Á. Farkas, E. Zajácz, Nectar production for the Hungarian honey industry, Eur. J. Plant Sci. Biotechnol. 1 (2007) 125–151. http://www.globalsciencebooks.info/Online/GSBOnline/images/0712/EJPSB_1(2)/EJPSB_1(2)125-151o.pdf.

[18] EC, Council directive 2001/110/EC of 20. December 2001. Relating honey, Official Journal of European Comminities 12 (1) (2001), 2002 L10/47-52, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32001L0110&from=EN.

[19] B. Szabó, E. Vincze, B. Czúch, Flowering phenological changes in relation to climate change in Hungary, Int. J. Biometeorol. 60 (2016) 1347–1356, https://doi.org/10.1007/s00484-015-1128-1.

[20] J. Pullens, M. W, B. Sharif, M. Trnka, J. Balek, A.M. Semenov, J.E. Olesen, Risk factors for European winter oilseed rape production under climate change, AGR FOREST METEOROL 272–273 (2019) 30–39, https://doi.org/10.1016/j.agrformet.2019.03.023.

[21] C.I. Mureşan, M. Cornea-Cipcigan, R. Suharoschi, S. Erler, R. Mărgăoan, Honey botanical origin and honey-specific protein pattern: characterization of some European honeys, LWT–Food Sci. Technol. 154 (2022) 112883, https://doi.org/10.1016/j.lwt.2021.112883.

[22] P.M. da Silva, L.V. Gonzagaa, F.C. Bilucaa, M. Schulza, L. Vitalib, G.A. Mickeb, A. Costaa, O. C, R. Fetta, Stability of Brazilian *Apis mellifera L.* honey during prolonged storage: physicochemical parameters and bioactive compounds, LWT–Food Sci. Technol. 129 (2020) 109521, https://doi.org/10.1016/j.lwt.2020.109521.

[23] R. Sawyer, Honey Identification, Cardiff Academic Press, 2010, 978-1-904846-53-6.

[24] M. Pendleton, Descriptions of melissopalynological methods involving centrifugation should include data for calculating relative Centrifugal Force (RCF) or should express data in units of RCF or gravities (g), Grana 45 (2006) 71–72, https://doi.org/10.1080/00173130500520479.

[25] S. Bogdanov, Harmonised methods of the international honey commission, International Honey Commission (2009) 1–63.

[26] N. Uršulin-Trstenjak, D. Puntarić, D. Levanić, V. Gvozdić, Ž. Pavlek, A. Puntarić, E. Puntarić, I. Puntarić, D. Vidosavljević, D. Lasić, M. Vidosavljević, Pollen, physicochemical, and mineral analysis of Croatian Acacia honey samples: applicability of identification of botanical and geographical origin, J FOOD QUALITY (2017) 11, https://doi.org/10.1155/2017/8538693. *Article ID* 8538693.

[27] N. Czipa, C.J.C. Phillips, B. Kovács, Composition of acacia honeys following processing, storage and adulteration, J. Food Sci. Technol. 56 (2019) 1245–1255, https://doi.org/10.1007/s13197-019-03587-y.

[28] MSZ 6943/3-80. (Hungarian protocol 1980b) Méz kémiai és fizikai vizsgálata. Savfok És pH Meghatározása. (Chemical and Physical Analysis of Honey: Determination of Acidity and pH.).

[29] M. Pospiech, Z. Javůrková, P. Hrabec, H. Čížková, D. Titěra, P. Štarha, S. Ljasovská, V. Kružík, T. Podskalská, J. Bednář, P.K. Burešová, B. Tremlová, Physico-chemical and melissopalynological characterization of Czech honey, Appl. Sci. 11 (2021) 4989, https://doi.org/10.3390/app11114989.

[30] MSZ-6943/6-81. (Hungarian protocol 1981) Méz kémiai és fizikai vizsgálata. Diasztáz-aktivitás meghatározása. (Chemical and Physical Analysis of Honey: Determination of Diastase Activity.).

[31] MSZ 6943/5-1989. (Hungarian protocol 1989) Méz fizikai és kémiai vizsgálata. Hidroximetilfurfurol tartalom (HMF) meghatározása. (Physical and Chemical Analysis of Honey: Determination of Hydroxymethylfurfural (HMF) Content.).

[32] J. White, Spectrophotometric method for hydroxymethylfurfural in honey, J Assoc Off Anal Chem 62 (1979) 509–514, https://doi.org/10.1093/jaoac/62.3.509.

[33] AOAC Official Methods of Analysis, AOAC official method 977.20 separation of sugars in honey liquid chromatographic method, in: Sugars and Sugar Products, 1995, p. 25 (chapter 44).

[34] A. Pisani, G. Protano, F. Riccobono, Minor and trace elements in different honey types produced in Siena County (Italy), Food Chem. 107 (2008) 1553–1560, https://doi.org/10.1016/j.foodchem.2007.09.029.

[35] StatsKingdom. www.statskingdom.com. (Accessed 19 January 2024).

[36] F. Grubbs, Procedures for detecting outlying observations in samples, Technometrics 11 (1969) 1–21, https://doi.org/10.1080/00401706.1969.10490657.

[37] T. Szczęsna, H. Rybak-Chmielewska, E. Waś, K. Kachaniuk, D. Teper, Characteristics of polish unifloral honeys. I. Rape honey (Brassica napus L. Var. Oleifera metzger), J APIC SCI 55 (2011) 111–119. https://www.researchgate.net/profile/Katarzyna-Jaskiewicz-6/publication/358446230_Characteristics_of_Polish_unifloral_honeys_I_Rape_honey/links/6202fa5d82638c2de5204c63/Characteristics-of-Polish-unifloral-honeys-I-Rape-honey.pdf.

[38] M. Dżugan, G. Zaguła, M. Wesołowska, P. Sowa, C. Puchalski, Levels of toxic and essential metals in varietal honeys from Podkarpacie, J ELEM 22 (2017) 1039–1048, https://doi.org/10.5601/jelem.2016.21.4.1298.

[39] Zs Sajtos, T. Varga, Z. Gajdos, P. Burik, M. Csontos, Zs Lisztes-Szabó, A. Jull, T, J, M. Molnár, E. Baranyai, Rape, sunflower and forest honeys for long-term environmental monitoring: presence of indicator elements and non-photosynthetic carbon in old Hungarian samples, Sci. Total Environ. 808 (2022) 152044, https://doi.org/10.1016/j.scitotenv.2021.152044.

[40] M.L. González-Miret, F. Ayala, A. Terrab, J.F. Echávarri, A.I. Negueruela, F.J. Heredia, Simplified method for calculating colour of honey by application of the characteristic vector method, Food Res. Int. 40 (2007) 1080–1086, https://doi.org/10.1016/j.foodres.2007.06.001.

[41] N. Pasias, K.G. Raptopoulou, G. Makrigennis, D.D. Ntakoulas, D. Lembessis, V. Dimakris, R. Katsinas, C. Proestos, Finding the optimum treatment procedure to delay honey crystallization without reducing its quality, Food Chem. 381 (2022) 132301, https://doi.org/10.1016/j.foodchem.2022.132301.

[42] P.R. Bevington, Data Reduction and Error Analysis for the Physical Sciences, McGraw-Hill Book Co., New York, 1969.

[43] E. Khansaritoreh, Y. Salmaki, T.A. Azirani, F. Henareh, K. Alizadeh, E. Ramezani, S. Zarre, G. Beckh, H. Behling, The sources and quality of Iranian honey, Heliyon (2021) e06651, https://doi.org/10.1016/j.heliyon.2021.e06651.

[44] Ł. Zieliński, S. Deja, I. Jasicka-Misiak, P. Kafarski, Chemometrics as a tool of origin determination of polish monofloral and multifloral honeys, J. Agric. Food Chem. 62 (13) (2014), https://doi.org/10.1021/jf4056715, 2973–298.

[45] H.F. Kaiser, The application of electronic computers to factor analysis, Educ. Psychol. Meas. 20 (1960) 141–151, https://doi.org/10.1177/001316446002000116.