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Physicochemical characteristics of Bulgarian bee honey: Part 1

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Abstract

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The present study investigates 59 representative samples of monofloral honey (Acacia 12 pcs., Coriander 12 pcs., Limetree 12 pcs., Rapeseed 11 pcs., Sunflower 12 pcs.) and 30 samples of multifloral honey, crop 2019, from various apiaries on the territory of the Republic of Bulgaria. The changes in the following physicochemical parameters (Active acidity (pH); Electrical conductivity, μS/sm; Water content, %; Refractive index; Fructose + Glucose (F+G) content, %) have been studied. The obtained average values of the above parameters conform to the requirements of Council Directive 2001/110/EC relating to honey and Regulation on requirements to bee honey intended for human consumption. Statistically significant differences have been observed between the physicochemical parameters of monofloral and multifloral honey. The values of the coefficients of determination (R^2) show that 77.8% of the variations in the variable for the parameter (pH) and from 22.4 – 36.5% in the other studied parameters are due to the effect of the type of honey.

Keywords: honey; physicochemical parameters; statistically significant differences

Inroduction

According to Council Directive 2001/110/EC – Annex 1 (2002) and the Bulgarian Regulation on the requirements to bee honey intended for human consumption (2002) honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature. Bee honey composition is influenced by various factors – plant origin, environmental characteristics, use of medications by bee-keepers, etc. (Shkenderov & Ivanov, 1983; Abu-Tarboush et al., 1993; Da Costa Leite et al., 2000; Kaškonienė et al., 2010; EL-Metwally, 2015). Bee honey contains a big amount of sugars and when consumed it supplies the body with a lot of energy (Rahman et al., 2010). Shkenderov & Ivanov (1983) reported that carbohydrate content in honey 95 – 99% of dry matter.

According to Bogdanov et al. (2004), there are over 22 sugars in honey, with fructose and glucose being in the greatest amount. The total amount of glucose + fructose reaches 75 – 80%. The ratio between them is typical of the different types of honey, in most cases it is over 1.0 and is the reason for the crystallization of honey (Shkenderov & Ivanov, 1983; Manikis & Thrasivoulou, 2001; Kaškonienė et al., 2010; Buba et al., 2013).

Bee honey from most plants contains under 5% of saccharose, sometimes (acacia, lavender, honeydew honey) – 10% (Shkenderov & Ivanov, 1983; Council Directive 2001/110/EC – Annex 2 (2002); Regulation on the requirements to bee honey intended for human consumption (2002).

In addition to easily digestible sugars, bee honey also contains amino acids, organic acids, aromatic substances, minerals, colorants (Shkenderov & Ivanov, 1983; Bogdanov et al., 1998; Redtke & Hadtke, 1998; Jasim et al., 2007). The amount of acids (total acidity) changes within a significant range depending on the type of honey (Shkenderov & Ivanov, 1983). These authors found that active acidity (pH) of honey varies from 3.2 to 6.5.

Water content of bee honey is within the limits between 13 and 23% and the duration of the product storage depends on it (Beckh, et al., 2004; Bogdanov, 2009; Buba et al., 2013; Akhtar et al., 2014; Kivrak et al., 2017; Krishnan et al., 2021). Some biologically active substances such as enzymes and compounds of hormonal nature, vitamins, etc., are also found in bee honey (Yilmaz & Yavuz, 1999; Qiu et al., 1999).

The information in the specialized literature accounts for the existence of differences relating to the chemical composition of and some physicochemical properties of the various types of bee honey, such as mineral and sugar content, electrical conductivity, pH and refraction coefficient (Bogdanov et al., 1987, 1999; Golob & Plestenjak, 1999; Sanjuan et al., 1997).

Based on the information mentioned in the studies of numerous authors when determining the physicochemical parameters of bee honey, the author's team thinks it is justifiable to expand investigations on that topic.

The objective of the present paper is to determine some physicochemical parameters in Bulgarian bee honey and to establish the statistically significant differences between the different types of honey. This is necessary for a good knowledge of certain quality parameters of different types of honey, the production conditions, proper storage, and possible changes in their composition to prevent their adulteration and satisfy the growing consumer requirements.

Material and Methods

Physicochemical analysis of honey samples of various origin

The study uses 89 representative samples from bee honey, crop 2019 from various apiaries on the territory of Bulgaria. Until the beginning of the study, the honey samples were stored in glass containers, in the dark, under room conditions.

The type of honey samples included in the study is determined in advance by pollen analysis in the Central Scientific Research Laboratory at Trakia University, Stara Zagora, Bulgaria, following Bulgarian State Standard (BSS) 3050/80 – Bee honey (1980). The groups of monofloral honey are determined according to the percentage of pollen grains in them. For coriander, rapeseed and sunflower honey not less than 45%, and for acacia and linden honey not less than 30% (Ivanov, 2006). Based on the pollen analysis of the copper samples participating in the experiment, the following groups were determined:

– monofloral honey – Acacia 12 pcs., Coriander 12 pcs., Linden 12 pcs., Rapeseed 11 pcs., Sunflower 12 pcs.

– multifloral honey 30 samples.

The physicochemical test was carried out at the Central Scientific Research Laboratory at Trakia University via the "Thermo Scientific Surveyor Plus" system following the harmonized methods of the International Honey Commission (Bogdanov et al., 1997) and the methods described in BSS 3050-80 and Regulation No. 48/2003.

The following physicochemical parameters have been determined:

– glucose (%), fructose (%) and saccharose (%) content – via highly effective liquid chromatography, and the detector used is differential rafractometer;
– electrical conductivity (**µS** /cm) by means of a Conductometer with an electrical conductivity cell;
– refraction and water content coefficient (%) by means of Abbe refractometer;
– active acidity (pH) by means of pH-meter;

Based on the results obtained concerning glucose and fructose content in honey samples the sum total of Glucose + Fructose has been calculated, a parameter included in Regulation on the requirements to bee honey intended for human consumption (2002).

Data analysis

Statistical data analysis includes obtaining the main statistics (mean values, Standard Deviations – SD, and Coefficient of variations) for the observed physicochemical parameters (Active acidity (pH); Electrical conductivity, µS/sm; Water content, %; Refractive index; Fructose + Glucose (F+G) content, %), a normality distribution verification of the datasets via Kolmogorov-Smirnov test, and multivariate ANOVA, applied to calculate the significant differences between the varied types of honey, based on mean values of the examined physicochemical parameters. The presence of significant differences was assumed by the Post Hoc multiple comparisons with Dunnett T3 test (depending on Levene's test of equality of error variances) at p-value < 0.05. The IBM SPSS Statistics 17.0 package was used to process the data (SPSS Statistics, 2007).

Results and Discussion

The basic statistical data (mean values and Standard Deviations) of the studied physicochemical parameters (Active

acidity (pH); Electrical conductivity, µS/sm; Water content, %; Refractive index; Fructose + Glucose (F+G) content, %) are presented in Table 1. Figure 1 visualizes the statistically significant differences between the different types of honey, based on the average value of the physicochemical parameters.

Limetree honey samples are characterized by high values of *Active acidity (pH)* – 4.12±0.507. In acacia, rapeseed, sunflower, and multifloral honey the values of the specified parameter are within the range from 3.2 to 6.5 according to Shkenderov & Ivanov (1983). Low Active acidity (pH) value is reported for coriander honey – 1.93±0.049. As is evident from Figure 1a and Table 1 for the Active acidity (pH) parameter, the most significant are the differences between coriander honey and all other types of honey. Significant differences are observed between sunflower honey and all other types of honey. Statistically significant are the differences between limetree honey and rapeseed honey. The coefficient of determination is $R^2 = 0.778$ (Table 1), which means that about 77.8% of the variations in the parameter pH depend on the types of honey. The remaining 22.2% of the variations are due to the effect of other factors not included in the study.

The analysis of the results in the studied honey samples concerning the parameter *Electrical conductivity* (Table 1) shows that the obtained values conform to the requirements of Regulation on the requirements to bee honey intended for human consumption (2002) and the Bogdanov et al. (1997) – in nectar honey not more than 800 µS/sm. It is evident from Figure 1b that Coriander honey differs significantly from all other types of honey. Significant differences have been registered between rapeseed honey and all other types except acacia honey. Limetree honey differs significantly from all other types of honey except multifloral honey. The coefficient of determination of the Electrical conductivity parameter is $R^2 = 0.365$ (Table 1), i.e. about 36.5% of the variations are due to the effect of the honey type.

Water content in all analyzed honey samples ranges from 16.67±0.429% in coriander honey to 19.07±0.858% in limetree honey. They are within the variation range for Bulgarian bee honey from 15 – 23% (Shkenderov & Ivanov, 1983) and conform to the criteria of the Regulation on the requirements to bee honey intended for human consumption (2002) and the Bogdanov et al. (1997) – content less than 20%. By the Water content parameter (Figure 1c), limetree honey differs significantly from all other types of honey except the rapeseed honey. Acacia honey and coriander honey differ from limetree and rapeseed honey. By that parameter rapeseed, honey significantly differs from multifloral honey as well.

Table 1. Basic statistics of observed physicochemical parameters of the varied types of honey

Type of Honey		Parameters				
		Active acidity (pH)	Electrical conductivity, µS/sm	Water content, %	Refractive index	Fructose and Glucose content, %
Acacia (*Robinia pseudoacacia*) (n = 12)	Mean	3.61^{a}	376.00 ^{a}	17.17 ^{a}	1.49 ^{c}	56.89 ^{a}
	SD	0.278	172.898	0.470	0.001	11.455
	CV	0.83	45.98	2.74	0.07	20.14
Coriander (*Coriandrum sativum*) (n = 12)	Mean	1.93 ^{ab}	490 ^{ab}	16.67 ^{b}	–	66.36 ^{b}
	SD	0.049	80.37	0.429	–	2.854
	CV	2.54	16.52	2.57	–	4.3
Limetree (*Tilia sp.*) (n = 12)	Mean	4.12 ^{bc}	639.67 ^{abc}	19.07 ^{abc}	1.49 ^{c}	61.90
	SD	0.507	163.419	0.858	0.002	17.531
	CV	12.31	25.55	4.5	0.13	28.32
Rapeseed (*Brassica napus*) (n = 11)	Mean	3.47 ^{bce}	243.09 ^{bcde}	18.72 ^{abd}	1.49	65.55
	SD	0.187	114.321	1.365	0.004	13.541
	CV	5.39	47.03	7.29	0.27	20.66
Sunflower (*Helianthus annuus*) (n = 12)	Mean	3.20 ^{abcde}	453.50 ^{bce}	17.58 ^{c}	1.49 ^{c}	76.20 ^{abd}
	SD	0.094	54.355	1.228	0.003	2.720
	CV	2.94	11.99	6.99	0.20	3.57
Multifloral (n = 30)	Mean	3.7 ^{bd}	669.47 ^{bd}	17.03 ^{cd}	1.49 ^{c}	66.90 ^{d}
	SD	0.459	512.614	1.492	0.004	7.725
	CV	12.41	76.57	8.76	0.27	11.55
	R^2	0.778	0.365	0.355	0.275	0.224

^{a,b} Equal superscripts within the columns represent significant differences at the level of significance $P < 0.05$; R^2 – coefficients of determination based on observed means through Dunnett T3 test; SD – Standard deviation, CV – Coefficient of variation

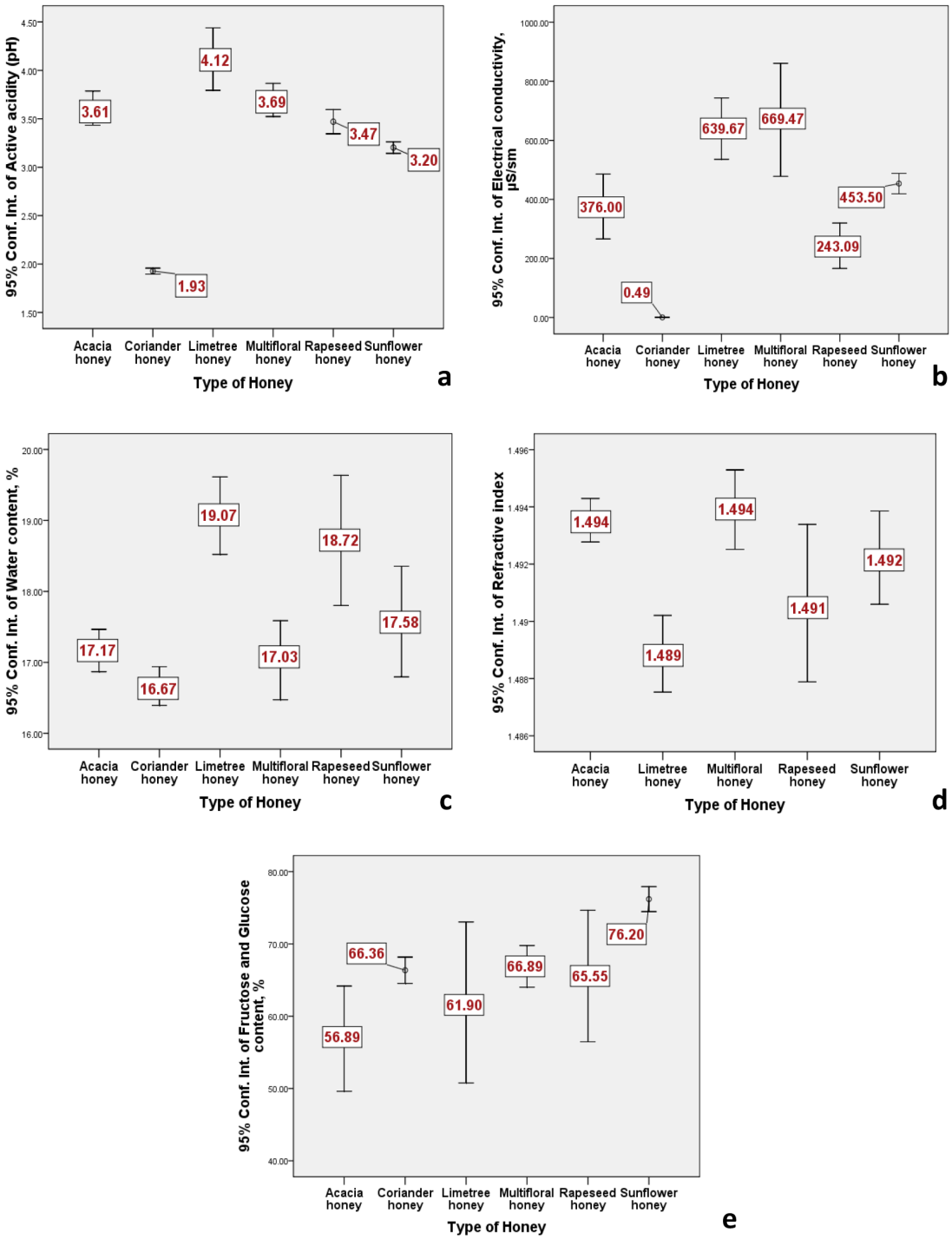

Fig. 1. Significant differences between various types of honey depending on mean values of observed physicochemical parameters

According to Table 1, about 35.5% of variances in the Water content parameter are result from the influence of the type of honey ($R^2 = 0.355$).

Based on the *Refractive index* parameter (Figure 1d) significant differences have been found mainly between limetree honey and the other studied types except for rapeseed honey. Not all other types of honey differ statistically from one another with regard to that parameter. From Table 1 it can be seen that the coefficient of determination is $R^2 = 0.275$, i.e. only 27.5% of the variations in the variable Refractive index could be accounted for by the effect of the type of honey.

The data from Table 1 show that with regard to Fructose + Glucose content the highest is the value of the sunflower honey samples – 76.20±2.720 %, and lower but close are the values according to that parameter in the multifloral, rapeseed, limetree and coriander honey – 66.90±7.725%; 61.90±17.531% and 66.36±2.854%, respectively. The obtained mean values about Fructose + Glucose content conform to the requirements of the Regulation on the requirements to bee honey intended for human consumption (2002) and the Bogdanov et al. (1997) for F + G content in nectar honey not less than 60 g/100 g (60%). Acacia honey shows the lowest content of reducing sugars 56.89±11.455%. The lower reducing sugar content in acacia honey according to Shkenderov & Ivanov (1983) is related to abundant nectar production of the acacia tree. Concerning the Fructose + Glucose content parameter (Figure 1e), sunflower honey differs significantly from acacia, coriander, and multifloral honey. With regard to the coriander, limetree, and rapeseed honey no statistically significant differences from the other types of honey have been found. The value of the coefficient of determination is $R^2 = 0.224$ (Table 1), which means that only 22.4% of the variations in the Fructose + Glucose variable are due to the effect of the type of honey.

The coefficients of variation (CV) except for the Electrical conductivity parameter in most of the types of honey and the Fructose + Glucose parameter for the limetree honey vary within low limits, which is evidence about data homogeneity in the separate groups (Table 1).

Conclusions

The established average values of the studied physicochemical parameters (Active acidity, Electrical conductivity, Water content, Refractive index, Fructose + Glucose content) for all analyzed honey samples are within the permissible limits according to the Council Directive 2001/110/EC of 20 December 2001 relating to honey and Regulation on requirements to bee honey intended for human consumption (Decree of the Council of Ministers 196/2002) for Bulgarian bee honey.

Statistically significant differences have been observed between the physicochemical parameters of monofloral and multifloral honey.

The values of coefficients of determination (R^2) show that 77.8% of the variations in the variable for the parameter (pH) and from 22.4 – 36.5% in the other studied parameters are due to the effect of the type of honey.

The coefficients of variation (CV) except for the parameter Electrical conductivity for most types of honey and the parameter Fructose + Glucose content for limetree honey vary within narrow limits.

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