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Survey of the mineral content and some physico-chemical parameters of Bulgarian bee honeys

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The quality control of honey requires a number of physical and chemical parameters to be determined in order to provide evidence of the origin and environmental purity of the product. 14 types of Bulgarian bee honeys were analyzed and the following parameters were determined: refraction index, thermophysical characteristics, color characteristics, lightness L^* and chroma C^*_{ab}. The contents of water, β-carotene, glucose, fructose, saccharose, oligosaccharides, essential and toxic trace elements were also found. The correlation between the refractive index and the water content of honey was determined as a criterion for the quality of honey. The fructose-glucose ratio was determined as a parameter related to the crystallization of honey. The criteria used were applied for the first time to Bulgarian honeys.

Relatively high content of potassium was found in the analyzed Bulgarian bee honeys, which makes them an important source of this essential element. No traces of the toxic elements As, Cd, Ni and Pb were found.

Key words honey, mineral content, sugar content, thermophysical and optical parameters

INTRODUCTION

Honey is a natural sweet substance that bees produce by transforming natural nectar of plants. It is well known for its valuable nutritional and medicinal qualities. Honey composition depends on its type and origin. Honey contains monosaccharides, macro- and micronutrients, antioxidants, free amino acids, organic acids, vitamins, enzymes and minerals [1].

The contents of water, glucose, fructose, saccharose, essential and toxic metal ions, as well the refractive index are major parameters in assessing the quality of honey. On the other hand, parameters with stronger impact for the consumers are the color, the crystallization state and the fermentation grade [2]. Trace element levels in honey are of particular importance as an indicator of environmental pollution [3], owing to the fact that bees are in contact with air, waters and plants. The determination of trace elements such as Ca, Mg, Zn, Mn is widely used in food authenticity studies [4].

The purpose of the present work was to determine several physical and chemical characteristics of samples of bee honey harvested from different regions in Bulgaria. Classical physico-chemical methods available in most laboratories dealing with food control were used. Parameters were looked for permitting an unequivocal differentiation between natural honeys and those adulterated by addition of sweeteners. The criteria used were applied for the first time to Bulgarian honeys.

MATERIALS AND METHODS

Samples

14 honey samples, harvested in 2008 and 2009, were purchased from supermarkets or directly from beekeepers and stored at room temperature in dark. The geographical and botanical origin of samples is indicated in Table 1.

The contents of glucose, fructose, saccharose, water, oligosaccharides, β-carotene, trace elements, the refractive index and the color parameters were determined according to methods proposed by the International Honey Commission [5].

Methods

The content of carbohydrates in honey was determined by high performance liquid chromatography (HPLC) using a Refractive Index detector, Waters. Peaks were identified on the basis of their retention times using an Aminex HPX-87H column. The temperatures of both column and detector (differential refractometer R401, Waters) were 30°C; the sample volume was 10 µl.

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Table 1. Geographical and botanical origin of honey samples.

Sample N	Geographical origin (town in Bulgaria)	Predominant botanical origin	Year of harvesting
1	Triavna	Honeydew	2009
2	Elena	Honeydew	2009
3	Asenovgrad	Lime (*Tilia*)	2009
4	Asenovgrad	Acacia (*Robinia pseudoacacia*)	2008
5	Asenovgrad	Thistle (*Cardus nutans*)	2008
6	Karlovo	Honeydew	2009
7	Galabovo	Multifloral	2009
8	Asenovgrad	Multifloral	2009
9	Sliven	Multifloral	2008
10	Montana	Sunflower (*Helianthus annuus*)	2009
11	Montana	Sunflower (*Helianthus annuus)*	2009
12	Montana	Sunflower (*Helianthus annuus)*	2008
13	Samokov	Multifloral	2009
14	Troian	Multifloral	2009

The refractive indexes of the samples were measured at 20ºC at λ=589 nm on an Abbé refractometer, Carl Zeiss Jena, Germany, calibrated with distilled water. Constant temperature in the refractometer was maintained using a thermostat. The water content was determined from the refractive indexes using the Wedmore table [6].

The honey samples were heated at a temperature of 30÷35°C to dissolve the sugar crystals and then were poured into a 10-mm thick dish. The *β*-carotene content was determined on a Lovibond PFX 880 instrument (Tintometer Ltd., UK). A standard light source was used and wavelengths from 420 to 710 nm were selected by means of 16 narrow-band interference filters of 20 nm bandwidth. The measuring system was programmed to interpolate peak wavelengths at 5 nm intervals.

The color parameters (index of lightness L^* and color coordinates x, y, a^*, b^* corresponding to the uniform color space CIELab [7], were determined on the Lovibond PFX 880 instrument as well. The parameter chroma (C^*) was calculated according to the formula $C^* = [(a^*)^2+(b^*)^2]^{1/2}$.

The differential scanning calorimeter SETARAM 141, (France) was used for thermal scanning of the honey samples cooled to –60ºC. Nitrogen was used as a purge gas at a flow rate of 50 ml min^{-1}. Samples were accurately weighed into hermetically sealed polymer-coated aluminium pans. An empty aluminium pan was used as a reference. The test was made in the following way: (i) Cooling the sample to –60ºC with a cooling rate of 5ºC/min; (ii) Holding for 5 min at –60ºC; (iii) Heating to 150 ºC with a heating rate of 5ºC/min; (iv) Holding for 5 min at 150 ºC.

The content of Li, Na, K, Ca, Mg, Cu, Fe, and Mn in the honey samples was determined by flame atomic absorption spectrometry using the Thermo M5 instrument (UK) in an air-acetylene flame under standard conditions. 3 g of honey were dissolved in 100 ml of distilled water and the obtained solutions were subjected to analysis. For calibration, aqueous standard solutions of the analytes at the mg l^{-1} level, prepared from titrisols (Merck, Germany), were used.

RESULTS AND DISCUSSION

The examined honey samples may be divided in three groups: multifloral honeys, blossom honeys of predominant botanical origin and honeydew honeys.

The color of bee honey is affected by the harvesting method and the storage duration. It depends on the content of pigments such as chlorophylls, carotenoids, flavonoids. The color parameters (L^*, a^*, b^*), established in CIELAB system, *x*, *y* parameters and chroma (C^*_{ab}) are presented in Table 2.

Table 2. Color parameters of the examined bee honey samples

	x	y	L^*	a^*	b^*	C^*_{ab}
Max value	0.61	0.5	87.29	29.67	84.26	85.54
Min value	0.35	0.38	3.47	-4.47	1.39	1.41
Average value	0.44	0.44	65.33	2.83	54.51	55.49

As can be seen, ten of the examined honey samples have negative values of the color parameter a^*. This indicates the presence of a large proportion of green color. Only four of the samples – 1, 6 (honeydew) and 7, 14 (multifloral) have positive values of the parameter a^*, indicating the presence of red color. Similar results have been obtained for orange honey from Greece [8].

Table 3. Values of fructose/glucose ratio (F/G), refractive index (n) and content of carbohydrates, water and β-carotene for the investigated honey samples

	Carbohydrates				Parameter			
	Fructose g/100 g	Glucose g/100 g	Saccharose g/100 g	Oligo-saccharides g/100 g	F/G	n	W, %	β-carotene, ppm
Max value	52.26	45.27	11.59	8.81	1.49	1.497	19	65.23
Min value	44.64	31.14	7.56	1.74	0.99	1.489	15.8	8.21
Average	48.68	37.88	9.56	3.54	1.29	1.4937	17.09	35.07

All honeys examined in the present work showed positive values of the parameter b^*, characteristic of the yellow colors. The value was highest for honeydew samples and lowest for sunflower and lime honey.

Linear dependences between chroma C^*_{ab} and the color parameter b^*, as well as between the content of β-carotene and the color parameter x were found as follows:

$$C^*_{ab} = 0.99b^* + 1.54 \qquad R^2=0.99$$

$$\beta=351.3x–120.7 \qquad R^2=0.97$$

Upon increasing the value of the color parameter x, the content of β-carotene also increases.

The results for the content of water, carbohydrates (glucose, fructose, saccharose and oligosaccharides), the refractive index (n) and the crystallization index (fructose/glucose ratio F/G) of the examined honey samples are presented in Table 3.

The content of oligosaccharides in the examined honey samples is between 2.1 and 8.81 g/100 g honey. Some authors have reported that honeydew honeys have about 35–40 g fructose per 100 g dry matter, while flower honeys show about 40–50 g fructose per 100 g dry matter [9]. In contrast, the flower honeys and honeydew honeys in our investigation have 40–53 g fructose per 100 g dry matter. All investigated samples satisfy the condition of EC Directive 110/2001 – the sum of glucose and fructose contents to be above 60 g/100 g of honey [9].

The fructose/glucose ratio (F/G) was calculated for all samples. From this parameter information about the crystallization state of honey may be derived: the crystallization ability of honey is low if the content of fructose exceeds that of glucose. Only one of the examined samples had a crystallization index equal to 1 and it was partially crystallized. The other samples had F/G>1 and they were fluid. The F/G ratio depends on the nectar source. Monofloral blossom honeys contain more glucose than honeydew honeys while multifloral and honeydew honeys have close glucose contents.

The moisture content of honey samples depends on the botanical origin of the sample, on the degree of ripeness, on the processing techniques and on the storage conditions. The water content is an important characteristic of honey quality: the high water content promotes the separation of a crystallized phase at the bottom and a liquid phase on top of the honey sample [10]. Moreover, the high water content leads to higher risk for spoilage of honey *via* fermentation [11]. According to EC Directive 110/2001, the water content of pure honey should be below 20 % [9].

All examined samples had water content between 15.8 % and 17.6 %, except for samples 2 and 6, having water contents of 18.25 and 19 %, respectively. The low water content of most of the samples points to an extremely low rate of the fermentation process. The higher water content of the latter samples may be attributed to (1) honey extraction in humid weather; (2) immaturity of the extracted honey or (3) adulteration of the honey sample with glucose. The glucose/water (G/W) ratio could be a better indicator for predicting honey crystallization. G/W values lower than 1.7 are typical for slowly crystallizing honeys. The G/W values of honeydew honeys are about 1.7 while those of multifloral and monofloral honeys are about 2.2 and 2.3, respectively.

In Table 4 the color parameters, the contents of water, glucose, fructose, saccharose and oligosaccharides of three commercial brands of bee honey containing sweeteners (isosweet or glucose-fructose syrup) are presented. By color parameters and β-carotene content these samples do not differ from the other examined honey samples.

The addition of isosweet to honey does not essentially increase the water content and consequently, does not affect the fermentation ability of the honey sample. This sweetener is enzyme-hydrolyzed saccharose – laevorotating mixture of glucose and saccharose. Its addition to honey leads to a considerable increase in the glucose content, so that the F/G index of honey

Table 4. Physico-chemical parameters of natural honey and honeys containing different sweeteners

Honey sample	n	W, %	β-carotene, ppm	x	Y	a*	b*	L^*	Oligo-saccharides, g/100 g	Saccha-rose, g/100 g	Fructose, g/100 g	Glucose, g/100 g	F/G
Natural honey (sample 9)	1.4970	15.8	21.44	0.41	0.43	-2.21	50.87	72.25	2.10	10.22	48.24	39.44	1.07
Commercial (Pepino) containing isosweet	1.4920	17.8	34.49	0.45	0.46	-0.17	49.51	48.18	3.75	13.77	35.23	47.26	0.75
Commercial (Orfeo) containing glucose-fructose syrup purchased	1.4907	18.2	17.47	0.40	0.42	-4.29	40.47	74.23	27.32	10.91	32.03	29.74	1.08

Table 5. Mineral composition of some Бulgarian honey samples (mean ± standard deviation, n=3)

Sample N	Element							
	K, mg g^{-1}	Na, μg g^{-1}	Ca, μg g^{-1}	Mg, μg g^{-1}	Fe, μg g^{-1}	Cu, μg g^{-1}	Mn, μg g^{-1}	Li, μg g^{-1}
1	1.23±0.05	34.6±1.5	76.8±1.0	36.1±1.0	3.05±0.05	0.97±0.02	1.34±0.05	0.10±0.02
2	1.21±0.05	22.3±1.2	84.2±1.0	35.6±1.0	3.24±0.05	0.96±0.02	1.79±0.05	<0.05
3	0.29±0.02	13.6±1.2	46.4±1.0	11.5±0.5	3.47±0.05	0.42±0.02	<0.15	<0.05
4	0.25±0.02	62.1±2.0	46.9±1.0	13.1±0.5	0.31±0.05	0.34±0.02	<0.15	<0.05
5	0.36±0.02	11.8±1.2	94.6±1.0	17.4±0.5	1.89±0.05	<0.05	<0.15	<0.05
6	1.64±0.05	22.3±1.5	127.7±1.5	109.1±1.5	5.01±0.05	0.99±0.02	15.6±1.0	0.57±0.05
7	1.82±0.05	25.4±1.5	99.3±1.0	32.6±1.0	<0.15	0.19±0.01	0.36±0.05	<0.05
8	0.40±0.02	15.0±1.2	82.1±1.0	21.4±1.0	0.93±0.05	<0.05	<0.15	<0.05
9	0.38±0.02	15.8±1.2	64.7±1.0	11.9±0.5	3.28±0.05	1.29±0.02	0.27±0.05	<0.05.
10	0.21±0.02	9.8±1.0	42.2±1.0	6.9±0.2	2.22±0.05	0.90±0.02	<0.15	<0.05
11	0.28±0.02	9.5±1.0	50.9±1.0	9.6±0.3	0.31±0.05	0.76±0.02	<0.15	<0.05
12	0.26±0.02	10.2±1.0	56.8±1.0	11.0±.0.5	3.33±0.05	1.06±0.02	<0.15	<0.05
13	0.30±0.02	20.6±1.5	33.1±1.0	7.5±0.2	1.05±0.05	2.26±0.03	<0.15	<0.05
14	0.26±0.02	15.3±1.2	22.8±1.0	5.2±0.2	0.56±0.05	1.74±0.02	<0.15	<0.05

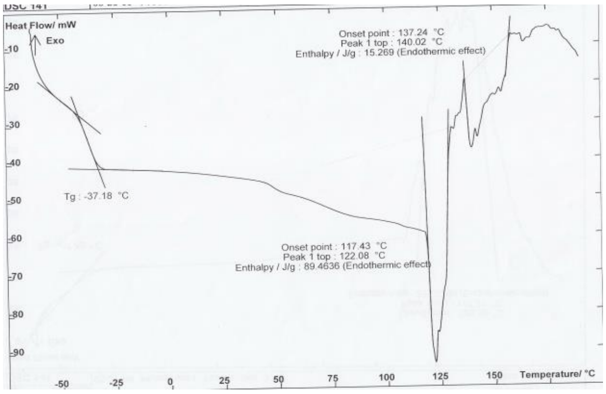
becomes less than unity (Table 4), which favors the crystallization ability of the sample.

The addition of glucose-fructose syrup does not influence the crystallization ability of honey, but elevates its water content and reduces the refraction index. Such honey samples are characterized with a higher content of oligosaccharides (up to 27.6 g per 100 g of honey).

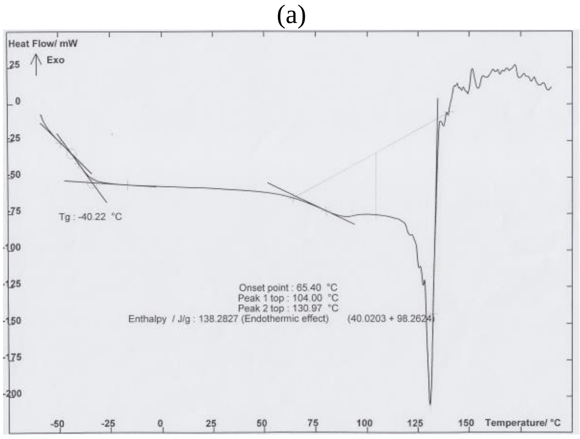
Differential scanning colorimetry was used for the first time for the identification of the composition of Bulgarian honeys**.** As a criterion the glass transition temperature (T_g) was used. The thermograms of pure bee honey and honey adulterated with glucose-fructose syrup or isosweet are presented in Figures 1a, b, c. Sample 9 was chosen as a representative of pure bee honey. Fig. 1 shows that T_g of natural honeys is about –37ºC, while for those adulterated by glucose/frucrose syrup it is –48ºC. The lack of glucose adulterant in this sample was checked by comparing its parameters with those of pure lime honey obtained by private producers (own production, guaranteed free from added glucose syrup).

The addition of glucose-fructose syrup to honey results in a decrease in the glass transition temperature T_g. T_g of a sample containing glucose-fructose syrup is by about 10ºC beneath that of pure honey. T_g of the isosweet containing sample, however, is close to that of pure honey, which is in the range between –33 and – 42ºC [12]. Isosweet addition leads, however, to a considerable change in ΔC_p (ΔC_p=-43.60) while samples of pure bee honey and those containing glucose-fructose syrup have ΔC_p values of -12.83 and 12.13, respectively.

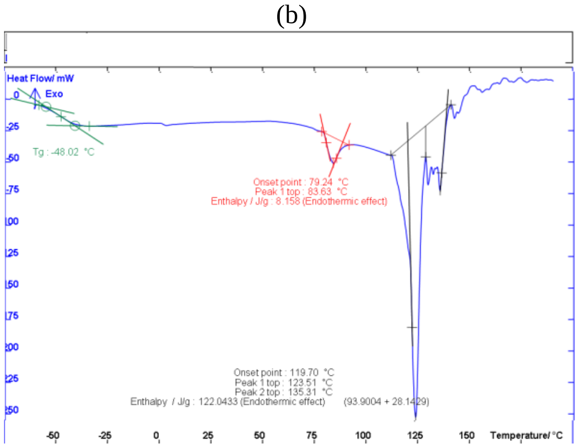
The content of Li, K, Na, Ca, Mg, Fe, Cu and Mn in the examined honey samples is presented in Table 5. As can be seen, the content of potassium is at the mg g^{-1} level in most of the analyzed samples



(a)



(b)



(c)

Fig. 1. Thermophysical parameters of pure and adulterated bee honey; a) pure bee honey (sample 9); b) bee honey containing isosweet; c) bee honey containing glucose-fructose syrup

and exceeds that of the other elements by approx. one order of magnitude. Its content is between 0.2 and 0.4 mg g^{-1} for the blossom honeys. Similar potassium content was registered by Madejczyk for rape honey [13]. The honeydew samples displayed a higher content of potassium – between 1.2 and 1.6 mg g^{-1}. Similar results were reported for honeydew honeys from Poland [14]. Hence, bee honey and particularly honeydew honey may be considered is an important source of the essential element K. Na, Mn, Ca, Cu and Fe are present at the µg g^{-1} level. The Mg content (between 7 and 110 µg g^{-1}) is similar to that of Macedonian bee honeys (12–117 µg g^{-1}) [15]. The Ca content is close to that in honey from other European regions, *e.g.*, Poland and Czech Republic [16–17], while the Cu and Mn contents are similar to those of Turkish honeys [18]. Li was detected in two of the honeydew samples only (1 and 6). The concentrations of the toxic trace elements As, Cd, Ni and Pb in the honey were below the corresponding limits of detection.

CONCLUSIONS

All examined Bulgarian honey samples correspond to the EC regulations. The honey samples collected from different regions of Bulgaria display similar characteristics; moreover, there are no significant differences between commercial honeys and honeys from private producers. The examined honey samples have a low water content, hence a low tendency for fermentation. The addition of glucose-fructose syrup to honey can be established by an increase in the water content and a decrease in the refractive index. The content of oligosaccharides in these samples is about 4 times higher than that in pure bee honey. The presence of isosweet in bee honey accelerates the crystallization of the honey samples and lowers the crystallization index.

The glass transition temperature of honey containing over 25% of glucose-fructose syrup is below –40ºC, which hampers its crystallization. Bulgarian bee honeys have a relatively high content of the essential element potassium and do not practically contain toxic trace elements**.** It follows from the obtained results that Bulgarian bee honeys are a high-quality environmentally pure product with excellent characteristics.

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ПРЕГЛЕД НА МИНЕРАЛНОТО СЪДЪРЖАНИЕ И ФИЗИКО-ХИМИЧНИТЕ ПАРАМЕТРИ НА БЪЛГАРСКИ ПЧЕЛЕН МЕД

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(Резюме)

Качественият контрол на пчелния мед изисква определянето на редица физични и химични параметри с цел оценка на натуралността и екологичната чистота на продукта. Анализирани са 14 типа български пчелен мед от различни области на България и са определени техните параметри като показател на пречупване, цветови параметри, светлост L^*. В изследваните образци е определено също така съдържанието на β-каротен, глюкоза, фруктоза, захароза, олигозахариди, водното съдържание, както и съдържанието на основни и токсични елементи. Като критерий за качеството на пчелния мед е определена корелацията между водното съдържание и показателя на пречупване. Фруктозо-глюкозното отношение е определено като параметър, свързан с кристализацията на пробите. В изследваните български медове е установено сравнително високо съдържание на калий, което ги прави важен източник на този есенциален елемент. Медовете практически не съдържат токсични елемент.