Significant parameters of Bulgarian honeydew honey

Znachimi harakteristiki na bulgarskiya manov med

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Abstract

Three main physico-chemical parameters (moisture content, pH, and electrical conductivity), 19 elements contents (K, Ca, Mg, P, S, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr, V, and Zn) and microscopic characteristics of 30 honeydew honey samples from Bulgaria were evaluated. The most abundant elements were K, P, Ca and Mg in the honeydew honey investigated and low concentrations of toxic elements and heavy metals were established. The electrical conductivity – one of the most important parameters for honeydew honey, exceeded 0.8 $mS*cm^{-1}$. The honeydew index in Bulgarian honeydew honey varied widely and its values were often below 3.

Keywords: Bulgarian honeydew honey, honeydew elements, macro- and microelements, physico-chemical parameters

Rezyume

Otseneni sa tri osnovni fisiko-himichni parametri (vodno sadarzhanie, pH i elektroprovodimost), sadarzhanieto na 19 elementa (K, Ca, Mg, P, S, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr, V i Zn) i mikroskopskata harakteristika na 30 probi bulgarski manov med. Nai-razprostranenite elementi v meda sa K, P, Ca i Mg. Ustanovena e niska kontsentratsiya na toksichni elementi i tezhki metali. Elektroprovodimostta - edin ot nai-vazhnite parametri sa manov med, previshava 0.8 $mS*cm^{-1}$. Indeksat za manovi elementi v izsledvaniya manov med varira v shiroki granits i negovite stoinosti sa chesto pod 3.

Klyuchovi dumi: Bulgarski manov med, fisiko-himichni poksateli, makro- i mikroelementi, manovi elementi

Introduction

Honeydew honey is made by honeybees from excretions of plant sucking insects (*Hemiptera*) on different plants, mostly trees (Bogdanov, 2009; Persano Oddo et.al., 2004). Honeydew honeys have specific physico-chemical, organoleptic and microscopic properties depending on the wide variety of insects and host plants. In Mediterranean region for example the main producing species of honeydew are *Abies alba, Pinus brutia, Castanea sativa,* different oaks (*Quercu*s ssp.) and willow-trees (*Salix* spp.) (Ricciardelli D'Albore, 1998); for Croatia – different oaks, fir and spruce, and for Macedonia, especially for the western part – *Pinus peuce* and *Quercus macedonicus* (Primorac et al., 2009). Under microscopic examination honeydew honeys are characterised by the presence of numerous honeydew elements (HDE) such as fungal spores, conidia and hyphae especially of sooty moulds, unicellular algae and wax elements. Pollen grains of nectarless plants are also common (Louveaux et al., 1978).

The physico-chemical characteristics of honeydew honey includes high values for electrical conductivity and pH (Persano Oddo and Piro, 2004). In Bulgaria honeydew honey is frequently associated with dark brown. Beekeepers offer honeydew honey in different regions, but the main production comes from the southeastern part of the country (Strandzha Mountain) where *Quercus* forests occur. The characteristic features of honeydew honey and its producers in the vicinity of Tsarevo town (the Strandzha Mountain) during the period 1999-2006 were investigated by Georgiev et al. (2008). Five species of plant aphids - honeydew producers were established. Four of them - *Lachnus roboris* (Linnaeus, 1758), *Lachnus pallipes* (Hartig, 1841), *Tuberculatus querceus* (Kaltenbach, 1843), *Tuberculatus annulatus* (Hartig, 1841) (Hemiptera: Aphididae) develop on oak leaves and shoots. According to these authors the population dynamic of the honeydew producers depend strongly on the specific climatic conditions and this affected the honeydew honey production. Simova et al. (2012) found a rapid differentiation between oak honeydew honey, blossom honey and honeydew honeys from fir and spruce. These authors considered that only the oak honeydew honey contained the cyclitol quercitol.

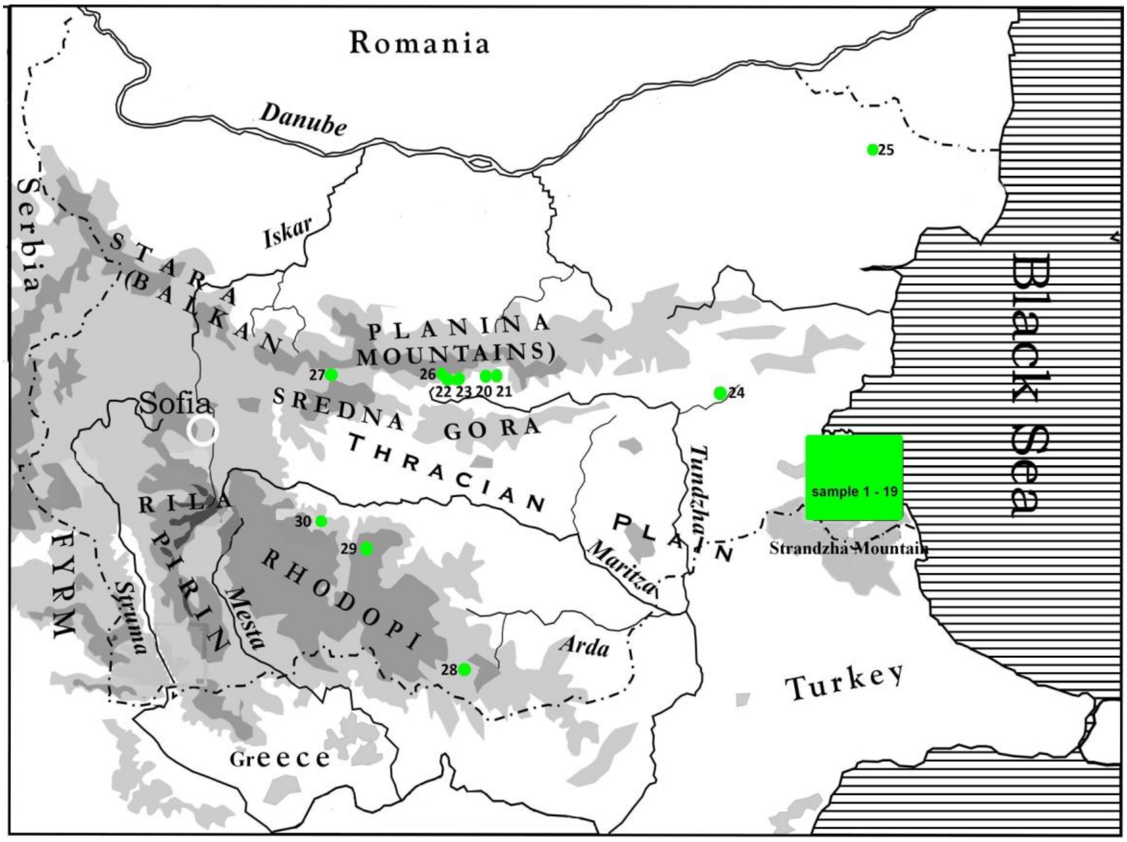
Data about the mineral content of honeydew honey in Bulgaria are sporadic (Atanassova et al., 2012; Nikolova et al., 2013; Yurukova et al., 2008) and for microscopic features are almost absent.

Higher antioxidant activity of honeydew honeys in comparison to other types of honey has been established during the last years, and has increased the interest to this type of honey and its identification (Bobis et al., 2008; Bogdanov, 2009; Wilczyńska, 2010; Dinkov, 2014).

The aim of the present study was to determine three physico-chemical parameters (electrical conductivity, moisture content and pH), the element content and microscopic features of Bulgarian honeydew honey and contribute to the knowledge of this type of honey.

Materials and Methods

Thirty samples of honeydew honey from different regions of Bulgaria were collected during the period 2006 – 2010. Most of them were taken directly from the beekeepers. Nineteen of the samples were from southeast Bulgaria (mainly from Strandzha Mountain) (Figure 1).



Sample 1 – 5 Burgas area, sample 6 – 19 Strandzha Mountain and southern Black Sea coast, sample 20 and 21 surroundings of Kazanlak, sample 22, 23 and 26 surroundings of Kalofer, sample 24 surroundings of Aitos, sample 25 Ludogorie, sample 27 surroundings of Teteven, sample 28 surroundings of Zlatograd, sample 29 surroundings of Peshtera, sample 30 surroundings of Belovo.

Figure 1. Map of Bulgaria with the areas of collection of the honey samples studied

Figura 1. Karta na Bulgaria s rayonite, ot koito sa sabrani izsledvanite probi med

Three main physico-chemical parameters were measured in this study: moisture content, electrical conductivity, pH and also 19 macro- and microelements contents. Organoleptic characteristics (colour and physical state) were described by sensorial analysis (honey was visually examined and tasted). Microscopic analyses were also performed.

During the routine physico-chemical analysis moisture content (%) was determined according to AOAC 969.38B (1992) using honey refractometer (Atago Co., Ltd., Tokyo, Japan). Electrical conductivity (EC) ($mS*cm^{-1}$ ± 1%) in 20% solution at 20°C

(MultiLine P3; WTW, Weilheim, Germany), and pH (20% solution, ± 0.01, Jenway pH-meter; Bibby Scientific Ltd., Staffordshire, UK) were also determined.

The International Honey Commission (2009) do not require any method for identification of chemical elements in honey. About 10 g fresh weight honey was treated with 15 cm^3 nitric acid (9.67 M) overnight. The wet-ashed procedure was continued with heating on a water bath, following by addition of 2 cm^3 portions of 30% hydrogen peroxide. This treatment was repeated until full digestion. The filtrate through a filter paper Filpap KA 2 (Czech Republic) was diluted with double-distilled water (0.06 $mS*cm^{-1}$) up to 25 cm^3. All solutions were stored in plastic flasks. Macroelements potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S), and microelements aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), strontium (Sr), vanadium (V), and zinc (Zn) were determined by atomic emission spectrometry with the inductively coupled plasma system (ICP-AES) of VARIAN VISTA-PRO. The detection limits were 0.002 $mg*l^{-1}$ for Mn and Sr, 0.004 $mg*l^{-1}$ for Cd, Co, Cr, Cu, Ni, and Zn, 0.02 $mg*l^{-1}$ for As and V, 0.03 $mg*l^{-1}$ for Pb, 0.04 $mg*l^{-1}$ for Al and Fe, and 0.5 $mg*l^{-1}$ for Ca, K, Mg, Na, P, and S. To quality control information could be added that triplicates of each honey sample were prepared independently. Triplicates of blanks – all reagents and all analytical procedures, but without biological material were also analyzed. Three measurements for each solution of the digested sample and blanks for the series were done. Finally, standard deviation in all cases was below 5%. The last concentration was calculated according to all measurements, dilution of digested solution, weight of each sample and blank. Triplicates of 2 plant reference materials were analyzed. The measured concentrations were in good agreement with the certified values (SD < 5%).

Methodological Limits of Determination for this series (three measurements for each solution of the digested sample, calculated according to dilution and weight of each sample and corresponding blank) were found as follows: <0.01 $mg*kg^{-1}$ for Cd, Co and Cr, <0.05 $mg*kg^{-1}$ for V, <0.08 $mg*kg^{-1}$ for Pb, and <0.1 $mg*kg^{-1}$ for As.

Melissopalynological data were obtained from all 30 investigated honey samples. The laboratory preparation for qualitative melissopalynological analysis followed the method of Louveaux et al. (1978). The method for quantitative analysis by Moar (1985) was followed. Tablets containing a known number of spores of *Lycopodium clavatum* were added. A minimum of 500 pollen grains of nectar producing species (Pn) were counted from each sample and all honeydew elements. Pollen grains of nectar producing species (Pn) and of nectarless plants (Pa) were separately counted. The ratio of HDE to Pn (the honeydew index) was calculated (Louveaux et al., 1978). The absolute concentration of Pn in 1 g of honey was derived from the ratio of Pn × *Lycopodium* spores added to the number of *Lycopodium* spores counted. The concentration of Pa and of the HDE was calculated in the same way. Pollen identification was carried out with the light microscope Meiji (Japan) at 400x and 800x magnification. The pollen reference collections of the Department of Botany at Sofia University and Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, Sofia (Bulgaria) were used for the identification of pollen. Pollen taxonomy is based on Beug (2004).

Results

The consistency of all investigated samples were liquid and the color of thel samples was amber-brown to dark brown with reddish shades. The results of the physico-chemical parameters - moisture content, pH and electrical conductivity were presented in Table 1. Moisture and pH varied in the range 15.9 % – 17.5% and 4.1 – 4.9 respectively. Electrical conductivity showed values above 0.8 mS*cm^{-1} in all cases.

Table 1. Physico-chemical parameters (moisture content, pH and electrical conductivity)

Tablitsa 1. Fisico-himichni parametri (vodno sadarzhanie, pH i elektroprovodimost)

Physico-chemical parameters	Min – Max	Mean values
Moisture content %	15.9 – 17.5	17.3 ± 1.4
pH	4.1 – 4.9	4.3 ± 0.2
El. conductivity mS*cm^{-1}	0.8 – 1.4	1.05 ± 0.146

The content of 19 macro- and microelements in the honeys were present in Table 2 and Table 3.

Table 2. Concentrations of macroelements in the honeys studied (mg*kg^{-1})

Tablitsa 2. Kontsentratsia na makroelementite v izsledvanite probi (mg*kg^{-1})

Element	Min - Max	Mean ± standard deviation
Ca	43 – 178	103 ± 33
Fe	1.1 – 13.1	3.0 ± 2.3
K	892.4 – 2114	1331 ± 288
Mg	25.7 – 182	83 ± 27
Na	7.3 – 42	17 ± 7
P	59.3 – 210	123 ± 27
S	25.3 – 104	53 ± 18

Potassium was the most abundant mineral component, considering all the investigated samples (with a mean value of 1331 ± 288 mg*kg^{-1}). Phosphorus was also well represented with a mean value of 123 ± 27 mg*kg^{-1}, followed by calcium 103 ± 33 mg*kg^{-1}, magnesium 83 ± 27 mg*kg^{-1} and sulfur 53 ± 18 mg*kg^{-1} (Table 2). Sodium and manganese had a mean value of 17 ± 7 and 12 ± 5 mg*kg^{-1} respectively (Table 2 and Table 3). The rest of the studied minerals were present in minor quantities and some of them could be detected in trace amount.

Table 3. Concentrations of microelements in the honeys studied (mg*kg^{-1})

Tablitsa 3. Kontsentratsia na mikroelementite v izsledvanite probi (mg*kg^{-1})

Element	Min - Max	Mean ± standard deviation
Al	0.4 – 4.4	1.3 ± 1.1
As	<0.01 – 0.32	
Cd	<0.01	
Co	<0.01	
Cr	<0.01 – 0.04	
Cu	0.2 – 1.1	0.55 ± 0.21
Ni	<0.01 – 0.92	
Pb	<0.08 – 0.25	
Sr	0.2 – 0.7	0.38 ± 0.10
V	<0.05	
Zn	0.28 – 4.6	1.2 ± 1.3
Mn	3.4 – 27	12 ± 5

The microscopic investigations showed the presence of various fungal elements and pollen grains of both nectarproducing and nectarless plants. Among the nectariferous plants pollen of *Paliurus, Trifolium,* other Fabaceae (*Dorycnium, Lotus, Melilotus, Vicia*), Brassicaceae and *Tilia* prevailed. Poaceae, *Plantago, Artemisia* and *Rumex* pollen was dominant among nectarless plants. The pollen grains of the trees *Quercus, Carpinus, Fagus* and *Pinus* were sporadic represented.

The values of HDE concentration in Bulgarian honeydew honey ranged widely between 895 - 8245 in 1g honey. The honeydew index varied also in high interval from 0.89 up to 9.46 (Figure 2).

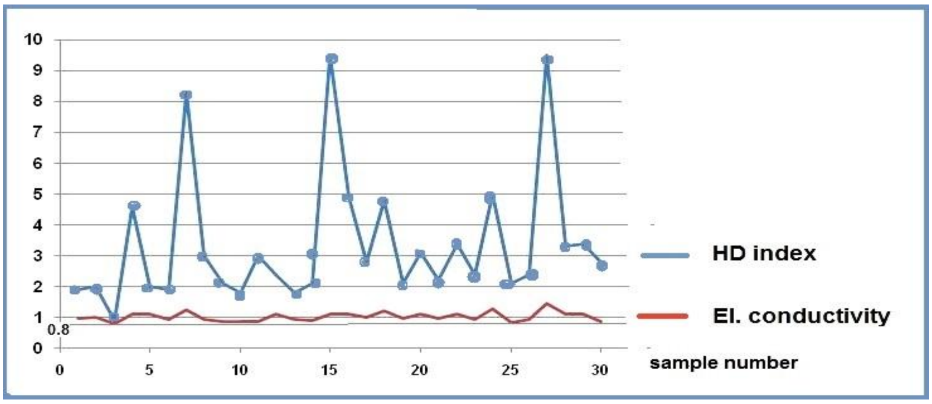


Figure 2. Fluctuation of the HD index and electrical conductivity

Figura 2. Fluctuatsii na HD indeksa i elektroprovodimostta

Pn varied over a wide range of 505 – 3050 in 1g honey and the concentration of Pa vas also variable 85 - 1900 in 1g honey, but in average only 477 (Table 4).

Table 4. Concentration of HDE, Pn and Pa in 1g honey

Tablitsa 4. Kontsentratsia na HDE, Pn i Pa v 1g med

	Min - Max	Mean values
HDE	895 – 8245	3320
Pn	505 – 3050	1218
Pa	85 – 1900	477

The microscopic picture of most of the honeydew samples was characterized by dark colored fungal elements (Figure 3.).

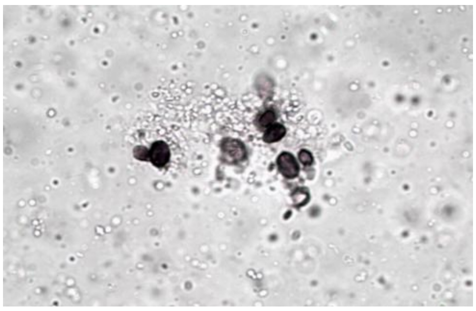


Figure 3. Dark colored fungal elements in Bulgarian honeydew honey (microscopic photography)

Figura 3. Tamni gabni elementi v bulgarskiya manov med (mikroskopska snimka)

Discussion

Data about element content together with physico-chemical parameters and microscopic characteristics of Bulgarian honeydew honeys are uncompleted (Yurukova et al., 2008), which determines the importance of this study.

The consistency and the amber-brown to dark brown color of the investigated honeydew honeys were identical to these of Croatian and Macedonian origin (Primorac et al., 2009). The color of honey is related to the floral origin, total polyphenols from plant pigment and pollen, mineral composition and hydroxymethylfurfural (Bertoncelj et al., 2007; Gonzalez-Miret et al., 2005). Georgiev et al. (2008) recorded growth of a dark colored saprophyte fungi from *Capnodium* and *Fumago* genera which feed on honeydew and caused blackening (black soot) on the tree leaves in the Strandzha Mountains. Dark colored fungal elements were identified also during the microscopic analyzes (Figure 3). This may affected to some extent the color of the honeys investigated as well.

Honey contains a number of acids which include amino acids and organic acids that determine the pH value. In the honeys investigated pH varied in the interval 4.1 – 4.9. The moisture content of the honeydew honeys varied in the range 15.9 % – 17.5%. This parameter depends on environmental conditions, season of production, maturity of honey and storage of the product, as well (Cantarelli et al., 2008; Karimov et al., 2014). Electrical conductivity, that depends primarily on the mineral content, showed values in the interval 0.8 – 1.4 $mS*cm^{-1}$. The results of the moisture content and pH were within the generally accepted for this type of honey (Persano Oddo and Piro, 2004). According to the European Honey Standard, the electrical conductivity of the honeydew type is one of the most important characteristic (Bogdanov et al., 2004), and should exceed 0.8 $mS*cm^{-1}$, which is established also in all honey samples from Bulgaria (Table 1).

Potassium was the most abundant mineral component, considering all the investigated samples (with a mean value of 1331 ± 288 $mg*kg^{-1}$), which agrees with other studies indicating that K is the most abundant element in all honey types (Cantarelli et.al., 2008; Escuredo et al., 2012; Lazarević et al., 2013). Mineral content is generally lower in blossom honeys compared to the honeydew honey, particularly for potassium and phosphorus content according to the investigations of different types of honey from northwestern Spain (Escuredo et al., 2012). The element content of Bulgarian unifloral honeys (Atanassova et al., 2012) showed that the maximum values of the macroelement potassium was recorded in chestnut, *Paliurus* honey and in the sample of honeydew honey (1628, 1198 and 1121 $mg*kg^{-1}$). P and Mg were higher in the sample of honeydew honey in comparison to all unifloral honeys investigated according to the same authors. Higher concentration of Mg (mean of three samples 60.26 ± 1.2 $mg*kg^{-1}$) and Mn (mean of three samples 6.24 ± 0.6 $mg*kg^{-1}$) in Bulgarian honeydew honey in comparison to other Bulgarian honey types was also established by Nikolova et al. (2013). The elements Cd, Co, Ni and toxic elements As and Cr in most cases were under the detection limits in the honey investigated. The highest concentrations of Al was in the honey produced in the area of Ludogorie in Northeast Bulgaria (Figure 1 sample 25), – 4.4 $mg*kg^{-1}$, the highest concentration of Pb was in the samples from Kazanlak and Kalofer town (Figure 1 sample 21 and 22) – 0.25 $mg*kg^{-1}$ and the highest concentrations of Ni was from the

Belovo town (Figure 1 sample 30) – 0.92 mg*kg^{-1}. The concentration of Al ranged in the interval 5.19 – 20.2 mg*kg^{-1} in Czech honeydew honey and Ni ranged in the interval 0.32 - 1.53 mg*kg^{-1} (Lachman et al., 2007) which is higher than in Bulgarian honeydew honey. The data of elements content in Bulgarian honeydew honey showed similar concentrations with Swiss honeydew honey (Bogdanov et al., 2007) for Fe, Pb, Ni and Zn but higher concentration for Mn and lower for Cu. The heavy metals as Pb, Cd and toxic elements Cr and As could reflected the presence of contaminants due to environmental contamination or pharmacological (antiparasitical or acaricidal) treatment or by incorrect procedures during the honey processing and conservation phases (Pisani et al., 2008).

Honeydew honey index should be higher than 3 in honeydew honey according to Louveaux et al. (1978). In the present study it varied widely often below the limit of 3. Nevertheless in many cases there was a correlation between the values of HD index and the values of electrical conductivity (Figure 2). The values of HDE concentration in Bulgarian honeydew honey was relatively low, as the concentration of Pa. These results coincided with the low presence of honeydew elements in honeydew honey from *Quercus frainetto* in Serbia (Jerković and Marijanović, 2010).

More microscopic analyses are necessary to establish the reasons for the low honeydew index and the fungal diversity in Bulgarian honeydew honeys.

Conclusions

The present study introduced and discussed the important physical and chemical characteristics together with the microscopic features of 30 honeydew honey samples from Bulgaria. The ranking of averages of all the analysed macroelements, heavy metals, and toxic elements in the honeydew honey was K > P > Ca > Mg > S >Na > Mn > Fe > Al > Zn > Cu, Sr, Ni, As, Cr, Pb, V, Cd, Co. The heavy metals Cd, Co, and the microelement V were in all cases below the detection limits. The conductivity values – one of the most important parameters, ranged in the interval 0.8 – 1.4 mS*cm^{-1} and were within the accepted for this type of honey. The analytical data indicated a good level of quality of the honeys investigated, especially with regard to the low concentration of toxic trace elements. The melissopalynological analyses showed high variations of the concentration values of Pn and Pa (505 – 3050 pollen grains in 1g and 85 – 1900 grains in 1g respectively). The honeydew index ranged in high interval (0.89 – 9.46) in a lot of cases below 3, which does not comply with the requirements of Louveaux et al. (1978).

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