INORGANIC CHARACTERISTICS OF POLYFLORAL HONEY IN PLOVDIV (BULGARIA)

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ABSTRACT

Representative honey samples, harvest of 2009, from different places of the town of Plovdiv and its district (Bulgaria), were characterized on the basis of their physico-chemical parameters and inorganic chemical data. Water content, pH, electrical conductivity, macroelements – Ca, K, Mg, P, S and microelements – Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr, V, Zn were determined after the Harmonised Methods of the International Honey Commission, and ICP-AES method in a certified laboratory. The results were discussed in order to evaluate the existence of data patterns and the possibility of differentiation of Plovdiv honey samples according to their site characteristics.

Keywords: honey, physico-chemical parameters, macro- and microelements, urban and industrial pollution

Introduction

Honey is believed to be good both for body and soul; as apart from being medically accepted, the importance of honey is also recognized by many religions. Honey bees gather their honey from two sources: nectar and honeydew (5). In Bulgaria it seems that nectar is the more wide distributed honey source. There are a number of varieties of honey available in the market or directly from the beekeepers today. According to the type of plant which is collected nectar, honey is polyfloral (from different plants) and unifloral (mostly from one species of plants). In the area of Plovdiv with its specific climate grow various melliferous plants: natural and planted trees, herbaceous species, crops, vegetables and garden-plants; therefore polyfloral honeys are the most. The composition of honey is complex and subject of many studies (1, 2, 3, 4, 7, 8, 13, 14, 15, 24, 27).

The aim of the present study was to determine 3 physico-chemical properties, content of 19 elements in honeys from the town of Plovdiv and its district. As honey is quite a practical food and due to the fact that different plant species, melliferous included, accumulated high concentration of heavy metals and toxic elements in their tissues, an attempt to assess health effects of honey was done.

Materials and Methods

Honey samples were collected directly from beekeepers in 5 regions of the Plovdiv area (**Fig. 1**).

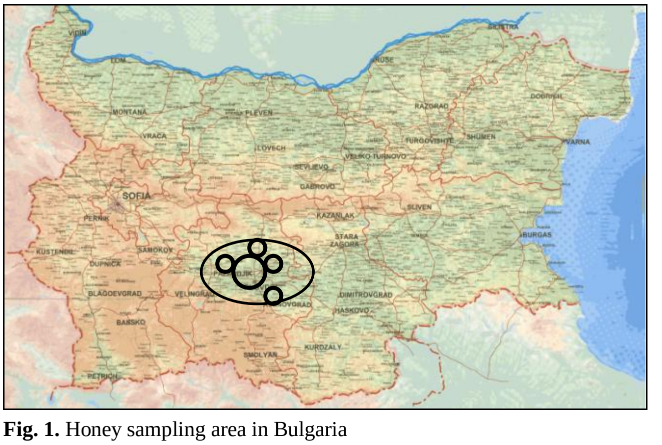

Fig. 1. Honey sampling area in Bulgaria

First region was the town of Plovdiv, affected by various human activities, with 7 sampling sites as follows: 1 – near church Ivan Rilski, 2 – Vegetable Crops Research Institute “Maritsa”, 3 – NW of the housing estate “Trakiya”, 4 – exBotanical Garden, 5 – railway station Trakiya, 6 – N end of the housing estate “Proslav”, 7 – Nature monument Youth Hill - exJendem Tepe (**Fig. 2**).

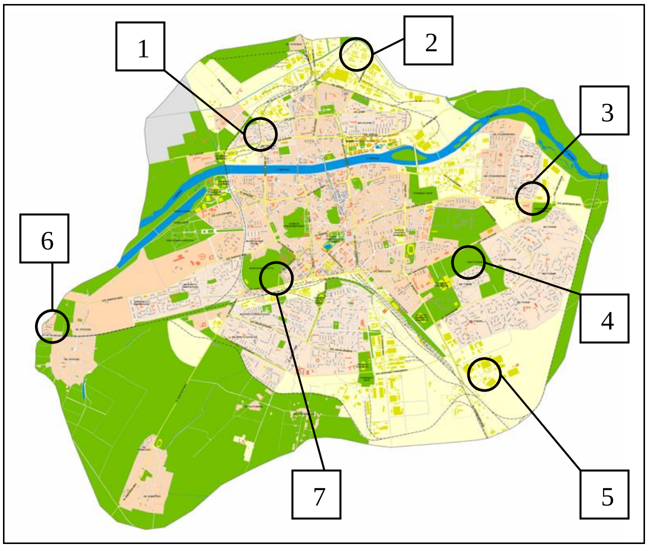

Fig. 2. Honey sampling sites in Plovdiv (Bulgaria)

Other 4 regions were located: 18 km W from Plovdiv, near town of Stamboliyski, 17 km NE from Plovdiv, in Rakovski district, 14 km E from Plovdiv in Sadovo and 13 km SE from Plovdiv in Asenovgrad region or 5 km W from a big non-ferrous smelter.

All honeys were harvest of 2009. Routine physico-chemical analysis included water content (honey refractometer Atago HHR-2N 12–30%, Japan), electrical conductivity (C, μS cm^{-1}, ± 1%) in 20% solution at 20°C (MultiLine P3, WTW, Germany), and pH (20% solution, ± 0.01, Jenway pH-meter 3310, England). There are no requirements in Harmonised Methods of the International Honey Commission (18) for the determination of elements in honey. About 10 g material was treated with 15 ml nitric acid (9.67 M) overnight. The wet ashed procedure was continued with heating on a water bath, following by addition of 2 ml hydrogen peroxide. This treatment was repeated till full digestion. The filtrate was diluted with double distilled water (0.06 μS cm^{-1}) up to 25 ml. All solutions were stored in plastic flasks. Macroelements (Ca, K, Mg, P, S) and microelements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr, V, Zn) have been determined by atomic emission spectrometry with inductively coupled plasma (ICP–AES) using SPECTROFLAME instrument (Germany). 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, 0.5 mg L^{-1} for Ca, K, Mg, Na, P and S. Analytical precision was checked with replicating (deviation between the duplicates was below 5% in all cases), blanks and stock standard solutions (1000 μg L^{-1} Merck) for the preparation of working aqueous solutions. The quality control was assured with plant reference material (NCS DC73348). The measured concentrations were in agreement with the recommended values. The value for each site is the average of 3 samples and for separate sample is the mean of 3 analytical determinations. The concentrations of elements are presented in mg kg^{-1} fresh weight.

Results and Discussion

The water content in polyfloral honeys was in the range of 15.6 (site 7) up to 22.0 (site Sadovo) g 100 g^{-1}, the pH values were 2.92 (site Sadovo) - 3.61 (site 6), and the electrical conductivity was between 225 (site Sadovo) and 578 (site 6) μS cm^{-1} (see **Table 1**). The heavy metals as Cd, Co and microelement V in all cases were under the detection limits (0.01, 0.01 and 0.05 mg kg^{-1}, respectively). Toxic Cr and As and heavy metal Pb were under the detection limits (0.01, 0.05 and 0.078 mg kg^{-1}) in most of cases; exception were some honey samples from the town of Plovdiv. Nickel varied more than one order of magnitude among the different honey samples, followed by Cu, Na, Zn and Fe (8 times), Al, Ca, K, Mg, Mn and Sr (4 times). The maximum values of 6 elements: Al, Ca, Cu, Mn, S and Sr were found in a honey from Rakovski district. The highest concentrations of Fe and Ni were in the honey produced in the area of Plovdiv town (site 7), whereas the highest content of Cu and P was accumulated in the honey sample from Sadovo district.

Water content in blossom honey was found to be in a range of 15 - 20 g 100 g^{-1} (5, 28). The honey samples from the area of Plovdiv were approximately similar to this range. In Switzerland pH in honeys was in average 4.5 ± 0.8 for blossom honey and 4.5 ± 0.26 for honeydew honey (6). The

Mean values (± SD) or range of studied indexes in the polyfloral honey of the Plovdiv area

TABLE 1

Index	**Water content** g 100 g^{-1}	**pH**	**Conductivity** µS cm^{-1}	**Al** mg kg-1	**As** mg kg-1	**Ca** mg kg-1	**Cd** mg kg-1	**Co** mg kg-1	**Cr** mg kg-1	**Cu** mg kg-1	**Fe** mg kg-1
Plovdiv town (n=7)	17.3 ± 1.6	3.35 ± 0.20	408 ± 114	0.46 ± 0.18	<0.05 - 0.11	61.1 ± 16.1	<0.01	<0.01	<0.01 - 0.2	0.06 ± 0.01	2.17 ± 1.58
18 km W, Stamboliyski district (n=3)	18.5 ± 2.0	3.31 ± 0.19	352 ± 81	0.33 ± 0.08	<0.05 - 0.06	77.7 ± 18.5	<0.01	<0.01	<0.01	<0.01 - 0.41	1.89 ± 0.58
17 km NE, Rakovski district (n=3)	17.5 ± 2.1	3.31 ± 0.09	357 ± 177	0.70 ± 0.36	<0.05	97.9 ± 71.8	<0.01	<0.01	<0.01	<0.01 - 0.23	0.89 ± 0.09
14 km E, Sadovo district (n=3)	19.7 ± 2.1	3.15 ± 0.24	303 ± 70	0.39 ± 0.05	<0.05	39.1 ± 11.2	<0.01	<0.01	<0.01	<0.01 - 0.43	2.39 ± 0.33
13 km SE, Asenov-graddistrict (n=3)	17.0 ± 1.0	3.33 ± 0.16	463 ± 50	0.84 ± 0.01	<0.05	136 ± 3	<0.01	<0.01	<0.01	0.23 ± 0.02	1.04 ± 0.43

Continued

TABLE 1

Index	**K** mg kg^{-1}	**Mg** mg kg^{-1}	**Mn** mg kg^{-1}	**Na** mg kg^{-1}	**Ni** mg kg^{-1}	**P** mg kg^{-1}	**Pb** mg kg^{-1}	**S** mg kg^{-1}	**Sr** mg kg^{-1}	**V** mg kg^{-1}	**Zn** mg kg^{-1}
Plovdiv town (n=7)	297 ± 112	13.4 ± 1.9	0.21 ± 0.10	10.7 ± 7.3	<0.01- 0.29	61.4 ± 13.9	<0.08 - 0.50	30.3 ± 8.0	0.19 ± 0.08	<0.05	0.56 ± 0.36
18 km W, Stamboliyski district (n=3)	345 ± 175	19.2 ± 1.9	0.22 ± 0.09	14.5 ± 2.5	<0.01- 0.06	68.2 ± 6.5	<0.08 - 0.38	38.4 ± 7.6	0.24 ± 0.03	<0.05	0.44 ± 0.25
17 km NE, Rakovski district (n=3)	364 ± 310	23.0 ± 18.7	0.27 ± 0.18	14.8 ± 3.3	<0.01- 0.04	63.4 ± 38.3	<0.08	35.8 ± 16.1	0.27 ± 0.18	<0.05	0.39 ± 0.35
14 km E, Sadovo district (n=3)	314 ± 181	16.2 ± 2.2	0.19 ± 0.07	15.7 ± 2.0	0.03 ± 0.01	112 ± 44	<0.08	26.8 ± 9.8	0.15 ± 0.02	<0.05	0.76 ± 0.21
13 km SE, Asenovgrad, district (n=3)	551 ± 98	23.1 ± 1.0	0.24 ± 0.05	13.1 ± 2.6	0.04 ± 0.03	66.9 ± 3.0	<0.08	41.1 ± 5.1	0.28 ± 0.02	<0.05	0.80 ± 0.24

approximately low pH in honey is due to the presence of organic acids that contribute to honey flavour and stability against microbial spoilage. Values of pH (above 4) were pointed out only for honeydew type of honey (25). The studied honey samples were quite acidic, especially one sample from Sadovo district. According to the European honey standard the electrical conductivity, as the best known and one of the most important honey characteristics (9), only of honeydew type should exceed 0.8 mS cm^{-1} (16). Recently the conductivity in blossom Swiss honeys was 0.74 ± 0.54 mS cm^{-1} and 1.04 ± 0.19 mS cm^{-1} in honeydew honeys (6). The studied blossom honeys from the Plovdiv area had similar values of electrical conductivity.

The differences of accumulated elements in honey types may be due to the different geographical origin of the honeys. The most pronounced ones, especially for trace elements, were those between honeydew and blossom type of honey (17, 19, 24). The descending order of all analysed macroelements, heavy metals and toxic elements in average was found as follows: K>Ca>P>S>Mg>Na>Fe>Zn>Ni>Al>Pb>Cu>Sr>Mn>As>Cr >V, Co, Cd. The potassium concentration, being the most abundant in various honey samples, was in accordance with the Food and Agriculture Organization of the United Nations. According to the literature (8) the content of nickel in honey was up to 0.051 mg 100 g^{-1}. The Czech, Slovak and Polish honeys had higher nickel levels than honeys originating from other parts of the world (20). The found maximum of Ni in this study was 0.29 mg kg^{-1} in one site of town of Plovdiv as there is the high motor traffic from the whole area. The heavy metals as Pb, Cd and toxic elements as Cr, As could reflected the presence of contaminants due to environmental contamination or pharmacological (antiparasitical or acaricidal) treatment of honey or by incorrect procedures during the honey processing and conservation phases (22). Data observed in this preliminary study of honeys in the area of Plovdiv showed approximately low content of heavy metals and toxic elements. Except the botanical origin of honey, the reason for the differences of such elements could be some geological and/or geochemical features. In Lithuanian honey some heavy metals varied in a wide range: Pb 2.9 –22.1 μg kg^{-1}, Cd 4.1 - 14.6 μg kg^{-1}, Cu 119.6 - 342.9 μg kg^{-1}, Zn 514.0 - 5639.0 μg kg^{-1} with conclusion that maximum values were found in the urban or industrial area, and the content of these microelements in Lithuanian honey are lower than in honey of other EU countries (26). In comparison the maximum concentrations found in this study were slightly higher for Cu (0.43 mg kg^{-1}) in the honey from Sadovo district and approximately one order of magnitude lower for Pb.

The Bulgarian hygiene norms for heavy metals and toxic elements in bee honey are missing (23). According to the published data from European countries and some Maximum Admitted Levels or Maximum Residue Limits (10, 11, 12, 21), the inorganic content of heavy metals and toxic elements in the studied polyfloral honeys from the area of Plovdiv was approximately lower.

At this stage applied one-way ANOVA (SPSS 10) did not provide any confirmation of significant statistic differences at the 99.0% confidence level among the measured parameters in the honey samples (values of the F-test are greater than 0.01) between different 5 regions in the selected Plovdiv area.

It is known that contaminants can reach the raw materials of bee products (nectar, honeydew, pollen, plant exudates) by air, water, plants and soil due to industry and motor traffic, and then be transported into the bee hive by the bees and after that in honey products (4). Lead (Pb) and cadmium (Cd) are considered the principle toxic heavy metals and were thus most frequently studied. Lead, contained in the air and originating mainly from motor traffic can contaminate air and then directly nectar and honeydew. Generally, Pb is not transported by plants. On the other hand, Cd originating from metal industry and incinerators, is transported from the soil to plants and can then contaminate nectar and honeydew. Only a small portion of Cd might reach honey by air, mainly in the vicinity of incinerators. In 2000 the MRL (Maximum Residue Limit) values of 0.1 mg/kg for Cd and 1 mg/kg for Pb were proposed for the EU. However, today there are no established MRL values for heavy metals in bee honey. Recently Pb contamination is diminishing, due to the decreased world-wide use of car-engine catalysts. Indeed, a decrease of the Pb honey concentration was documented, e.g. in Switzerland (4).

Unpublished data (Yurukova, in preparation) proved that in the Plovdiv area the content of some toxic heavy metals were accumulated quite different in the bees from the regions located NE and SE from the town of Plovdiv. The bees from the Asenovgrad district and 5 km from a non-ferrous big smelter contained up to one order of magnitude higher concentrations of As, Cd, Ni and Pb.

All substances found in the composition of the honey are elements of the modern pharmacotherapy. A composition of honey is a complex of medicinal-prophylaxis substances with diverse characteristics which determine its multispectral medicinal application. In this aspect an exclusively important is the role of melissopalinology, which provides an opportunity to determine the pollen composition of honey in order to apply it purposefully to the corresponding diseases. The analysed honey in Plovdiv area seemed to be polyfloral-nectariferous and with good quality.

The presented data are from a stage of a long-lasting research that will give an opportunity for juxtaposition and comparison of our results from different years with published information from similar studies of inorganic content of bee honey produced in urban and industrial conditions.

Conclusions

The concentrations of heavy metals and toxic elements in the studied honey samples, labelled as polyfloral-nectariferous honey from the beekeepers, were approximately low. Potassium was the most abundant in the honey samples. The contamination levels found do not present a health hazard in the whole area of Plovdiv.

The statistical analysis proved insignificant differences for the honey inorganic chemistry in the different regions of the Plovdiv area, due to the polyfloral type of honey.

The spatial and seasonal fluctuations of electrical conductivity, heavy metals, toxic elements and macroelements accumulated in polyfloral honeys, as well as microscopic analysis, should be a subject of our future studies for confirmation of their high quality as food.

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