Pollen and Inorganic Characteristics of Bulgarian Unifloral Honeys

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Abstract

Atanassova J., Yurukova L., Lazarova M. (2012): **Pollen and inorganic characteristics of Bulgarian unifloral honeys.** Czech J. Food Sci., **30**: 520–526.

The melissopalynological characteristics, three main physicochemical parameters (water content, pH, and electrical conductivity), and 19 macro- and microelements contents of 15 honey types from throughout Bulgaria that were collected from 2006 to 2009 were evaluated. The main honeys studied came from *Robinia pseudoacacia* L., *Helianthus annuus* L., *Brassica* spp., *Tilia* spp., and *Vicia* spp. The botanical origins of unifloral honey samples were identified as *Lotus* spp., *Coriandrum sativum* L., *Daucus*-type, *Stachys*-type, *Salix* spp., *Prunus* spp., *Castanea sativa* Mill., *Paliurus spina-christi* Mill., *Sophora japonica*, and *Amorpha* spp. Based on the physicochemical parameters and elements contents, one sample with high a percentage of *Trifolium* spp. pollen was identified as honeydew honey.

Keywords: unifloral honey; melissopalynology; physical-chemical parameters; macro- and microelements

Unifloral honey is a type of honey predominantly produced from the nectar of a single plant species. Unifloral honeys have an important commercial value because they are regarded as more valuable, often offered for sale at higher prices than the multifloral honeys (Persano Oddo & Bogdanov 2004). Botanical origin strongly influences the quality of honey and all its physical and chemical characteristics (Přidal & Vorlová 2002; Bogdanov 2009; Primorac *et al.* 2011). It can be difficult to differentiate between polyfloral and unifloral honeys because multiple factors influence the honey composition. The European Directive (European Commission 2002) specifies some compositional limits for blossom and honeydew honeys, but it does not establish any legal criteria for unifloral honeys (Persano Oddo & Bogdanov 2004). The classification of unifloral honeys needs to include microscopic, physicochemical, and elemental analyses (Bogdanov *et al.* 2004; Persano Oddo & Piro 2004), however, the limits vary from country to country and may lead to some difficulties in the international trade in unifloral honey.

Melissopalynological investigations are rare in Bulgaria, and so are physicochemical and elemental analyses of honey (Ivanov & Chervenakova 1984; Lazarova & Bozilova 2001; Atanassova & Kondova 2004; Atanassova *et al.* 2004, 2009). The goal of the present study was to define the melissopalynological and physicochemical parameters – water content, pH, and electrical conductivity (EC) as well as the elemental characteristics of Bulgarian unifloral honeys, and to compare them with the data obtained from other European regions.

Partly supported by the National Science Fund, Ministry of Education, Youth and Science, Bulgaria, Project No. N TK-B-1611.

MATERIAL AND METHODS

More than 200 honey samples were obtained, most of them directly from beekeepers all over Bulgaria, having been collected during the years of 2006–2009. Only 36 samples were identified as unifloral honeys according to their melissopalynological characteristics (Table 1) and they are the focus of the present paper. The samples were examined for their physicochemical and elemental properties. Organoleptic characteristics (colour and physical state) were described by sensorial analysis (Table 1).

Melissopalynolgical analysis. The method described by Louveaux *et al*. (1978) was followed in the laboratory preparations and qualitative melissopalynological analysis. Along with the pollen identification, all honeydew elements detected (HDE) (algae and fungal elements) were counted. The frequency of the pollen types was expressed as percentage of the pollen sum (PN), which includes pollen grains only from nectar producing plants. To be considered unifloral, a honey sample should contain at least 45% of the corresponding pollen (Pd) (Louveaux *et al*. 1978). The pollens of some plant species are known to be overrepresented in the honey (e.g. *Castanea sativa* Mill.), while others are underrepresented (e.g. *Robinia pseudoacacia* L), therefore different percentage values mark the honey as unifloral (Louveaux *et al*. 1978; Persano Oddo & Piro 2004). For the quantitative analysis, the method described by Moar (1985) was followed. Tablets containing a known number of spores of *Lycopodium* spp. were added to the sample. The absolute pollen concentration in 1 g of honey was derived from the ratio of total pollen × *Lycopodium* spores added to the number of *Lycopodium* spores counted. The frequency classes of honeys were identified according to Maurizio (1939). The pollen concentration of the Pd was calculated in the same way (Table 1). The ratio HDE/PN was also calculated (Louveaux *et al*. 1978). Pollen identification was made by light microscopy and compared to the reference collections and with Beug (2004).

Physicochemical analysis. The routine physicochemical analysis included water content (honey refractometer Atago HHR-2N 12–30%; Atago Co., Ltd., Tokyo, Japan), EC (mS/cm, ± 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) (Table 2). The Harmonised Methods of the

Table 1. Organoleptic and melissopalynological characteristics of the honeys studied

Numbers	Dominant pollen type	Colour	Physical state	Pd (%)	Concentration of Pd	Frequency class
1–6	*Robinia*	water white–light yellowish	liquid	34.7–53.5	1 193 (mean of 6 samples)	I–II
7	*Trifolium*	brownish	liquid	52.9	1 381 (1)	II
8	*Lotus*	light yellowish	liquid	49.6	4 482 (1)	II
9–10	*Vicia*	water white	liquid	51.8–45.0	1 588 (mean of 2 samples)	II
11	*Sophora*	light yellowish	liquid	74.8	1 767 (1)	II
12	*Amorpha*	light yellowish	liquid	63.8	2 118 (1)	II
13–17	*Tilia*	light amber–dark amber	liquid	36.8–87.8	1 769 (mean of 5 samples)	II
18–23	*Helianthus*	bright yellow–dark yellow	fine crystallised–large granulated	45.1–78.9	2 473 (mean of 6 samples)	II
24–29	*Brassica*	milky white–creamy white	fine crystalised	86.5–93.4	5 043 (mean of 6 samples)	II
30	*Prunus*	water white	liquid	78.8	1 659 (1)	II
31	*Paliurus*	dark yellow	liquid	67.7	3 890 (1)	II
32	*Stachys*	light yellow	fine crystalised	48.8	4 926 (1)	II
33	*Castanea*	dark amber	liquid	97.0	10 104 (1)	III
34	*Coriandrum*	amber	liquid	44.8	3 661 (1)	II
35	*Daucus*	dark yellow	fine crystalised	46.2	1 111 (1)	II
36	*Salix*	amber	fine crystalised	54.2	1 328 (1)	II

International Honey Commission (2009) do not specify any method for the identification of elements in honey. About 10 g of material was treated with 15 ml nitric acid (9.67M) overnight. The wet-ashing was continued with heating in a water bath, followed by the addition of 2 ml hydrogen peroxide. This treatment was repeated until reaching full digestion. The filtrate (through filter paper Filpap KA 2; Filpap, Štětí, Czech Republic) was diluted with double-distilled water (0.06 μS/cm) up to 25 ml. All solutions were stored in plastic flasks. Macroelements K, Ca, Mg, P, S, and microelements Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr, V, and Zn were determined by atomic emission spectrometry with the inductively coupled plasma system (ICP-AES) of VARIAN VISTA-PRO (Tables 3a and 3b). The detection limits were 0.002 mg/l for Mn and Sr, 0.004 mg/l for Cd, Co, Cr, Cu, Ni, and Zn, 0.02 mg/l for As and V, 0.03 mg/l for Pb, 0.04 mg/l for Al and Fe, and 0.5 mg/l for Ca, K, Mg, Na, P, and S. Analytical precision was checked by replications and blanks and by stock standard solutions (1000 μg/l Merck) for the preparation of working aqueous solutions.

RESULTS AND DISCUSSION

According to the literature, over 100 plant species have been recorded as being used by bees to produce unifloral honey in Europe (Persano Oddo & Piro 2004). The data summarised in this study provide a good description of five Bulgarian unifloral honeys: black locust (*R. pseudoacacia*) – 6 samples, lime (*Tilia* spp.) – 5 samples, sunflower (*Helianthus annuus* L.) – 6 samples, rape (*Brassica*, most probably *B. rapa* L. ssp. *oleifera*, *B. napus* L. ssp. *oleifera*) – 6 samples, and vetch (*Vicia* spp.) – 2 samples. Further investigations are necessary to confirm the established characteristics of the other types of unifloral honeys represented by single samples only: coriander (*Coriandrum sativum* L.), willow (*Salix* spp.), *Prunus* spp. (including plums, cherries, peaches and apricots), chestnut (*C. sativa*), garland thorn (*Paliurus spina-christi* Mill.), *Daucus*-type (Apiaceae family), *Stachys*-type (Lamiaceae family), birdsfoot trefoil *(Lotus* spp.), japanese pagodatree (*Sophora japonica* L.), and amorpha (*Amorpha* spp.). One of the samples (7) with high percentage values of *Trifolium* spp. pollen was identified as honeydew honey.

Robinia honey is one of the most valued and purchased honeys on the Bulgarian market but it is difficult to identify. Six samples were identified as *Robinia* honey and were characterised by their light colour and liquid consistence, as were other Fabaceae honeys (*Lotus*, *Vicia*, *Sophora*, and *Amorpha*), with the exception of sample 7 (Table 1). The investigated *Robinia* honeys had the lowest concentration of the Pd and the lowest

Table 2. Main physicochemical parameters of the honeys studied

Number	Type of honey	Water content (%)	pH	Electrical conductivity (mS/cm)
1–6	*Robinia* (mean of 6 samples)	16.9	3.23	0.159
7	Honeydew (1)	17.5	4.28	0.961
8	*Lotus* (1)	15.5	3.22	0.338
9–10	*Vicia* (mean of 2 samples)	19.0	3.32	0.261
11	*Sophora* (1)	15.5	3.36	0.323
12	*Amorpha* (1)	19.0	3.20	0.200
13–17	*Tilia* (mean of 5 samples)	17.1	4.07	0.689
18–23	*Helianthus* (mean of 6 samples)	18.9	3.32	0.359
24–29	*Brassica* (mean of 6 samples)	19.7	3.33	0.181
30	*Prunus* (1)	15.2	3.19	0.185
31	*Paliurus* (1)	17.0	4.14	1.046
32	*Stachys* (1)	17.0	3.32	0.443
33	*Castanea* (1)	18.8	5.65	1.804
34	*Coriandrum* (1)	15.4	4.46	0.469
35	*Daucus* (1)	18.0	3.24	0.454
36	*Salix* (1)	20.0	3.61	0.399

total pollen concentration, belonging to class I and II (Table 1). Pollen in *Robinia* honey is usually underrepresented, because of the low pollen production (Ricciardelli d'Albore 1998). The recommendations of Louveaux *et al*. (1978), and BSS (1990) were followed in this study, namely that 30% of *Robinia* pollen is considered sufficient to classify the honey as unifloral. *Vicia* honey and *Prunus* honey were also declared as *Robinia* honey by the beekeepers, probably because of its identical colour and physical state (Table 1). The mean values of the water content, pH, and EC were the lowest in this study (Table 2), coinciding with the results of Persano Oddo and Piro (2004).

Three types of unifloral honey come from crop plants: sunflower, rape, and coriander. The rape honey was creamy white to milky white with fine crystals. The concentration of *Brassica* pollen was 5043/g on the average, higher than in the *Helianthus* honey (2473/g). Electrical conductivity of rape honeys was on the average 0.181 mS/cm, and of *Helianthus* honey on the average 0.359 mS/cm, which is close to the values cited by Persano Oddo and Piro (2004). For *Brassica* honey, the maximum water content (19.7% on average) was established, followed by *Helianthus* honey (18.9% on average). Water content in blossom honey was found to be in the range of 15–20% (White 1975; Bogdanov 2009). The unifloral honey samples from different sites in Bulgaria revealed a similar range.

The colour of *Tilia* honey ranged from light to dark-amber. The mean value of the pollen concentration (Pd) was 1767/g. We accepted 30% lime pollen as sufficient to classify the honey as unifloral (Louveaux *et al*. 1978; BSS 1990). The mean values of pH and EC had maxima (Table 2) coinciding with the data of Bogdanov (2009). In Switzerland, pH in honeys was on the average 4.5 ± 0.8 for blossom honey and 4.5 ± 0.26 for honeydew honey (Bogdanov & Gfeller 2006). The relatively low pH of honey is due to the presence of organic acids, which contribute to the honey flavour and stability against microbial spoilage. The values of pH above 4.5 were mentioned only for honeydew honey (Bogdanov 2009).

Castanea sativa honey is specific for southwest areas of the country where small groves of sweet chestnut occur. One sample only (33) was identified as unifloral chestnut honey (declared by the beekeeper as honeydew honey). Many authors have noted that *Castanea* pollen is strongly overrepresented in honey, and requires at least 90%, with more than 10 000/g pollen concentration, for accepting this honey as unifloral. The high pollen concentration in sample 33 is the only one assigned to class III in this study (Table 1). The low ratio of HDE/PN (0.06) proved its nectar origin. The high EC (1.804 mS/cm) and pH (5.65%) coincide with the values given by Persano Oddo and Piro (2004) and Bogdanov (2009).

Sample 7 was declared by the beekeeper as honeydew honey, and its organoleptic characteristics corresponded to this type of honey in contrast to the palynological data (Table 1). The ratio HDE/PN was low (0.66). According to the high pH and EC (4.28% and 0.961 mS/cm, respectively), the elevated mineral contents of six elements (Mg, Mn, Na, P, Cu, Cr), and especially the high concentration of Mn, it could be assumed that sample 7 was honeydew honey. According to the European Honey Standard, the EC of the honeydew type is one of the most important characteristics (Bogdanov *et al*. 2004), and should exceed 0.8 mS/cm. The average values of macro- and microelements in different unifloral honeys can be summarised as follows: The lime honeys were characterised on average by the highest contens of Ca, K, Mg, Mn, S, Sr, and Zn. The minimum average values of Ca, Fe, Mg, Mn, P, S, and Zn were found in *Robinia* honeys. The lowest concentrations of Al and K were found in the *Brassica* honeys (Table 3). Metals Ni and Mn varied by more than two orders of magnitude in different honey samples, followed by Cu (over 45 times). The heavy metals Cd, Co, and the microelement V were in all cases below the detection limits. The elements Zn, K, Mg, and Fe varied 25–30 times, Al, P, S, and Ca 6–12 times. The maximum values of the biogenic macroelements K and S were recorded in chestnut honey. According to the literature (Bogdanov *et al*. 2008), the content of Ni in honey was up to 0.051 mg/100 g. The Czech, Slovak, and Polish honeys had higher Ni levels than the honeys originating from other parts of the world (Lachman *et al*. 2007). Maximum of Ni found in this study was in sample 8 (1 mg/1 kg), probably because of the high motor traffic in the area of the Plovdiv town. The heavy metals Pb and Cd and the toxic elements Cr and As could reflect the presence of environmental contamination or pharmacological treatment of bees, or incorrect procedures used for the honey preservation (Pisani *et al*. 2008). In Lithuanian honey, some heavy metals showed a wide range of values: Pb 2.9–22.1 μg/kg, Cd 4.1–14.6 μg/kg, Cu 119.6–342.9 μg/kg, Zn 514.0–5639.0 μg/kg, lead-

Table 3. Concentrations of macro-and microelements in the honeys studied (mg/kg fresh weight)

Number	Honey type	Ca	K	Mg	P	S	Na	Al	Fe	Mn	Zn
1–6	*Robini*a	32	126	6.0	24	12	8.11	0.80	0.83	0.11	0.22
7	Honeydew	92	1121	97	124	41	16.3	0.47	1.73	12.7	0.47
8	*Lotus*	62	223	11	43	22	7.99	0.56	4.37	0.10	0.56
9–10	*Vicia*	33	196	10	68	20	12.2	0.41	1.33	0.12	0.41
11	*Sophora*	78	264	15	71	32	9.62	0.69	3.04	0.34	1.17
12	*Amorpha*	22	103	4.8	30	13	7.22	0.24	0.58	0.06	0.08
13–-17	*Tilia*	77	792	21	49	24	7.50	0.39	1.62	2.45	1.04
18–23	*Helianthus*	71	247	14	41	20	7.58	0.36	1.93	0.36	0.61
24–29	*Brassica*	46	105	11	28	19	8.49	0.36	1.01	0.17	0.25
30	*Prunus*	33	146	6.2	26	12	7.50	0.37	0.35	0.10	0.17
31	*Paliurus*	62	1198	17	67	51	11.8	0.75	1.58	0.97	0.83
32	*Stachys*	57	403	20	71	32	8.43	0.41	0.65	1.71	0.44
33	*Castanea*	66	1628	16	32	20	9.55	0.64	0.59	3.73	0.20
34	*Coriandrum*	44	564	7.1	31	23	14.2	0.49	1.33	0.15	0.27
35	*Daucus*	110	339	15	41	25	8.63	0.35	1.33	0.15	0.23
36	*Salix*	37	464	13	50	20	11.0	1.58	2.17	0.26	0.25

		As	Cd	Co	Cr	Cu	Ni	Pb	Sr	V
1–6	*Robinia*	< 0.1–0.16	< 0.01	< 0.01	< 0.01–0.01	< 0.01–0.15	< 0.01–0.08	< 0.08–0.15	0.15	< 0.05
7	Honeydew	0.320	< 0.01	< 0.01	0.020	0.45	0.14	< 0.08	0.39	< 0.05
8	*Lotus*	< 0.1	< 0.01	< 0.01	< 0.01	< 0.01	1.00	< 0.08	0.17	< 0.05
9–10	*Vicia*	< 0.1	< 0.01	< 0.01	< 0.01	< 0.01–0.43	0.05	< 0.08	0.12	< 0.05
11	*Sophora*	0.112	< 0.01	< 0.01	0.013	0.07	0.06	0.31	0.23	< 0.05
12	*Amorpha*	< 0.1	< 0.01	< 0.01	0.012	< 0.01	0.14	< 0.08	0.11	< 0.05
13–17	*Tilia*	< 0.1	< 0.01	< 0.01	< 0.01–0.01	0.12	< 0.01–0.92	< 0.08–0.19	0.33	< 0.05
18–23	*Helianthus*	< 0.1–0.17	< 0.01	< 0.01	< 0.01–0.01	< 0.01–0.07	< 0.01–0.98	< 0.08	0.21	< 0.05
24–29	*Brassica*	< 0.1–0.40	< 0.01	< 0.01	< 0.01–0.01	< 0.01–0.02	< 0.01–0.04	< 0.08	0.15	< 0.05
30	*Prunus*	0.220	< 0.01	< 0.01	< 0.01	< 0.01	0.57	< 0.08	0.13	< 0.05
31	*Paliurus*	< 0.1	< 0.01	< 0.01	< 0.01	0.07	< 0.01	< 0.08	0.27	< 0.05
32	*Stachys*	< 0.1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.08	0.19	< 0.05
33	*Castanea*	< 0.1	< 0.01	< 0.01	< 0.01	0.09	< 0.01	< 0.08	0.40	< 0.05
34	*Coriandrum*	< 0.1	< 0.01	< 0.01	< 0.01	0.05	< 0.01	< 0.08	0.18	< 0.05
35	*Daucus*	< 0.1	< 0.01	< 0.01	< 0.01	0.08	< 0.01	< 0.08	0.34	< 0.05
36	*Salix*	0.268	< 0.01	< 0.01	< 0.01	0.23	< 0.01	< 0.08	0.13	< 0.05

ing to the conclusion that the maximum values were found in the urban or industrial areas, and that the overall content of these microelements in Lithuanian honey is lower than in the honeys of other EU countries (Staniškienė *et al.* 2006). In comparison, the maximum concentrations found in this Bulgarian study were slightly higher for Cu (0.45 mg/kg) in the honeydew honey, and almost three times lower for Zn (1.861 mg/kg) in the *Tilia* honeys. The most pronounced difference in the trace elements contents was that between honeydew and blossom honeys (Ivanov & Chervenakova 1984; Feller-Demalsy *et al.* 1989; Sevlimli *et al.* 1992). The differences in the contents of metals between the individual monofloral honey samples could be related to different compositions of organic compounds and their concentrations in the pollen. Some constituents such as phenolic compounds and flavonoids contained in the pollen could form complexes with metals (Lachman *et al*. 2010). This can explain higher levels of Al, As, Cu e.g. in *Salix* type honey.

The ranking of averages of all the analysed macroelements, heavy metals, and toxic elements in the *Robinia* honey was K > Ca > P > S > Na > Mg > Fe > Al > Zn > Sr > Mn, Ni, Cu, Cr, As, Pb, V, Co, Cd. In the *Tilia* honey, the ranking was: K > Ca > P > S > Mg > Na > Mn > Fe > Zn > Al > Sr > Cu; in the studied *Helianthus* honeys and the *Brassica* honeys, the order was similar to that of the *Robinia* honey, except with Mn occupying the last place. Potassium concentration, the highest one in various honey samples, was in accordance with the standards set by the Food and Agriculture Organization of the United Nations. No hygienic norms have been set for heavy metals and toxic elements in honey in Bulgaria. According to the data published by the European countries and some Maximum Admitted Levels, or Maximum Residue Limits (Čelechovská & Vorlová 2001; Matei *et al*. 2004; Bratu & Georgescu 2005), however, the contents of heavy metals and toxic elements in the studied honeys from Bulgaria were low and did not present any health hazard

CONCLUSIONS

Unifloral honeys are rare in Bulgaria, because it is a common practice of the small honey producers to harvest honey only two times during the apicultural period. The botanical origin of honeys declared by the producers often did not correspond to our results of melissopalynological and physicochemical analyses.

The present study showed that the most common unifloral honey types in different regions of Bulgaria during 2006–2009 were black locust, lime, sunflower, and rape honeys. The ranking of average pollen concentrations for the honeys studied was *Brassica* > *Helianthus* > *Tilia* > *Robinia*. The highest pollen density was established for chestnut honey. The lime honey was found to have the highest average pH and EC and the maximum contents of Ca, K, Mg, Mn, S, Sr, and Zn. The highest EC (1.804 mS/cm) and pH (5.65) were observed in a single honey sample of chestnut. Based on the physico-chemical parameters and elements contents, one of the samples with a high percentage (59%) of *Trifolium* spp. pollen was identified as honeydew honey.

The present study showed low contents of heavy metals and toxic elements in all the honeys studied. The strongly varying contents of macro- and microelements could not be related only to different botanical origins of the honeys, but it also reflects different geographical origins as well as the environmental contamination of the localities in the respective geographical regions.

Acknowledgements. The authors are grateful to Dr. P. Zhelev for collecting chestnut honey, to the staff of the Analytical Laboratory at the Institute of Biodiversity and Ecosystem Research, BAS for the laboratory work, and to Prof. Petra Mudie for improving the English text.

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Received for publication February 2, 2012
Accepted after corrections April 17, 2012

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