

HONEY AS A SOURCE OF BIOACTIVE COMPOUNDS

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Abstract

The total phenolic content and the content of flavonoids and metals – K, Ca, Mg, Zn, Fe, Mn and Cu, were determined in honey of different botanical origin supplied mostly by domestic producers, in particular from north-eastern Poland. The daily intake of the analyzed compounds supplied with honey was determined for Poland on a per capita basis.

Phenolic compound levels in the analyzed honey ranged from 86 to 1141 mg kg⁻¹, and the highest concentrations were noted in buckwheat honey, followed by honeydew and heather honey. The majority of the analyzed honeys were characterized by low total flavonoid levels, and the highest flavonoid concentrations were noted in buckwheat honey at 27.8 mg kg⁻¹ on average. The average flavonoid content of the analyzed samples was determined at 10.5 mg kg⁻¹. Summer multifloral honey and linden honey were most abundant in metals, and linden honey was characterized by the highest concentrations of K.

The results of this study and honey consumption statistics indicate that honey, in particular light-colored botanical varieties, is a poor dietary source of phenolic compounds and flavonoids. Honey is also deficient in metals. It is a source of approximately 0.02% of the recommended daily allowance (RDA) for calcium, 0.01% RDA for magnesium and 0.03% AIs of potassium.

MIÓD JAKO ŹRÓDŁO ZWIĄZKÓW BIOAKTYWNYCH

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Słowa kluczowe: związki fenolowe, flawonoidy, metale, miód.

Abstrakt

Określono poziomy stężenie związków fenolowych ogółem i flawonoidów oraz metali: K, Ca, Mg, Zn, Fe, Mn i Cu w botanicznych odmianach miodu pochodzącego w większości z pasiek krajowych, przede wszystkim z regionu północno-wschodniej Polski. Przedstawiono szacunkowe dzienne pobranie tych składników z porcją miodu spożywaną przez statystycznego mieszkańca Polski.

Poziomy związków fenolowych w badanych miodach były zróżnicowane i wynosiły od 86 do 1141 mg kg⁻¹, najwyższe oznaczono w miodach gryczanych, nieco niższe w spadziowych i wrzosowych. Większość miodów charakteryzowała się niskimi poziomami flawonoidów ogółem, najwięcej zawierały ich miody gryczane, średnio 27,8 mg kg⁻¹. Z kolei średnia zawartość flawonoidów w badanych miodach wyniosła 10,5 mg kg⁻¹. Najbogatszym źródłem metali okazały się miody letnie: wielokwiatowy letni oraz lipowy, z najwyższym udziałem K.

Zestawiając uzyskane wyniki z danymi dotyczącymi spożycia miodu stwierdzono, że miód, a szczególnie jego jasne odmiany botaniczne, wnoszą do dziennej diety niewielką ilość związków fenolowych oraz flawonoidów. Miody są również ubogim źródłem metali. Pobranie Ca z miodem stanowiło około 0,02% RDA, Mg około 0,01% RDA oraz K około 0,03% AIs.

Introduction

The therapeutic properties of honey have been valued for many centuries. The health benefits offered by honey can be attributed to the specific chemical composition of different honey types. Natural honey is a rich source of natural antioxidants which effectively minimize the risk of coronary heart disease, immune system disorders and cancer (Linus Pauling Institute). Honey also contains bioactive phenolic compounds, enzymes and minerals. The group of non-enzymatic antioxidants present in honey includes phenolic acids, flavonoids (flavanones and flavanols), carotenoids and organic acids. The antioxidant properties of honey are attributed mainly to the presence of phenolic compounds. Dark-colored varieties are believed to have the most potent antioxidant properties, although research results are not always conclusive (LACHMAN et al. 2010, WILCZYŃSKA 2010, ESCUREDO et al. 2013). The total phenolic content of honey differs significantly across varieties. It was determined in the range of 17.6 to 189.5 mg per 100 g in nine types of Polish honey (WILCZYŃSKA 2010) and 59.9 to 121.4 mg per 100 g in heather and buckwheat honey (JASICKA-MISIAK et al. 2012).

The predominant flavonoids in European varieties of honey are pinocembrin, pinobanksin, chrysin, hesperetin, galangin, myricetin, kaempferol and quercetin. In Spanish honey, the presence of around 18 flavonoids was determined with total concentrations of 500 to 2000 µg 100 g⁻¹ (ANKLAM 1998). Polish buckwheat honey is a rich source of myricetin and quercetin. The flavonoid profiles of honeys of different botanical origin vary considerably between studies, and there is scant information about the total flavonoid content of particular varieties (JASICKA-MISIAK et al. 2012). Selected polyphenols can be used as specific markers with characteristic UV spectra (such as

hesperetin, a marker of citrus honey), and they facilitate quick identification of monofloral honey varieties (TOMÁS-BARBERÁN et al. 2001).

Phenolic acids that can be potentially used as markers for identifying the botanical origin of honey include p-coumaric, hydroxybenzoic, ellagic, syringic, ferulic, gallic and abscisic acid (AMIOT et al. 1989, BOGDANOV et al. 2004). Polish honey is also a rich source of those compounds. Buckwheat honey contains mainly p-hydroxybenzoic, ferulic, p-coumaric and vanillic acid, whereas heather honey is a potent source of abscisic, ellagic, rosmarinic and p-coumaric acid (BIESAGA, PYRZYŃSKA 2009, JASICKA-MISIAK et al. 2012, WILCZYŃSKA 2012).

The authenticity and health benefits of different honey types is determined based on their polyphenol profiles and mineral composition. Trace elements have been long used as indicators of the geographic and botanical origin of honey, and honey is a reliable environmental marker (CAROLI et al. 1999, WIECZOREK et al. 2006, ATANASSOVA et al. 2009). BOGDANOV et al. (2007) analyzed trace elements of anthropogenic and natural origin in honey. The elements of anthropogenic origin are mainly Pb, Cd and Zn. The presence of Cu, Cr and Ni could also be attributed to environmental pollution, whereas Fe and Mn occur naturally in the soil.

The average mineral content of floral honey is 0.2% (max. 0.5%). The most abundant trace element is potassium. Honey varieties may differ in their content of Mg, Ca, Al, Fe, Mn, Zn, B, Cu, Co, Cr, Ni, Cd and P (NOZAL NALDA et al. 2005, WIECZOREK et al. 2006). The correlations between the content of trace elements and types of honey were determined by NOZAL NALDA et al. (2005).

The authenticity of different honey types is ascertained by infrared spectroscopy and fluorescence spectroscopy (ETZOLD, LICHTENBERG-KRAAG 2007). It should be noted, however, that the time-consuming palynological analysis continues to be the reference method for identifying polyfloral honey types. Its results point to considerable pollen diversity across spring and summer varieties of honey harvested from the same region in different years, which explains the significant variations in bee products and the mineral content of honey (ATANASSOVA et al. 2009).

European suppliers have to document the botanical and geographic origin of their products to register traditional and regional honey in the EU protection system. For the consumers, certificates of authenticity testify to the product's high quality (desirable sensory characteristics and health benefits) which, in turn, contributes to higher sales. In Poland, consumers buy 65% of honey directly from apiaries, which testifies to the product's authenticity (*Pszczelarstwo...* 2012).

The objective of this study was to determine the total phenolic content, flavonoid content, metal concentrations (K, Ca, Mg, Zn, Fe, Mn and Cu) and

differences in the concentrations of the analyzed chemical compounds in honey of different botanical origin. Also, the daily intake of the analyzed bioactive compounds supplied with honey was determined for Poland on a per capita basis.

Materials and Methods

Honey samples

Honey of different botanical origin was supplied directly by Polish apiaries. The majority of samples were obtained from the Region of Warmia and Mazury, including the Department of Apiology at the University of Warmia and Mazury in Olsztyn (samples 1–5, 7 and 8). Information about the supplied honey varieties was provided by the apiaries. Manuka honey was purchased online, and honeys produced in and outside the EU were purchased in a supermarket. The purchases were made in 2011–2012 (Table 1), and the acquired samples were dark-stored at the temperature of 10°C. Total phenolic concentrations and flavonoid concentrations were determined in 23 products, metal levels were identified in four botanical varieties, including two spring (acacia and polyfloral) and two summer (linden and polyfloral) varieties.

Determination of total phenolic content

Honey was dissolved in distilled water to produce a solution with the concentration of 0.1 g/ml. The solution was filtered, 2 ml of the filtrate was transferred to 50 ml flasks and combined with 8 ml of distilled water, 2.5 ml of the Folin-Ciocalteu reagent and 7.5 ml of 20% Na₂CO₃. The mixture was supplemented with distilled water and stirred. After 1 hour, absorbance was measured in the Varian Cary 50 spectrophotometer at $\lambda = 765$ nm. The honey solution was replaced with distilled water in the reference sample. The total phenolic content was expressed as the equivalent of gallic acid in mg per kg of honey (LACHMAN et al. 2010).

Determination of total flavonoid content

The total flavonoid content of honey was determined colorimetrically with aluminum trichloride (analytically pure AlCl₃, Fluka) according to the method proposed by ÖZKÖK et al. (2010) with own modifications. 3 g of honey was dissolved in 10 ml of 80% water methanol solution and the mixture was

Table 1
Total phenolic and flavonoid content of the analyzed honey samples expressed as gallic acid and quercetin equivalents

Sample	Botanical variety	Origin	Harvest date	Color*	Phenolic compounds [mg kg ⁻¹]	Flavonoids [mg kg ⁻¹]
1	linden	Mazury	summer 2012	yellow	409 ± 24	15.1 ± 3.2
2	buckwheat	Mazury	summer 2012	dark brown	1141 ± 45	32.3 ± 4.1
3	honeydew	Mazury	summer 2012	brown	737 ± 14	11.5 ± 2.0
4	heather	Mazury	summer /fall 2012	amber, brown	517 ± 18	3.4 ± 1.3
5	buckwheat	Mazury	summer 2012	dark brown	1104 ± 42	23.4 ± 3.5
6	bean	Lublin	summer 2012	white, pale yellow	181 ± 29	3.2 ± 1.2
7	mixed (multifloral, rapeseed)	Mazury	spring 2012	white, pale yellow	175 ± 9	2.8 ± 0.9
8	willow	Mazury	spring 2012	white, pale yellow	281 ± 11	9.6 ± 2.1
9	linden	Mazury	summer 2012	amber, yellow	309 ± 25	8.4 ± 3.2
10	linden	Mazury	summer 2012	amber, yellow	252 ± 12	4.3 ± 2.6
11	multifloral	Mazury	spring 2011	yellow	217 ± 10	1.8 ± 0.8
12	honeydew	Mazury	summer 2012	brown	588 ± 43	21.8 ± 3.1
13	multifloral	Mazury	spring 2012	pale yellow	176 ± 13	2.0 ± 0.7
14	multifloral	Podlasie	summer 2012	yellow	307 ± 24	5.8 ± 1.1
15	manuka	New Zealand**	2012***	amber, orange	634 ± 39	22.3 ± 3.2
16	multifloral	Lublin	summer 2012***	yellow	255 ± 10	4.7 ± 1.8
17	linden	Mazury	summer 2012	yellow	296 ± 7	7.5 ± 2.1
18	mixed	EU and non-EU**	2012***	pale yellow	179 ± 13	3.3 ± 0.5
19	multifloral	Mazury	summer 2012	amber	292 ± 12	14.4 ± 2.9
20	acacia	Mazury	spring 2012	cream, pale yellow	86 ± 7	1.1 ± 0.5
21	mixed (linden, multifloral)	Mazury	summer 2012	yellow	167 ± 10	8.5 ± 1.7
22	linden	Mazury	summer 2012	yellow	259 ± 30	9.4 ± 1.5
23	mixed (buckwheat, linden)	Mazury	summer 2012	brown, medium-dark	878 ± 22	25.6 ± 4.1

* visual evaluation, **foreign honey, ***date of purchase

filtered. 1 ml of the filtrate was transferred to a centrifuge tube, it was combined with 3 ml of 99.8% methanol, 0.2 ml of 10% water solution of aluminum trichloride and 0.2 ml of 1M potassium acetate and supplemented with 5.6 ml of distilled water. The mixture was stirred and left to stand for 45 minutes at room temperature. Absorbance was measured in the Varian Cary

50 spectrophotometer at $\lambda = 415$ nm in two replications for each sample. The reference (blind sample) for every honey sample was prepared by replacing 0.2 ml of 10% aluminum trichloride solution with distilled water. The total flavonoid content was expressed as the equivalent of quercetin which was used to plot the calibration curve (MALWADE 2013).

Metal concentrations

Metal concentrations – K, Ca, Mg, Zn, Fe, Mn and Cu – in honey samples were determined by flame atomic absorption spectroscopy (FAAS) according to the method proposed by WIECZOREK et al. (2006).

Statistical analysis

The results were processed statistically in the GraphPad Prism v.4.01 software (GraphPad Software, San Diego, California, USA). Significant differences between the analyzed honey types were verified by the unpaired t-test with Welch correction and the Kruskal-Wallis test.

Results and Discussion

Total phenolic content

The total content of phenolic compounds and flavonoids in the analyzed honeys is presented in Table 1. Phenolic concentrations varied significantly in the range of 86 to 1141 mg kg⁻¹. The lowest average phenolic content was determined in acacia honey. Buckwheat honey was the richest source of phenolic compounds whose content was several to more than ten times higher (acacia honey) in comparison with other honey types. Similar phenolic compound concentrations in 7 buckwheat honey varieties were noted by JASICKA-MISIAK et al. (2012) in the range of 983 to 1214 mg kg⁻¹. The relatively leveled phenolic content of buckwheat honey could be an indicator of phenolic compound concentrations in mixed honey. Brown-colored buckwheat and linden honey (sample 23) was characterized by high phenolic content, which points to a small share of linden honey in the product. JASICKA-MISIAK et al. (2012) observed somewhat higher phenolic compound concentrations (599 to 762 mg kg⁻¹) in 14 varieties of heather honey in comparison with heather honey harvested in the region of Mazury (Table 1).

The botanical variety declared by suppliers was difficult to confront with the actual pollen content of honey. A palynological analysis revealed significant differences in the composition of the evaluated honey types, which explains the variations in their phenolic content (ATANASSOVA et al. 2009). In general, brown-colored honey had a higher phenolic content than lighter varieties which were white to pale yellow in color.

In comparison with domestic honey, manuka honey was characterized by higher phenolic content which was similar to that of honeydew honey but nearly twice lower than that of buckwheat honey. Honeys produced in and outside of the EU, including bean honey, spring multifloral honey and Polish mixed honey, were characterized by low levels of phenolic compounds.

Flavonoids

The total flavonoid content of the analyzed honey types was determined in the range of 1.1 to 32.3 mg kg⁻¹ with an average of 10.5 mg kg⁻¹. In Polish honey, the highest flavonoid concentrations were reported in buckwheat honey, followed by buckwheat and linden honey and honeydew honey (Table 1). Similar flavonoid levels were reported in manuka honey from New Zealand (22.3 mg kg⁻¹). A comparison of our results with other authors' findings indicates that the highest flavonoid concentrations in Polish products are similar to the average values reported in foreign honey varieties. Minimum flavonoid concentrations were similar regardless of geographic origin. Honey produced in Burkina Faso was characterized by a higher average flavonoid content at 25.7 mg kg⁻¹ (in the range of 1.7 to 83.5 mg kg⁻¹) (MEDA et al. 2005), and the average flavonoid concentrations in Turkish honeydew honey were determined at 22.8 mg kg⁻¹ (in the range of 4.8 to 54.8 mg kg⁻¹) (ÖZKÖK et al. 2010). Average flavonoid levels were also higher in Spanish honey of botanical origin (ESCUREDO et al. 2013).

Metals

The average mineral concentrations in the analyzed honey samples are presented in Table 2. The levels of different minerals were compared by the Kruskal-Wallis test to reveal varietal differences. Similarly to other samples of European honey (YILMAZ, YAVUZ 1999, LATORRE et al. 1999, ATANASSOVA et al. 2009), the predominant element was potassium. The highest concentrations of potassium were noted in summer multifloral and linden honey, whereas spring multifloral honey was least abundant in this mineral.

Table 2

Metal concentrations in honey. Concentrations are given in mg kg^{-1} ,
p-values refer to the Kruskal-Wallis test

		K	Ca	Mg	Mn	Zn	Fe	Cu
Spring multifloral	Mean	233	58.4	11.6	0.37	2.26	1.9	0.04
	SD	94	7.8	3.1	0.25	1.4	0.7	0.03
Acacia	Mean	383	54.1	13.2	0.41	1.65	3.6	0.06
	SD	150	27.6	6.0	0.30	0.59	1.3	0.07
Linden	Mean	742	86.6	14.3	0.39	2.63	3.1	0.06
	SD	340	14.3	6.0	0.34	1.6	0.9	0.03
Summer multifloral	Mean	782	68.7	24.0	1.25	6.20	4.0	0.04
	SD	305	4.6	8.5	0.76	1.5	1.7	0.02
p		0.007	0.024	0.125	0.187	0.052	0.050	0.695

The second most prevalent element in the tested honey varieties was calcium. Calcium concentrations were similar to those reported by YILMAZ and YAVUZ (1999) and ATANASSOVA et al. (2009). The highest Ca levels were noted in linden honey. Significant differences in Ca concentrations ($p < 0.05$) were determined between linden and spring multifloral honey, linden and summer multifloral honey, and spring multifloral and summer multifloral honey. Calcium concentrations varied over a relatively narrow range of values.

Magnesium concentrations in spring multifloral, acacia and linden honey were characterized by very low variation, and similar results were reported in a study of Bulgarian honey (ATANASSOVA et al. 2009). The average Mg concentrations reached 24.0 mg kg^{-1} only in summer multifloral honey.

Summer multifloral honey was characterized by significantly higher Zn concentrations in comparison with the remaining botanical varieties. Fe levels were relatively similar, but significant differences were observed between varieties. Zn and Fe concentrations determined in this study were somewhat higher than those noted by BOGDANOV et al. (2007) and MATUSEVICIUS et al. (2010), but Zn levels were more than ten-fold lower than in multiflower and linden honey analyzed by PRZYBYŁOWSKI and WILCZYŃSKA (2001).

The tested honey samples were characterized by similar manganese and copper levels (Table 1). The average Mn content of the evaluated samples was nearly identical to that of Swiss acacia honey and similar to that of multifloral varieties (Bogdanov et al. 2007). Honey of botanical origin produced in north-eastern Poland had a low Cu content which corresponded to minimum Cu concentrations reported by BOGDANOV et al. (2007) and MATUSEVICIUS et al. (2010).

Dietary intake of total phenolic compounds, flavonoids and metals (K, Ca and Mg) from honey

The estimated daily intake of selected bioactive ingredients consumed with one serving of honey in Poland and Greece is compared in Table 3. The presented data indicates that even dark-colored honey is a poor source of phenolic compounds in the daily diet (2 to 8 mg). In comparison with honey, berries are a much more abundant source of phenolic compounds. Even a small serving of berries whose phenolic content is equivalent to that of 16 g of strawberries (Institute of Agricultural and Food Economics – National Research Institute) supplies 76 to 126 mg of total phenolic compounds (BOJARSKA et al. 2006). The most popular fruit juice varieties – orange, apple and black currant – are also characterized by a high phenolic content which was determined at 27, 16 and 50 mg per 100 ml, respectively (MICHALAK-MAJEWSKA et al. 2009).

Table 3
Daily intake of phenolic compounds, flavonoids and metals (K, Ca, Mg) from consumed honey

Honey consumption [g/person/day]	Average intake from honey [mg/person/day]						
	phenolic compounds			flavonoids, average n=22	K	Ca	Mg
	polish honey n=22	dark-colored honey n=6	light-colored honey n=16				
Poland 1.67*	0.68	1.38	0.40	0.017	0.9	0.1	0.03
(2011): 2.33**	0.96	1.93	0.56	0.023	1.2	0.2	0.04
Greece 9.58***	3.92	7.93	2.30	0.096	5.1	0.6	0.15

* estimated based on annual per capita honey consumption in Poland in 2011 (0.61 kg/person/year), ** estimated based on per capita honey consumption in Poland in 2011, adjusted for exports and imports (0.85 kg/person/year), *** in terms of the highest honey consumption in the EU – Greece (3.5 kg/person/year).

Honey is a poor dietary source of flavonoids (Table 3). In the treatment of vascular diseases, cancer and neurodegenerative diseases (Parkinson's disease, Alzheimer's disease), the recommended daily flavonoid intake should exceed 13 mg (Linus Pauling Institute). Regardless of daily honey consumption, the estimated daily flavonoid intake does not exceed 0.02 mg in Poland and 0.1 mg in Greece. Plants are the richest source of flavonoids in the diet, and even regular and increased consumption of honey will not deliver similar flavonoid intake levels. Dark-colored fruit is abundant in anthocyanidins, and high concentrations of flavones (apigenin) and flavonols (isorhamnetin) are found in parsley, but the main sources of dietary flavonoids are tea, citrus fruit, berries, apples and vegetables (celery, lettuce) (USDA Database. 2011).

The intake of K, Ca and Mg from an average daily serving of honey is presented in Table 3. For adult consumers, the RDA for calcium is 1000-1300 mg, and the RDA for magnesium – 310–420 mg. Therefore, the daily intake of the above minerals from honey accounts for 0.02% RDA for calcium and 0.01% RDA for magnesium (Dietary Reference Intakes). Honey is also deficient in potassium whose intake with a daily serving of honey accounts for 0.03% RDA for K (Adequate Intakes, AIs).

Conclusions

1. Polish dark-colored honey (buckwheat honey and honeydew honey) has a much higher total phenolic and flavonoid content than honey of other botanical varieties and mixed honey.
2. Polish honey, in particular summer varieties, is most abundant in K, Ca and Mg.
3. The dietary intake of total phenolic compounds, flavonoids and metals from an average and increased (to the European maximum) daily serving of honey is low.

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