

PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF DIFFERENT TYPES OF POLISH HONEY – A SHORT REPORT

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Thirty two samples of different types of Polish honeys were investigated in order to assess their total phenolic content by Folin-Ciocalteu method and their potential antioxidant activity by 1,1 diphenyl-2-picrylhydrazyl (DPPH) and ABTS methods. Results of the study showed that the total phenolic content and antioxidant activity differed widely among different honey types. The highest phenolic content and antioxidant activity had dark honeys, while the lowest antioxidant activity and phenolic content were found in pale honeys. Statistical analysis showed that the total phenolic content of honey was correlated with its antioxidant activity.

INTRODUCTION

Honey is a natural food product produced when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. The definition of honey stipulates a pure product that does not allow for the addition of any other substance.

From ancient times honey has been used in healing purposes. Numerous studies have demonstrated that honey is a source of energy and may be used for the treatment in many diseases: colds, skin wounds and various gastrointestinal diseases [The National Honey Board, 2003]. This beneficial role is contributed to both antibacterial and anti-inflammatory properties of honey, regarding high osmolarity, acidity and content of hydrogen peroxide. The antibacterial property of honey is well known and documented as well [Molan, 1992a,b; Taomina *et al.*, 2001, Weston, 2000]. However, since some of diseases have been recognized as being a consequence of free radical damage, it seems that part of the therapeutic role of honey is due to its antioxidant activity. Honey contains a number of components known to act as antioxidants, *i.e.* vitamin C, vitamin E, enzymes and phenolic compounds, therefore it may be used as a natural source of free radical scavenging compounds.

Antioxidant capacity of honey depends on the floral and geographical origin, climatic conditions and processing, storing and handling of honey. The greatest influence on the antioxidant activity of honey has been contributed to its botanical origin [Al-Mamary *et al.*, 2002; Beretta *et al.*, 2005; Frankel *et al.*, 1998; Gheldof *et al.*, 2002]. Many studies have shown that antioxidant activity is strongly correlated with phenolics content, as well as that the antioxidant activity of dark honeys is higher than of the light honeys [Aljadi & Kamaruddin, 2004;

Al-Mamary *et al.*, 2002; Beretta *et al.*, 2005; Hołderna-Kędzia & Kędzia, 2006].

Available literature indicates that until now there have been just few researches to determine the phenolic content and antioxidant activity of Polish honeys [Hołderna-Kędzia & Kędzia, 2006]. In the present study we investigated the above mentioned parameters of different types of honey: acacia (*Robinia pseudoacacia*), lime (*Tilia spp.*), multifloral, rape (*Brassica napus*), dandelion (*Traxacum officinalis*), heather (*Calluna vulgaris*), buckwheat (*Fagopyrum*) and honeydew (32 samples in all). Additionally correlation between all the analysed parameters was evaluated.

MATERIALS AND METHODS

Chemicals and instruments

All the chemicals and reagents used were of analytical grade. DPPH (1,1 diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, Folin-Ciocalteu reagent, gallic acid, phosphate-buffered saline and methanol were obtained from Fluka, Germany. Potassium persulfate and sodium carbonate were purchased from POCH, Poland. For absorbance measurements an UV-VIS spectrophotometer Unicam UV2-100 was used.

Samples

All the honey samples were obtained directly from beekeepers from different locations across Poland during summer of 2009. The floral origin of samples was specified by beekeepers regarding hive location and available floral sources and confirmed by sensory analysis. The overall characteristics of honey samples is presented in Table 1. The honeys were stored at room temperature in dark before analysis.

TABLE 1. Characteristic of honey samples.

Sample no.	Floral origin	Geographical origin	Harvest date	Colour*
1	rape	Pomerania	July 2009	cream
2	rape-dandelion	Pomerania	July 2009	cream to pale yellow
3	rape-dandelion	Pomerania	July 2009	cream to pale yellow
4	lime	Pomerania	July 2009	amber
5	accacia	Pomerania	July 2009	pale yellow
6	honeydew	Pomerania	August 2009	dark brown with green tinge
7	buckwheat	Pomerania	July 2009	dark brown
8	multifloral	Pomerania	July 2009	amber
9	rape	Pomerania	July 2009	cream
10	dandelion	Pomerania	July 2009	cream
11	honeydew	Małopolska region	July 2009	dark brown with green tinge
12	buckwheat	Małopolska region	July 2009	dark brown
13	multifloral	Małopolska region	July 2009	amber
14	accacia	Małopolska region	July 2009	yellow
15	rape	Małopolska region	July 2009	cream
16	heather	Małopolska region	August 2009	dark brown
17	honeydew	Bieszczady	July 2009	dark brown with green tinge
18	dandelion	Małopolska region	July 2009	pale yellow
19	accacia	Wielkopolska region	July 2009	yellow
20	multifloral	Wielkopolska region	July 2009	amber
21	honeydew	Bieszczady	July 2009	dark amber
22	multifloral	Pomerania	July 2009	cream
23	multifloral	Wielkopolska region	July 2009	pale amber
24	nectar-honeydew	Bieszczady	July 2009	dark amber
25	heather	Wielkopolska region	August 2009	pale brown
26	multifloral	Masuria	July 2009	cream
27	rape	Pomerania	July 2009	cream
28	multifloral	Pomerania	July 2009	dark yellow
29	lime	Pomerania	July 2009	amber
30	buckwheat	Pomerania	August 2009	dark brown
31	rape	Pomerania	July 2009	cream
32	heather	Pomerania	August 2009	dark amber

*visual observation

Methods

To determine the total phenolic content (TPC) of honeys, the method of Meda *et al.* [2005] was employed. Honey solutions with a concentration of 1 g/10 mL of distilled water were centrifuged (3000×g) and filtered. After that, 0.5 mL of the resultant solution were mixed with 2.5 mL of 0.2N solution of Folin-Ciocalteu reagent and 2 mL of sodium carbonate solution (75 g/L). After incubation in dark and at room temperature for 2 h, the absorbance of the reaction mixture was measured at $\lambda=760$ nm using a UV-VIS spectrophotometer Unicam. The standard curve was produced for gallic acid within the concentration range from 20 to 200 mg/L ($R^2=0.9998$, $y=0.1254x-0.1424$) The total phenolic content was expressed as mg gallic acid equivalents per 100 g of honey sample (mgGAE/100 g).

The scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]) radical of honey was estimated according to the procedure described by Turkmen *et al.* [2005] with some modifications. A honey sample (2 g) was dissolved in 10 mL of distilled water, centrifuged (3000×g) and filtered. Then, 0.75 mL of the solution were mixed with 2.25 mL of 0.1 mmol/L methanol solution of DPPH 1,1-diphenyl-2-picrylhydrazyl. The control test was made with distilled water in place of honey solution. The reaction mixtures were vortex-mixed well and incubated for 60 min at a room temperature in the dark. Absorbance was measured at $\lambda=517$ nm against methanol, using a UV-VIS spectrophotometer Unicam. Antioxidant activity was expressed as a percent of inhibition of DPPH radical and calculated from the equation:

$$AA[\%] = (\text{Abs}_{\text{contr}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{contr}} \times 100\%.$$

The antioxidant activity of honey samples in the reaction with stable ABTS radical cation was determined according to Baltrusaityte *et al.* [2007]. ABTS^{•+} was obtained in the reaction of a 2 mmol/L stock solution of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt in phosphate-buffered saline with potassium persulfate. The mixture was left to stand for 24 h. Prior to analysis the ABTS^{•+} solution was diluted with phosphate buffer saline to produce a solution with absorbance of 0.80 ± 0.03 at $\lambda = 734$ nm. A honey sample (2 g) was dissolved in 10 mL of distilled water, centrifuged (3000×g) and filtered. Then, 0.1 mL of the solution were mixed with 6 mL of ABTS^{•+} solution, vortex-mixed well and after 15 min absorbance was measured at a wavelength of 734 nm. The control test was

made with distilled water in place of honey solutions. Antioxidant activity was expressed as percent inhibition of ABTS^{•+}, calculated from the same equation as for DPPH.

Statistical analysis

Correlation coefficients (*r*) to determine correlations between particular parameters were calculated using MS Excel Software (CORRELATION statistical function).

RESULTS AND DISCUSSION

Thirty-two honey samples of different floral origin were analysed in order to assess their antioxidant activity and find relationship between the antioxidant activity and the total phenolic content. The results obtained showed (Table 2) that all the samples tested were antioxidantively active, however

TABLE 2. Total phenolics content, radical scavenging activity and antioxidant activity of the analysed Polish honeys.

Sample no.	Floral origin	Total phenolics content (mgGAE/100 g)	Radical scavenging activity DPPH (%)	Antioxidant activity ABTS (%)
1	rape	33.42	43.91	12.80
2	rape-dandelion	32.33	39.45	15.02
3	rape-dandelion	37.42	56.24	15.30
4	lime	47.14	63.48	17.80
5	accacia	40.55	25.58	12.93
6	honeydew	64.59	83.51	9.60
7	buckwheat	110.41	68.88	22.11
8	multifloral	45.98	42.53	17.66
9	rape	35.87	43.14	20.86
10	dandelion	45.44	48.23	16.97
11	honeydew	71.88	79.51	22.81
12	buckwheat	87.28	56.39	19.05
13	multifloral	53.05	71.03	11.96
14	accacia	32.54	35.90	2.78
15	rape	41.17	55.16	26.43
16	heather	109.53	100.00	21.42
17	honeydew	67.13	73.33	19.34
18	dandelion	63.02	46.03	13.99
19	accacia	39.99	27.14	2.29
20	multifloral	45.25	43.97	12.21
21	honeydew	58.24	72.54	24.43
22	multifloral	37.05	40.95	11.70
23	multifloral	43.11	48.73	4.83
24	nectar-honeydew	71.84	23.81	21.63
25	heather	189.52	100.00	23.41
26	multifloral	44.93	56.83	22.65
27	rape	27.84	59.25	16.72
28	multifloral	44.23	78.47	23.15
29	lime	45.46	65.66	27.33
30	buckwheat	180.07	100.00	30.55
31	rape	17.57	39.15	16.72
32	heather	164.28	100.00	31.51

the total phenolic content, free radical scavenging activity and antioxidant activity varied greatly among the honey types.

The total phenolic content varied from 17.57 mg GAE/100 g (sample 31, rape honey) to 189.52 mg GAE/100 g (sample 25, heather honey). General observation can be made that dark honeys (heather and buckwheat, samples: 7, 12, 16, 25, 30, 32) were characterised by considerably higher phenolic content than the other samples. The phenolic content in pale honeys (rape, acacia, samples: 1, 2, 3, 5, 14, 15, 27, 31) was *ca.* 40 mg GAE/100 g, while in dark honey it was significantly ($p=0.05$) higher. This trend was generally similar to the relationship found for some Slovenian, Burkina Faso and Italian honeys [Bertoncelj *et al.*, 2007; Blasa *et al.*, 2006; Meda *et al.*, 2005].

The radical scavenging activity of honey varied from 23.81% to 100% in the DPPH[•] reaction system. The results of DPPH radical scavenging activity showed that dark honeys tended to be highly active in the reaction with DPPH[•]: in samples 16, 25, 32 (heather honey) and 30 (buckwheat honey) the radical scavenging activity, calculated as percent of inhibition, reached 100%. The lowest radical scavenging activity, at a level of *ca.* 25%, was reported for two samples of acacia honeys (5, 19) and nectar-honeydew honey (sample 24).

In the ABTS^{•+} reaction system the radical scavenging activity of honeys was much lower than in the DPPH[•] reaction system and varied between 2.29% and 31.51%. The lowest radical scavenging activity was determined for sample 19 (acacia honey), and the highest one for sample 32 (heather honey). Generally we found that honey samples, which were more effective in the DPPH[•] reaction system, showed higher inhibition in the ABTS^{•+} system, too.

It is very difficult to compare the results obtained with findings of other researchers because of differences in typical types of honeys and way of results' presentation, but the relationships observed are similar. Most of the workers noticed a relationship between colour and antioxidant activity of honey samples. Usually the dark honeys (forest, honeydew, buckwheat) had the highest antioxidant activity and the pale honeys (*i.e.* acacia honeys) had the lowest antioxidant activity [Beretta *et al.*, 2005; Bertoncelj *et al.*, 2007].

The correlation between phenolics content and antioxidant activity of the honey samples examined is presented in Table 3. Statistical analysis showed that there was a strong positive correlation between the antioxidant activity and total phenolic content ($r=0.74$ for TPC/DPPH[•], $r=0.55$ for TPC/ABTS^{•+},

$p=0.05$). This means that phenolics are one of the main components responsible for the antioxidant activity of honeys. This correlation was in agreement with the findings of other authors. Meda *et al.* [2005] found that correlation between radical scavenging activity and the total phenolic content was at a level of $p=0.5$, while other authors found it stronger [Bertoncelj *et al.*, 2007; Berreta *et al.*, 2005; Blasa *et al.*, 2006]. The linear correlation between two assays ($r=0.59$, $p=0.05$) was observed. A stronger correlation ($r=0.715$) between the antioxidant activity determined with DPPH[•] and ABTS^{•+} radicals was observed by Baltrusaityte *et al.* [2007]. The differences in the antioxidant activity of honeys suggest that the kinetics of radical scavenging reaction in the two systems may differ significantly. The time of incubation may influence the scavenging efficiency as well. These assumptions provide interesting ideas for further investigation, for instance aiming at standardization of methods for the evaluation of antioxidant properties of honeys or at finding potential relationships between honeys antioxidant activity and their floral origin.

CONCLUSIONS

In the present study we showed that all the honey samples contained phenolic compounds and possessed antioxidant activity. The total phenolic content and antioxidant activity varied between honey types. The highest total phenolic content and antioxidant activity were determined for dark honeys: buckwheat, honeydew and heather honeys, while the pale honeys showed a lower antioxidant activity and total phenolic content. A significant positive correlation was found between the total phenolic content and antioxidant activity of the honeys.

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TABLE 3. Correlation between total phenolics content, radical scavenging activity and antioxidant activity of the analysed Polish honeys (correlation coefficients (r) value).

	Phenolics content (mg GAE/100 g)	Radical scavenging activity DPPH	Radical scavenging activity ABTS ^{•+}
Phenolics content (mg GAE/100 g)	1	0.74	0.55
Radical scavenging activity DPPH	0.74	1	0.59
Radical scavenging activity ABTS ^{•+}	0.55	0.59	1

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