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COLOUR, PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY OF POLISH HONEYS

Introduction

Honey is a natural food product produced by honeybees, *Apis meliflera*, from nectar and honeydew. From ancient times honey is used in healing properties. Numerous studies demonstrated that honey has a high nutritional value and possess both antibacterial and antioxidant activities. Honey contains a lot of enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, carotenoids, organic acids, aminoacids, proteins, flavonoids and polyphenols, therefore honey 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 antioxidant activity of honey has its botanical origin [2, 3, 6, 7, 9]. Many studies have shown, that antioxidant activity is strongly correlated with phenolics content, as well as antioxidant activity of dark honeys is higher than light honeys [1, 2, 3, 10].

Available literature indicates that until now there has been just few researches to determine the phenolics content and antioxidant activity of Polish honeys. In the present study we investigated the above mentioned parameters of different types of honey: acacia (*Robinia pseudoaccacia*), 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 analyzed parameters was evaluated.

Materials and methods

Chemicals and instruments

All the chemicals and reagents used were of analytical grade. DPPH (1,1 diphenyl -2-picrylhdrazyl), Folin-Ciocalteau reagent, gallic acid and methanol were obtained from Fluka, Germany. For absorbance measurements a UV-VIS spectrophotometer Unicam UV2-100 was used and for determination of colour parameters ($L^*a^*b^*$) the Minolta Chromameter CR-400 was used.

Samples

All the honey samples were obtained directly from beekeepers from different location across Poland during summer 2009. The floral origin of samples was specified by beekeepers regarding hive location and available floral sources and confirmed by sensory analysis. Whole characteristic of honey samples contain Table 1. Honeys were stored in room temperature in dark before analysis.

Table 1. Characteristic of 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	acacia	Pomerania	July 2009	Pale yellow
6	honeydew	Pomerania	August 2009	Dark brown with green
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
12	buckwheat	Małopolska region	July 2009	Dark brown
13	multifloral	Małopolska region	July 2009	amber
14	acacia	Małopolska region	July 2009	yellow

Sample no	Floral origin	Geographical origin	Harvest date	Colour*
15	rape	Małopolska region	july 2009	cream
16	heather	Małopolska region	august 2009	dark brown
17	honeydew	Bieszczady	july 2009	dark brown with green
18	dandelion	Małoplska region	july 2009	pale yellow
19	acacia	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-honyedew	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

The antiradical activity of honey was estimated according to procedure described by Turkmen et al. [11] with some modifications. The 2 g of honey sample was dissolved in 10 ml of distillated water, centrifuged and filtered by paper filter. Then 0,75 ml of the solution were mixed with 2,25 ml of 0,1mM methanol solution of DPPH 1,1–diphenyl-2-picrylhydrazyl (Fluka, Germany). The control test was made with distillated water in place of honey solution. The reaction mixtures were vortex-mixed well and left in room temperature in the dark for incubation during 60 min. Absorbance was measured at $\lambda = 517$ nm against methanol, using UV-VIS spectrophotometer Unicam. Antioxidant activity was expressed as a percent of inhibition of DPPH radical and calculated from the equation:

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

To determine the total phenolic content of honeys, the method of Meda et al. [10] was employed. Honey solution with concentration of 1g/10ml was centrifuged, filtered by paper filter. After that 0,5 ml obtained solution were mixed with 2,5 ml 0,2N solution of Folin-Ciocalteau reagent (Fluka, Germany) and 2 ml of sodium carbonate solution (75g/l, POCH, Poland) was added. After incubation in dark and room temperature for 2 h, absorbance of the reaction mixture was measured at λ = 760 nm using UV–VIS spectrophotometer Unicam. The standard curve was produced for gallic acid within the concentration range from 0 to 200 mg/l. The total phenolic content was expressed as gallic acid equivalents in mg/100g of honey sample (mgGAE/100g).

Colour characteristic were assessed by CIE $L^*a^*b^*$ method where L^* lightness and two colour coordinates a^* and b^*, were defined by means of Minolta CR-400 Chromameter. Honey samples were placed in glass container and covered in a plate. The initial pretreatment of the honey samples did not alter their colour. The measured layer was 1 cm thick. L^*, a^* and b^* parameters were measured against grey background, the same for all samples and were directly obtained from the apparatus.

Statistical analysis

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

Results and discussion

The results obtained (Table 2) that the total phenolic content (GAE/100g), free radical scavenging activity and colour varied greatly among the honey types.

Table 2. A compilation of data from 32 honeys obtained in Poland

Sample no	Total phenolic content mgGAE/100g	Radical scavenging activity [%]	colour		
			L^*	a^*	b^*
1	33.42	43.91	61.67	–1.68	11.38
2	32.33	39.45	69.65	–1.22	12.73
3	37.42	56.24	66.14	–1.12	15.09
4	47.14	63.48	19.03	6.82	–3.26
5	40.55	25.58	16.34	11.05	–5.42
6	64.59	83.51	16.93	9.73	–4.2

Sample no	Total phenolic content mgGAE/100g	Radical scavenging activity [%]	colour		
			*L**	*a**	*b**
7	110.4	68.88	20.20	10.42	–0.59
8	45.98	42.53	21.97	5.08	1.5
9	35.87	43.14	54.39	–0.04	9.77
10	45.44	48.23	37.46	1.31	16.32
11	71.88	79.51	21.19	8.31	–3.63
12	87.28	56.39	19.10	9.32	–4.14
13	53.05	71.03	17.01	10.21	–5.04
14	32.54	35.90	17.66	10.22	–5.35
15	41.17	55.16	42.58	1.42	8.69
16	109.53	100.00	19.72	8.05	–0.27
17	67.13	73.33	17.28	9.81	–4.65
18	63.02	46.03	55.74	–0.65	21.22
19	39.99	27.14	21.68	7.32	–2.04
20	45.25	43.97	35.24	3.26	5.89
21	58.24	72.54	24.74	6.79	–2.31
22	37.05	40.95	70.25	–1.03	10.46
23	43.11	48.73	23.98	4.55	–1.38
24	71.84	23.81	32.23	3.68	5.49
25	189.52	100.00	29.04	9.33	7.28
26	44.93	56.83	65.80	–1.55	12.71
27	27.84	59.25	65.79	–0.95	12.81
28	44.23	78.47	37.43	0.9	14.84
29	45.46	65.66	27.53	2.74	4.43
30	180.07	100.00	24.46	4.01	–0.97
31	17.57	39.15	74.26	–1.07	8.54
32	164.28	100.00	38.85	4.52	6.21

Total phenolic content (mg GAE/100g) varied from 17,57 (sample 31, rape honey) to 189,52 (sample 25, heather honey) using a standard curve of gallic acid (R^2 = 0,999). Generally it can be say, that dark honeys (heather, honeydew and buckwheat, samples 7, 12, 16, 25, 30, 32) had much more higher phenolics content than other samples. These results are generally similar to the average values found for some Slovenian, and Italian honeys [4, 5, 10].

The results of DPPH radical scavenging activity show the same dependence: samples 16, 25, 30, 32 had radical scavenging activity, calculated as percent of inhibition, at level of 100%. The lowest radical scavenging activity had two sam-

ples of acacia honeys (5, 19) and nectar-honeydew honey (sample 24). It is very hard to compare obtained results to results of other researchers because of differences in way of presentation, but observed correlations are the same. Most of the workers noticed that the dark honeys (forest, honeydew, buckwheat) had highest antioxidant activity and the pale honeys (i.e. acacia honeys) had the lowest antioxidant activity [3, 4].

The colour characteristic contained in table 2 shows, that highest values of parameter L^* (about 60) have rape, multifloral and dandelion honeys (samples 1–3, 15, 22, 26, 27, 31), which also were found to be a lightest by visual comparison. The lowest L^* value (about 20) have buckwheat, heather and honeydew honeys (samples 7, 12, 17, 21, 25, 30), which were found to be darkest. Gonzales-Miret et al. [8] classified honey samples into 2 groups regarding their lightness value: light honeys with $L^* > 50$ and dark honeys with $L^* < 50$. Considering this classification, rape, dandelion and some multifloral Polish honeys can be class among light honeys and other types among dark honeys. Analyzing the parameters a^* and b^* it can be said, that the honey samples had yellow, red and green components. Negative a^* values (green components) were noticed in samples of rape, dandelion and multifloral honeys (samples 1–3, 10, 18, 22, 25, 26 and 31).

The relationship between colour lightness, phenolic content and antioxidant activity is presented in Table 3.

Table 3. Correlation matrix (correlation coefficients (*r*) value)

	Phenolics content mg GAE/100g	**Radical scavening activity AA [%]**	***L****
Phenolics content mg GAE/100g	1.00	0.74	–0.35
Radical scavening activity AA [%]	0.74	1.00	–0.30
*L**	–0.35	–0.30	1.00

Statistical analysis showed, that there is a strong positive correlation between antioxidant activity and total phenolics content (r = 0,74). This means, that phenolics are one of the main components responsible for the antioxidant activity of honeys. We found also week negative correlation between lightness and total phenolics content as well as between lightness and radical scavenging activity. It can be said that than the lower is L^* value (the darkest is honey), the highest phenolics content and antioxidant activity of honey is noted. Many researchers found the same correlation: honeys with dark colours have a higher total phenolics content and consequently, higher antioxidant capacity [3, 6].

Conclusions

In the present study we establish that all the honey samples contained phenolics compounds and possess antioxidant activity. The total phenolics content and antioxidant activity varied between types. The highest total phenolics content and antioxidant activity possessed dark honeys: buckwheat, honeydew and heather honeys, while the pale honeys showed lower antibacterial activity and total phenolics content. A significant positive correlation was found between the total phenolics content and antioxidant activity as determined by the DPPH radical scavenging activity.

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BARWA, AKTYWNOŚĆ PRZECIWUTLENIAJĄCA ORAZ OGÓLNA ZAWARTOŚĆ POLIFENOLI W MIODACH PSZCZELICH RÓŻNYCH ODMIAN

Streszczenie

Celem pracy było porównanie aktywności przeciwutleniającej miodów pszczelich różnych odmian z ich barwą oraz zawartością polifenoli. Badaniami objęto 32 próbki miodów różnych odmian. Ogólną liczbę polifenoli oznaczono metodą Fiolin-Ciocalteau, aktywność przeciwultleniającą oznaczono metodą z użyciem roztworu DPPH (1,1–diphenyl-2-picrylhydrazyl). Dodatkowo określono parametry barwy *L*a*b**.

Stwierdzono, iż poszczególne odmiany miodów wykazywały zróżnicowaną aktywność antyoksydacyjną oraz ogólną zawartość polifenoli, przy czym miody ciemne wykazywały większą aktywność antyoksydacyjną niż miody jasne. Miody ciemne zawierały także większą ilość polifenoli. Analiza statystyczna wykazała, iż aktywność antyoksydacyjna jest proporcjonalna do ogólnej zawartości polifenoli.