SUPPLEMENTARY MATERIAL

Antioxidant activity of honey supplemented with bee products

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Abstract

The aim of this work was to evaluate the influence of supplementation of multiflower honey with bee products on the phenolic compound content and on antioxidant activity. Average total phenolic and flavonoids contents in the multiflower honeys were 36.06 ± 10.18 mg GAE/100 g and 4.48 ± 1.69 mg QE/100 g, respectively. The addition of royal jelly did not affect significantly the phenolic compound content and antioxidant activity. Supplementation of honey with other bee products, i.e. beebread, propolis, pollen, resulted in significant increase in the total phenolic and flavonoids contents, and in antiradical activity and reducing power, with the largest effect found for addition of beebread. Significant linear correlations between the total phenolic and flavonoids contents and antiradical activity and reducing power were found.

Key words: honey, bee products, antioxidant activity, antiradical properties

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Experimental

1. Materials

The material were multifloral honeys supplemented with various bee products: royal jelly, beebread, propolis and pollen. The honeys were obtained from the following apiaries from southern Poland: Apiary "Barć Kamianna", Łabowa, Lesser Poland, Apiary "PH Barć", Biszczka, Lublin Province, Beekeeping Farm "Sądecki Bartnik", Stróże, Lesser Poland, Apiary "Pasięka pod Pilskiem", Korbielów, Silesia, Beekeeping Farm "Łysoń", Sułkowice, Lesser Poland. The honeys from each of these apiaries were multifloral honeys and honeys supplemented with various bee products, however, not each manufacturer offered all the kinds of samples, therefore the number of samples was 22.

2. Sample preparation

Water solutions of the samples in concentration of 20 g/100 ml were prepared. The sample solution for analysis was filtered through a filter paper.

3. Analysis procedures

3.1. Determination of total phenolic content

Total phenolic content was determined according to the method developed by Singleton and Rossi (1965). An aliquot of 0.5 ml of honey solution was mixed with 2.5 ml of 0.2 N solution of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), and then 2 ml of sodium carbonate (POCH, Poland) solution (7.5 g/100 ml) was added. Following incubation for 2 hours, absorbance of the reaction mixture was measured at $\lambda = 760$ nm against an ethanol as a sample blank. The total phenolic content was expressed as gallic acid equivalent in mg GAE/ 100g of honey on a basis of a standard curve produced for gallic acid (Sigma-Aldrich, Germany) within a concentration range from 0 to 20 $\mu\text{g}/\text{ml}$.

3.2. Determination of total flavonoid content

The total flavonoids content was established in the reaction with aluminum chloride using the method described by Ardestani & Yazdanparast (2007). Aliquots of 4 ml of deionised water and 0.3 ml of sodium nitrate(III) (POCH, Poland) solution (15 g/100 ml) were added to 1 ml of honey solution. This was followed by addition of 0.3 ml of aluminium chloride (POCH, Poland) solution (10 g/100 ml) and then 4 ml of sodium hydroxide (POCH, Poland) solution (4 g/100 ml). The sample was made up to 10 ml with deionised water, stirred and left to stand for 15 min. The total flavonoid content was calculated on the basis of the standard curve for quercetin (Sigma-Aldrich, Germany) solutions in the concentration range from 0 to 50 $\mu\text{g}/\text{ml}$ and expressed as quercetin equivalent in mg QE/ 100g of honey.

3.3. Determination of antiradical activity

Determination of antiradical activity in the reaction with a DPPH[•] (1,1-diphenyl-2-picrylhydrazyl) radical was performed using the procedure described by Blois (1958). An aliquot of 1 ml of honey

solution was mixed with 3 ml of 0.1 mM methanol solution of DPPH[•] (Sigma-Aldrich, Germany). The reaction mixture was vortex-mixed and left in the dark at room temperature for incubation during 30 min. Absorbance was measured at $\lambda = 515$ nm against methanol. Results were expressed as a percentage of inhibition of DPPH[•] radical calculated according to the following equation:

$$\%inhibition\ of\ DPPH^{\bullet} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \cdot 100\%$$

where: $Abs_{control}$ is the absorbance of DPPH[•] solution with water instead the tested sample.

The antiradical activity in the reaction with a ABTS^{•+} cation radical was determined in accordance with Baltrušaitytė et al. (2007) with some modification (Socha et al., 2011). The ABTS^{•+} was obtained in the reaction of a 2 mM stock solution of 2,2'-azino-di(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma-Aldrich, Germany) in phosphate-buffered with potassium persulfate (POCh, Poland) solution (70 mM). The mixture was left to stand for 24 hours. Prior to analysis, the ABTS^{•+} solution was diluted with phosphate buffer to produce a solution with an absorbance of 0.80 ± 0.03 at $\lambda = 734$ nm. The 0.1 ml of honey solution was mixed with 6 ml of the ABTS^{•+} cation radical solution and after 15 minutes absorbance was measured at a wavelength of 734 nm. Results were expressed as a percentage of inhibition of ABTS^{•+} radical calculated according to the following equation:

$$\%inhibition\ of\ ABTS^{\bullet+} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \cdot 100\%$$

where: $Abs_{control}$ is the absorbance of ABTS^{•+} solution with water instead the tested sample.

3.4. Determination of reducing power (FRAP) assay

Reducing power (FRAP) assay was also determined by spectrophotometric method (Socha et al., 2011). Working FRAP reagent was prepared as required by mixing 3.3 ml of acetate buffer (pH 3.6) with 0.33 ml of TPTZ (2,4,6-tripyridyl-S-triazine) (Sigma-Aldrich, Germany) solution in 40 mM HCl and 0.33 mL FeCl₃ (POCh, Poland) solution (20 mM), incubated at 37 °C during 5 min. Then 0.33 ml of honey solution was added and the reaction mixture was incubated at 37 °C during 15 min. The absorbance of a resulting blue-coloured, ferrous-tripyridyltriazine complex was measured at a wavelength of 593 nm against a blank sample. The reducing power was calculated on the basis of the standard curve of aqueous solutions of known ferrous sulphate concentrations in the range of 0 – 1200 µM and expressed in mM Fe(II)/ 100g of honey.

Measurement of absorbance was performed using UV/Vis spectrophotometer (V-630, Jasco, Japan).

3.5. Statistical analysis

Coefficients of linear correlations between the parameters describing antioxidant activity were calculated using the Pearson correlation test. In order to demonstrate the similarities and differences in the characteristics of the individual samples and groups of products, the principal component analysis (PCA) was performed. All calculations were done using Statistica v. 10.0 software.

4. References

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Figure S1. Principal component analysis of honeys antioxidant activity with distribution of analyzed samples. MH – multifloral honey, RJH – honey with royal jelly, POH – honey with pollen, BBH – honey with bee bread, PRH – honey with propolis.