Antioxidant Activities and Total Phenolics of Acacia Honey

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Abstract: The antioxidant activities and total phenolic content of 30 samples of acacia honey from Croatian territory were analysed. Phenolics were determined by the modified Folin-Ciocalteu method, antiradical activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and potential antioxidant activity using the ferric reducing antioxidant power (FRAP) method. In all samples, physicochemical parameters (water content, electrical conductivity, total reducing sugars, sucrose content, acidity, hydroxymethylfurfural content, prolin content, optical rotation, diastase activity and invertase activity) were measured according to Croatian legislation and International regulatory standards. Honey can be considered as a dietary supplement as it contains some important components including α-tocopherol, ascorbic acid, flavonoids and phenolics. The composition and properties of honey are dependent on floral origins, climatic conditions of the produced area, processing and storage methods. The results of physicochemical analyses showed that all the values of investigated parameters are in agreement with the current legislation. Phenolic content ranged from 31.72 mg/kg to 80.11 mg/kg, antiradical activity expressed as IC_{50} ranged from 61.28% to 253.47% and antioxidant activity expressed as FRAP value from 6.95 to 142.43. A positive correlation was observed between total phenolic content and antioxidant activity, indicating that phenolic compounds are mainly responsible for the antioxidant power of acacia honey.

Keywords: antioxidant activity; honey; phenolics; physicochemical parameters

INTRODUCTION

Honey is a natural product made by honeybees (*Apis mellifera*) which have a highly variable sensorial and physicochemical characteristics due to climatic and environmental conditions and diverse origin of plants from which it is harvested. Having that in mind, different honey types have diverse phenolic content and consequently different antioxidant activity. In addition, processing, handling and storage of honey may influence its composition (Gheldof & Engeseth 2002; Turkmen *et al.* 2005). Due to its potential and proven positive medicinal properties, honey is particularly recommended for children and sportsmen and it can help improve the organism efficiency of the elderly and invalids (Blasa 2006). Because of that it is quite important to determine the antioxidative potential of honey and how can this parameter be of any help in differentiation of various honey types. Bertoncelj *et al*. (2007) found that of all honey types they investigated, acacia and lime showed low total phenolic content and consequently lower antioxidant capacity than other honey types (fir, spruce and forest). According to those results, our data are in similar range for acacia honey samples. Because there are temporarily no published research papers dealing with the problem in Croatian honey, the aim of our study was to determine the antioxidant activity and total phenolic content of 30 acacia honey samples harvested in Croatia. Besides that, in all of the honey samples, physicochemical parameters were measured according to Croatian legislation and International regulatory standards.

MATERIALS AND METHODS

Thirty honey samples were obtained directly from beekeepers from different locations across Croatia. The floral origin of the samples was specified as acacia by the beekeepers. Before and during the analyses, samples were stored in glass containers at room temperature. All of the chemicals and reagents used were of analytical grade and purchased from Sigma (St. Louis, USA) and Merck (Darmstadt, Germany).

In all samples, the physicochemical parameters were determined (water content, electrical conductivity, total reducing sugars, sucrose content, acidity, hydroxymethylfurfural content, prolin content, optical rotation, diastase activity and invertase activity using the IHC-proposed methods – IHC 2002). With all these analyses, the criteria laid down by the Regulations of the International Honey Commission (IHC 2002) were applied. The obtained results were evaluated using Croatian and international honey-profiling criteria (Croatian Regulations 2000). The Folin-Ciocalteu method as modified by Beretta *et al*. (2005) and Bertoncelj *et al.* (2007) was used to determine total phenolic content. Antioxidant activity of thirty acacia honey samples was determined by FRAP (the ferric reducing/antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays, also according to Beretta *et al*. (2005) and Bertoncelj *et al*. (2007). All analyses were performed in triplicate and the data were expressed as means ± standard deviations (SD).

RESULTS AND DISCUSSION

Table 1 show summarisation of results of physicochemical analyses for all analysed honey samples. The results of physicochemical analyses show that all the average values of investigated parameters lie within the limits set by Croatian legislation indicating the use of good practices by beekeepers in Croatia. Phenol content, FRAP values and antiradical power (DPPH) of acacia honey samples (n = 30) are shown in Table 2. The evaluated results (Table 2) were in close agreement with the results reported by Beretta *et al*. (2005) and Bertoncelj *et al*. (2007). The significant correlation was found between the antioxidant activity as determined by the FRAP assay and also as determined by the DPPH assay and the phenolic content (R^2 = 0.77;

Table 1. Physicochemical parameters of the accacia honey samples (n = 30)

Statistics	w (water) (%)	Electrical conductivity (mS/cm)	w (total reducing sugars) (%)	w (sucrose) (%)	Acidity (mmol/kg)	Diastase activity (DN)	Invertase activity (IN)	w (HMF) (mg/kg)	w (proline) (mg/kg)	Optical rotation (°)
Mean ± SD	17.13 ± 1.06	0.20 ± 0.25	64.74 ± 3.41	5.42 ± 2.19	8.63 ± 1.31	15.35 ± 6.39	8.36 ± 4.62	10.17 ± 9.14	133.91 ± 71.40	–13.93 ± 2.21
Range	15.36–19.48	0.11–1.52	57.74–73.50	1.87–9.85	6.94–12.19	3.43–34.22	0.04–4.62	0.19–36.48	35.47–376.30	–17.5 (–8.00)
CV (%)	6.19	127.84	5.27	40.41	15.18	41.63	55.26	89.87	53.32	–17.00

Table 2. Phenol content, FRAP values and antiradical power (DPPH) of accacia honey samples (n = 30)

Statistic	Phenol content ($mg_{gallic\ acid}$/ kg_{honey})	FRAP value (μM Fe(II))	DPPH -IC_{50} (mg/ml)
Mean ± SD	43.66 ± 6.45	72.87 ± 15.44	111.05 ± 45.10
Range	31.72–80.11	6.95 ± 142.43	61.28–253.47
CV (%)	14.77	21.19	40.61

r = 0.88 and R^2 = 0.87; r = 0.93) indicating that phenolic compounds appear to be responsible for the antioxidant activity of acacia honey.

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