

THE ANTIOXIDANT ACTIVITY OF SELECTED ROMANIAN HONEYS

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Four types of *Apis mellifera* honey collected in the eastern region of Romania were screened for their total phenolic content by the Folin-Ciocalteu method, for anti radical power as assessed by DPPH radical scavenging assay, for the FRAP assay-ferric reducing antioxidant power. The antioxidant effect over a substrate sensitive to lipid per oxidation and also the presence of some pigments with antioxidant action in honey-ABS 450 were evaluated. All assays revealed the following order of the obtained values: Lime honey>poly-flower honey>Sea buckthorn honey>Acacia honey.

Keywords: antioxidant activity, FRAP assay, DPPH free radical, total phenol content, ABS 450.

1. Introduction

Nowadays there is a growing demand for bio-organic and natural products in the human diet, both due to the negative effects of synthetic food additives on human health and to the increased consumer perception of this problem. Many researches revealed that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some berry plants biosynthesize phytochemicals possessing antioxidant activity and may be used as a natural source of free radical scavenging compounds (Blasa, 2007; Javanmardi, 2002; Miliuskas, 2004; Sacchetti et al., 2005; Yu, Zhou and Wang, 2005).

Honey serves as a source of natural antioxidants, which are effective in reducing the risk of heart disease, cancer, immune system decline, the autism disease, gastrointestinal disorders, asthma, infected and chronic wounds, skin ulcers, cataracts etc. (The National Honey Board, 2009). Since some of these diseases are a consequence of oxidative damage, it seems that part of the therapeutic properties of honey is due to its antioxidant capacity. Additionally, the presence of hydrogen peroxide, as well as minerals in honey, may lead to the generation of highly reactive hydroxyl radicals as part of the antibacterial system (McCarthy, 2001; Molan, 2009); thus, it is evident that mechanisms must be available in honey to control the formation and removal of these reactive oxygen species. Furthermore, honey, as a source of antioxidants, has been proven to be effective against deteriorative oxidative reactions in food, caused by light, heat and some metals, such as enzymatic browning of fruit and vegetables (Chen, 2000), lipid oxidation in meat (Takeshi Nagai, 2006), and inhibit the growth of foodborne pathogens and food spoilage organisms (Mundo, 2004; Taormina, 2001).

The composition of active components in plant's nectar depends on various factors, such as plant source, climatic and geographical conditions. Therefore it can be reasonably expected that honey properties and biological activity from different locations should be different.

The purpose of the present study was to evaluate the antioxidant ability of some types of honey with different floral source, using several assays and to correlate the results between these methods.

2. Materials and methods

2.1. Materials

Honey samples: from eastern region of Romania where *Apis mellifera* species grow naturally, were collected in May when these species were flowery. They are named: Lime, Sea buckthorn, Acacia and poly-flower honeys.

All solvents were of analytical grade purity. All other chemicals were obtained from Sigma Chemical Co. and Merck. DPPH reagent was purchased from Sigma.

2.2 Total polyphenolic content –TP

To study the total polyphenolic content, the 5% hydro-alcoholic extracts of honey samples (96°ethanol: water=1:1) were prepared.

Then 1 ml of the hydro-alcoholic extract was introduced into a 50 ml volumetric flask, 5 ml of Folin Ciocalteu reagent and after 5 min of stirring 4.5 ml of (7.5%) Na₂CO₃ were added. After refilling with distilled water to the mark and through agitation the reaction mixture was kept for 2 hours in a dark place. After 2 hours, the absorbance was measured using a spectrophotometer (Portable Datalogging Spectofotometer, HACH DR/2010) $\lambda=760$ nm against the blank.

Total phenol content was expressed as mg Gallic acid equivalent in kg of honey as average from three parallel determinations.

Most frequently, the total phenol content is expressed in mg Gallic acid, results being reported in mg Gallic acid/l.

2.3. FRAP-the ferric reducing antioxidant power assay

The procedure described by Benzie and Strain (1996) was used with modifications. FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

A standard solution to obtain a calibration curve was prepared. The standard solution composition was: 0.3511g Mohr's salt were introduced into a 1000 ml volumetric flask and 10 ml acid solution was added (the ratio HCl:water=1:1). This stock solution was prepared to obtain different concentrations (from 0.1% to 1%) suitable to be used for the calibration curve.

In a 25 ml volumetric flask 5 ml of (0.2%) ammonium ferric alum, 2 ml hydroalcoholic extract of honey (with different concentrations), 5 ml of sodium acetate, 5 ml of 0.15% o-phenanthroline were introduced and the mixture was stirred.

The reduction of ferric complex (Fe³) to ferrous form (Fe²)-which has an intense blue colour, in the presence of antioxidants from honey was monitored by measuring the change in absorption at 510 nm, after 2 hours.

2.4. DPPH-(1,1 diphenyl-2-picrylhydrazyl) radical-scavenging effect assay

A volume of 3900 μ l methanolic solution containing DPPH radicals (6×10^{-5} mol/l) with 100 μ l honey extract were mixed. Each mixture was shaken vigorously and kept in a dark place until a stable absorption value was obtained at 515 nm. The diminution of the absorbance was determined at 515 nm at time 0, after 1 minute and every 15 minutes until the reaction ran to a constant value. The exact initial concentration in DPPH (C_i) was calculated using Brand-Williams' equation:

$$A_{1\ 515\ \text{nm}} = 12.509 \cdot C_i - 2.58 \cdot 10^{-3} \quad (1)$$

A_{1 515 nm} is the absorbance of a solution, containing 10 μ l methanol in 3900 μ l solution. The initial concentration, C_i was derived from the following equation 1:

$$C_i = (A_{1\ 515\ \text{nm}} + 2.58 \cdot 10^{-3}) / 12.509 \quad (2)$$

C_f can be expressed as:

$$C_f = (A_{2\ 515\ \text{nm}} + 2.58 \cdot 10^{-3}) / 12.509 \quad (3)$$

where A_{2 515nm} is the absorbance of stable DPPH in solution, i.e. the final absorbance of the antiradical reaction.

Finally, the percent of stable, unreacted DPPH radicals left in solution can be determined using the following expression:

$$C_f = (A_{2515\text{ nm}} + 2.58 \cdot 10^{-3}) / 12.509 \quad (4)$$

2.5. The evaluation of the antioxidant effect of honey over an oily substrate

The objective was to evaluate the antioxidant capacity of lime, acacia, sea-buckthorn and poly-flower honeys in delaying lipid oxidation in oil.

For this study, the sunflower oil peroxide values kept at 60° C/96 hours in the presence of a 1.5 % sample bearing antioxidant capacity were determined.

The peroxide values were determined using different volumes of honey extracts such as 0.5 ml; 1 ml; 1.5 ml; 2 ml; 2.5 ml and 5 ml.

The most significant values were obtained in 1.5 ml hydro-alcoholic extract of honey bee when over 10 ml vegetable oil was added.

The peroxide value was related to the sodium thiosulfate amounts consumed by the iodine liberated from the potassium iodine by the oil peroxides. The results were expressed in Milliequivalents O₂ / kg oil.

2.6. The color intensity - ABS 450

Many studies have analyzed the correlation between the antioxidant activity of honey and the color intensity.

A method applied with success was the analysis of the color intensity –ABS 450 when the presence of some pigments bearing antioxidant activity was determined (e.g., carotenoid pigments, flavonoids, Maillard reaction products).

A quantity of 25 g of honey sample was mixed with 50 ml distilled water (T=45-50° C) and after filtration-the optical density at λ=450 nm and λ= 720 nm was measured.

The color intensity-ABS 450 was calculated as the difference between A₄₅₀ and A₇₂₀.

2.7. Statistical analyses

All analyses were carried out in triplicate and the data were expressed as means ± standard deviations (SD).

3. Results and discussions

3.1. Total polyphenolic content –TP

The results of the total polyphenolic content for the four types of honey are represented in Figure 1. This figure reveals that the polyphenolic content increases according to the floral source in the following order: Acacia honey < Sea buckthorn honey < Poly-flower honey < Lime honey.

The differences between other honey samples(Lachman J.et al.,2010; Bertoncej, 2007) could be attributed to natural variations in composition(sugar, mineral and water content), to different locations in Romania and also to different floral sources of nectar.

3.2. FRAP-the ferric reducing antioxidant power assay

A very significant correlation was observed between the total polyphenolic content and the Fe²⁺ content formed in the presence of the honey antioxidants.

The amount of generated Fe²⁺ under the effect of the antioxidants was determined by plotting the standard curve. The antioxidant activity is expressed in mg Fe²⁺/kg of honey.

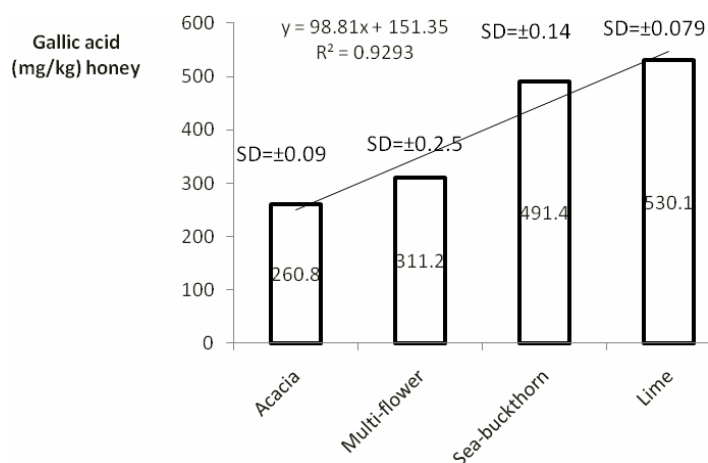


Figure1. The variation of total polyphenolic content for different types of honey samples

The obtained results justify the noticeable antioxidant capacity of lime honey, followed by, poly-flower honey, sea buckthorn honey and by acacia honey as the Figure 2 suggests.

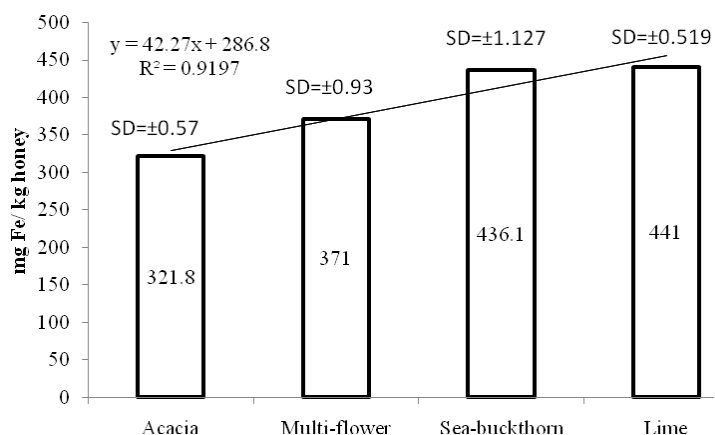


Figure2. The Fe²⁺ formed in the presence of the honey bee antioxidants

The differences between other honey samples (Bertoncelj, 2007) could be attributed to natural variations in composition, to different locations in Romania and also to different floral sources of nectar.

3.3. DPPH-(1,1 diphenyl-2-picrylhydrazyl) radical-scavenging effect assay

The Lime honey value was significantly higher than the other types of honey when the free radical scavenging ability was evaluated. The free radical scavenging activity of Lime honey extract was increased in response to increasing extract dose up to 10 mg/mL. In the Figure 3, the correlation between the total phenol content-Fe²⁺ formed and the radical scavenging effect can be seen.

There were some differences among the types of honey. The antioxidant activity for different types increased in the order: acacia < Sea buck-thorn < multiflower < lime. This order was similar to the results obtained by Bertoncelj, et al. (2007).

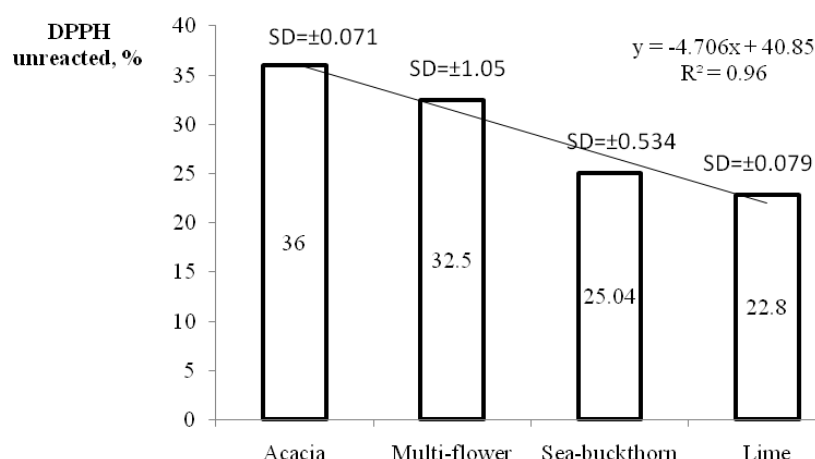


Figure 3. The anti radical activity of honey extracts measured using the DPPH reagent

3.4. The evaluation of the antioxidant effect of honey over an oily substrate

Analysing the results in Figure 4, it can be concluded that all samples revealed good lipid peroxidation inhibition measured in the peroxide values. Lime honey presented, in all assays, better antioxidant activity (lower peroxide values) than the other honey samples.

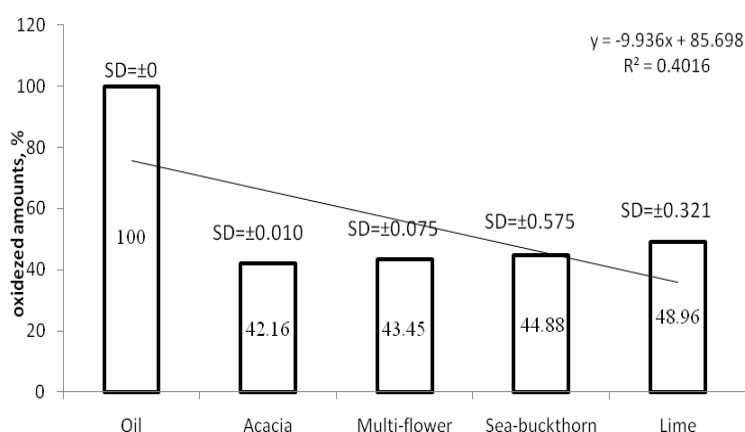


Figure 4. The oxidized amounts of honey extracts over an oily substrate

3.5. The color intensity - ABS 450

A high correlation was found between total antioxidant activities of different types of honey and their total phenol contents, indicating that phenolics are the components responsible for the antioxidant effects of Lime honey but, obviously, other factors are involved. These might be different phenolic compositions or the presence of non-phenol antioxidants such as ascorbate, α -tocopherol, and β -carotene. These findings are in agreement with those reported by Wang et al. (1996), Guo et al. (1997) and Velioglu et al. (1998), who found a high correlation between the total antioxidant activities of some fruits and their total phenol contents. Lime honey has been shown to contain some phenol acids, such as Gallic, ferulic, caffeic, benzoic, and cinnamic acids besides some of other unknown phenol compounds.

In fact, the increase of the color intensity seems to be related to an increase in the antioxidant properties and in phenol content.

Table 1. The absorbance of honey extracts in order to establish their color intensity

Type of honey	E ₁ , λ=450 nm	E ₂ , λ=720 nm	E ₁ -E ₂ ,
Lime	0.711	0.176	0.535
Multi-floral	0.395	0.138	0.257
Sea buckthorn	0.276	0.086	0.190
Acacia	0.09	0.033	0.057

4. Conclusions

The results of the investigations prove the existence of interdependence between the total phenol content – antioxidant activity – anti radical activity – antioxidant capacity over an oily substrate –the color intensity- ABS 450.

All tests revealed the same order of the values obtained from the 4 types of honey studied: Lime honey>Poly-floral honey>Sea buckthorn honey>Acacia honey.

The honey colour intensity is directly related to the polyphenol content.

Through the content of its composites bearing antioxidant effect, the honey could contribute to decreasing/preventing the oxidative stress.

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