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Investigation of Antioxidant Activity of Armenian Honey

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ABSTRACT

During the past decades, a lot of research has been conducted on measuring the antioxidant capacity of honey produced in different countries. Nevertheless, there are no data on the antioxidant capacity of Armenian honey. So, this study aims to evaluate total antioxidant capacity of Armenian honey. In total, 14 multi-floral honey samples were investigated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and the ferric reducing antioxidant power (FRAP) assay. The results of the current study have indicated that all honey samples have high antioxidant potential.

Introduction

Nowadays, there is a growing demand for bio-organic and natural products in the human diet (Dobre et al., 2010). Antioxidants, introduced by diet and presenting high bioactivity, can act on different cell targets, protecting consumers' health (Di Marco et al., 2018). In general, antioxidants are nutritive and non-nutritive agents that can retard biologically negative chemical reactions in foods and live organisms. These compounds are thought to protect humans from disease, in part, through their ability to scavenge oxidants and free radicals absorbing molecular damage that might otherwise compromise the function of essential lipids, proteins, and nucleic acids (Schramm et al., 2003). Among natural products with similar health-promoting effects, the bee products have generated considerable interest (Balkanska et al., 2017). Honey is one of the popular bee products, which has different biological properties, including antioxidant effects (Boulanouar et al., 2017).

Honey serves as a source of natural antioxidants, which are useful 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. Since some of these diseases are the consequence of oxidative damage, it seems that part of the therapeutic properties of honey is due to its antioxidant capacity (Ferreira et al., 2009, Moniruzzaman et al., 2012, Pontis et al., 2014).

Many studies have shown that antioxidant activity of honey can be attributed to the wide range of compounds such as phenolic acids, flavonoids, glucose oxidase, catalase, ascorbic acid, enzymes and carotenoids. Moreover, the antioxidant activity of honey can vary due to the botanical and geographical origin, climatic conditions and handling procedures (Boulanouar et al., 2017, Wilczynska, 2010).

During the past decades, there has been a lot of research on measuring the antioxidant activity of honey produced in different countries (Balkanska et al., 2017, Di Marco et al., 2018, Ferreira et al., 2009, Pontis et al., 2014). Nevertheless, there are no data on the antioxidant capacity of Armenian honey.

The excellent climatic conditions for beekeeping can promote high-quality honey production in Armenia (Mkrtchyan et al., 2015). Therefore, this study aims to evaluate the total antioxidant capacity of Armenian honey, which will provide an understanding of its functional properties.

Materials and methods

Totally 14 multi-floral honey samples were obtained directly from beekeepers in 4 regions of Nagorno-Karabakh Republic (Shushi, Askeran, Hadrut, Martakert) and Kotayq region of Armenia (Abovyan city) (Table 1).

Determination of the antioxidant activity of honey samples was carried out in the analytical laboratory of the Center for Ecological-Noosphere Studies of NAS, RA.

The antioxidant activity of each honey sample was determined based on the scavenging activity against the free radical 2,2-

diphenyl-1-picryl-hydrazyl (DPPH radical scavenging assay) (Molyneux, 2004, Saric et al., 2013). Stable DPPH radical reached the absorbance maximum at 517 nm, and its color was purple. The change of this color into yellow was the result of the pairing of an unpaired electron of a DPPH radical with the hydrogen of the antioxidant, thus generating reduced DPPH-H. Increase in the antioxidant resulted in the absorbance decrease, which was proportional to the concentration and antioxidant activity of the compound. The absorbance was measured in a spectrophotometer at 517 nm. Besides, the absorbance of the blank and control samples was measured. Results were shown as EC50 values, i.e. the concentration of an antioxidant that causes 50% inhibition of DPPH (Saric et al., 2013).

Table 1: Study sites and codes of investigated honey samples

Honey production regions		Sample code
Shushi region	Village Khalifali	AH-1
	Village Hinshen	AH-6
	Village Eghtsahogh	AH-8
	Village Qirsavan	AH-13
Askeran region	Village Jivani	AH-3
	Village Karmir	AH-4
	Village Qrasni	AH-5
	Village Patara	AH-15(19)
Hadrut region	Village Drakhtik	AH-22(25)
Martakert region	Village Varankatagh	AH-18
	Village Zardakhach	AH-21
	Village Vaghuhas	AH-26
	Village Chapar	AH-28(13)
Kotayq Region	Abovyan city	KH-1

Since there is no official method for honey's antioxidant activity determination, additionally FRAP (ferric reducing/antioxidant power) method was used for assessing the antioxidant activity of honey produced in Kotayq region of the Republic of Armenia (KH-1). The FRAP assay was carried out in the Laboratory of Cellular and Molecular Nutrition of Tuscia University (Italy).

The FRAP assay was adapted for 96-well plates and an automatic reader (Infinite F200, Tecan, Salzburg, Austria). The method is based on the reduction of the Fe3+-2,4,6-tripyridyl- s-triazine (TPTZ) complex to its ferrous form at a low pH. Briefly, 160 lL of FRAP assay solution (consisting of 20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer at pH 3.6) was prepared daily, mixed with 10 lL of the sample, standard or blank, and dispensed into each well of the 96-well plate. The absorbance was measured at 595 nm at 37ºC after 30 minutes of incubation. The final results were expressed as mmol Fe^{2+} equivalents/ g of dry weight (DW) (Costantini et al., 2014).

Generally, the FRAP method is used for the quantitative determination of antioxidants, whereas the DPPH method determines the qualitative presence of antioxidants.

Each antioxidant activity assay was done twice from the same extract to determine their reproducibility. Statistical analyses of all assay results were done using the Microsoft Excel program.

Results and discussion

The DPPH method with the stable organic radical 1,1-diphenyl-2-picrylhydrazyl is used for determination of free radical scavenging activity, usually expressed as EC50, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. The lower is the EC50 value for honey the higher is its antioxidant activity (Molyneux, 2004). The results obtained for the scavenging ability of honey (EC50) were in the range of 5,4 - 22,2 mg/ml (Fig.).

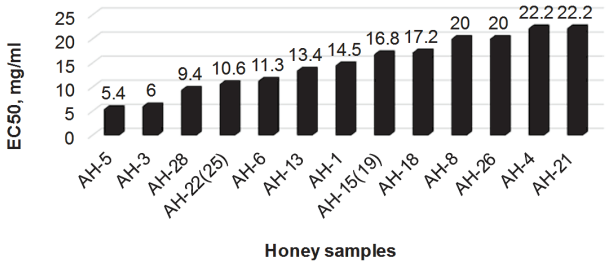

Fig. Antioxidant activity of honey samples

The detected antioxidant activity in studied samples showed a decreasing order of AH-21, AH-4 > AH-26, AH-8 > AH-18 > AH-15(19) > AH-1 > AH-13 > AH-6 > AH-22(25) > AH-28 > AH-3 > AH-5. So, the antioxidant activity was the lowest in the AH-21 and AH-4 honey samples, produced in village Zardakhach (Martakert region) and village Karmir (Askeran region), respectively. According to DPPH assay, the highest antioxidant capacity was observed in AH-5 honey, produced in Village Qrasni (Askeran region). Relatively high antioxidant capacity was obtained for AH-3 honey from village Zardakhach (Martakert region). Overall, the DPPH assay results emphasized the high antioxidant capacity of Armenian honey.

For determination of the antioxidant capacity of KH-1 honey sample, not only the DPPH method but also the FRAP assay was used. The results of two methods are introduced in Table 2.

Table 2: Antioxidant activity of honey produced in Kotayq region

Honey sample	Antioxidant activity	
	DPPH assay (EC50)	FRAP assay (mmol Fe^{2+}/g)
KH-1	12.5	3.31

Comparison of the results of both DPPH and FRAP assays allows to conclude that the KH-1 honey sample showed high antioxidant capacity.

Conclusion

It is the first study on evaluating the antioxidant capacity of Armenian honey. The results of the present study indicated that all honey samples showed high antioxidant potential. Nevertheless, for the overall evaluation of the antioxidant activity, future studies are needed to identify and quantify individual flavonoids and phenolic acids in honey.

References

Balkanska, R., Marghitas, L. A., Pavel, C. I. (2017). – Antioxidant Activity and Total Polyphenol Content of Royal Jelly from Bulgaria. International Journal of Current Microbiology and Applied Sciences, 6(10), -pp. 578-585.

Boulanouar, B., Mounir, H., Ahmed, B., Abdelaziz, G. (2017). – Total phenolic, flavonoid contents and antioxidant activities of honey and propolis collected from the region of Laghouat (South of Algeria). Int. J. Pharmacogn. Chinese Med., 1(2): 000110.

Costantini, L., Luksic, L., Molinari, R., Kreft, I., Bonafaccia, G., Manzi, L., Merendino, N. (2014). – Development of gluten-free bread using tartary buckwheat and chia flour rich in flavonoids and omega-3 fatty acids as ingredients. Food Chemistry, 165, -pp. 232-40.

Di Marco, G., Gismondi, A., Panzanella, L., Canuti, L., Impei, S., Leonardi, D., Canin, A. (2018). – Botanical influence on phenolic profile and antioxidant level of Italian honeys. J FOOD Sci. Thecnol., 55(10), -pp. 4042-4050.

Dobre, I., Gâdei, G., Patrascu, L., Elisei, A. M., Segal, R. (2010). – The antioxidant activity of selected Romanian honeys. The Annals of the University of Dunarea de Jos of Galati. Fascicle VI. Food Technology, 34(2), -p. 67.

Ferreira, I. C., Aires, E., Barreira, J. C., Estevinho, L. M. (2009). – Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. Food Chemistry, 114(4), -pp. 1438-1443.

Mkrtchyan, G.S., Pepelyan, H.G. (2015). – Honey production, Yerevan, -p.144 (in Armenian).

Molyneux, P. (2004). – The use of the stable free radical diphenilpicrylhydrazil (DPPH) for estimating antioxidant activity. J. Sci .Technol., 26(2), -pp. 211-219.

Moniruzzaman, M., Khalil, M. I., Sulaiman, S. A., Gan, S. H. (2012). – Advances in the analytical methods for determining the antioxidant properties of honey: A review. African Journal of Traditional, Complementary and Alternative Medicines, 9(1), -pp. 36-42.

Pontis, J.A., da Costa, L.A.M.A, da Silva, S.J.R, Flach, A. C. (2014). – Phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil, Food Science and Technology (Campinas), 34(1), -pp. 69-73.

Saric, G., Markovic, K., Vukicevic, D., Lez, E., Hruskar, M., Vahcic, N. (2013). – Changes of antioxidant activity in honey after heat treatment. J. Food Sci. Vol. 31, – N 6, -pp. 601–606.

Schramm, D. D., Karim, M., Schrader, R. H. (2003). – Honey with high levels of antioxidants can provide protection to healthy human subjects. Agricultural and Food chemistry 51, -pp. 1732−1735.

Wilczynska, A. (2010). – Phenolic content and antioxidant activity of different types of Polish honey - a short report. Polish journal of food and nutrition sciences. Vol. 60, – N 4, -pp. 309-313.