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Determination of the Total Antioxidant Potential in Iranian Honey, as well as their Radical Scavenging activity

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ABSTRACT

Honey is one of the oldest, widely used food products And it has been used since long time ago both in medical and domestic needs, but only recently its antioxidant property has come to lime light. In this research, thirty four Iranian honey samples were analyzed for determining the total antioxidant potential, as well as their radical scavenging activity. Evaluation of antioxidant capacities were carried out using FRAP and DPPH by spectrophotometric method. The results showed that high variety of antioxidant power in Iranian honeys. Obtained results of FRAP method showed the superiority of Summer crops honey of Boinzahra and Thyme honey of Zanjan Results of DPPH analysis showed that superiority of Thyme honey of Zanjan, Astragal honey of Garab. The current finding will provide useful information for evaluation of food quality and medicine quality of Iranian natural honeys and help us to classify the Iranian natural honeys and choosing the specific honey for our specific health – promoting effects.
Keywords: Honey, Antioxidant, FRAP, DPPH, IRAN

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INTRODUCTION

Honey has been used since the earliest times. It is widely appreciated as the only concentrated form of sugar available worldwide [1] and is also used as a food preservative [2, 3]. Lately, the physiological functioning of different foods including honey has received much attention. Antioxidants, for example, play an important role in food preservation and human health by combating damage caused by oxidizing agents e.g., oxygen Natural and synthetic antioxidants have a long history as preservatives in food [4]. Antioxidant activity has recently been determined in various foodstuffs by many scientists and research groups around the world [5, 6]. Scientists have found that oxygen may contribute to human aging and illness even it is vital to life. Cells form by-products called "free radicals" when oxygen is metabolized. Free radicals travel through the cell, disrupting the structure of other molecules and resulting in cellular damage. Such damage is believed to contribute to aging and various health problems. Humans protect themselves from the harmful effect of free radicals, partly, by taking antioxidants from high-antioxidant foods [7].Where they specifically retard deterioration, rancidity or discoloration due to oxidation caused by light, heat and some metals. These antioxidants, which act as preservatives because of their antioxidative activity [8, 2, 9]. Include both enzymatic (e.g., catalase, glucose oxidase) and non-enzymatic substances (e.g., organic acids, Maillard reaction products, amino acids, proteins, flavonoids, phenolics, a-tocopherol, flavonols, catechins, ascorbic acid and carotenoids) [4]. Many authors have studied the phenolic and flavonoid contents of honey to determine if a correlation exists with floral origins [10, 11] and also to determine the presence of antimicrobial activity [12]. It has been demonstrated that some amino acids also have antioxidant properties [13]. Many methods for determining the antioxidative activity in honey have been used, e.g., determination of active oxygen species (the superoxide anion, peroxyl and hydroxyl radicals), their radical scavenging ability [14, 15] the1,1-diphenyl-2-picrylhydrasyl

(DPPH) antioxidant content [16] and enzymatic or non-enzymatic measurements of lipid peroxidation inhibition [17]. Antioxidant capacity of honey depends on the floral and geographical origin, climatic conditions and processing, storing and handling of honey. The greatest influence on the antioxidant activity of honey has been contributed to its botanical origin [18, 19]. A growing number of evidence about honey antioxidant (AO) activity, a parameter useful to evaluate biological function and possible therapeutic potential, has been accumulated [20].

MATERIALS AND METHODS

Thirty-four honey samples were collected for this study (Table 1). Honey samples were collected from local producers famous for pure honey production. Honey samples (n = 34) considered in this study were of the different floral sources: Thyme, Astragal, Cedar, Barberry, Ucalyptus, Dill and other sample. The different samples were collected between June 2012 and February 2013 from different geographical regions (Eastern, western, South, North and central parts) of Iran. Qualitative microscopic analysis and frequency determination of the classes of pollen grains in the honey samples were done as described. All the samples were stored between 0 and 4 _C.

FRAP (ferric reducing antioxidant power) assay

The FRAP assay was carried out as previously described [21] with some minor modifications. The method is based on the ability of the honey sample to reduce the ferri form of 2,4,6-tri(2-pyridyl)-1,3,5-triazine complex (Fe3+-TPTZ) to ferro, coloured form (Fe2+-TPTZ) at acidic pH. Reduction is monitored by measuring the changes of absorbance at 593 nm.The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 Mm HCl, 2.5 mL of 20 mM FeCl3 and 25 mL of 0.3 M acetate buffer, pH 3.6. It was prepared daily and kept in the dark at 37 C. Aliquots of 400 mL of honey solution were mixed with 3.6 mL of FRAP reagent and the absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubation at 37 C for 10 min . The total antioxidant capacity of samples was determined against a standard solutions of $FeSO_4.7H_2O$ (1-10mM) and the results were mentioned mM Fe (II) for each of the samples. Measurements for all methods used in this research were done in three replications for each sample.

Radical scavenging activity (DPPH)

Stable DPPH radical reaches the absorbance maximum at 517 nm and its color is purple. The change of this color into yellow is a result of pairing of an unpaired electron of a DPPH radical with the hydrogen of the antioxidant, thus generating reduced DPPH-H. Adding an antioxidant results in the decrease of absorbance, this is proportional to the concentration and antioxidant activity of the compound. The scavenging activity of honey samples for the radical2,2-diphenyl-1 picrylhydrazyl (DPPH) was measured as described [22] with some modifications. Honey samples were dissolved in methanol at a concentration of 2.65–170 mg/ml, and 0.75 ml of each sample was mixed with 1.5 ml of DPPH (Fluka Chemie, Switzerland) in methanol (0.02 mg/ml), with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and the absorbance then measured at 517 nm. The IC_{50} value was reported for scavenging activity for each honey samples. The inhibition percentage of DPPH radical was calculated by the following formula, where A_C is absorbance of blank sample and A_S is absorbance of sample solution.

$$\% \text{ Inhibition} = [(A_C - A_S)/ A_C] \times 100$$

RESULTS AND DISCUSSION

Table 1. Antioxidant Capacity of 34 honey samples (average value±standard deviation)

SAMPLE NO	Floral origin	Geographical origin in IRAN	color	FRAP (mmol Fe2II/100g± SD)	RSA: IC50 (mg/ml ± SD)
1	Cedar	Lar	Saddle Brown	5.37±0.25	23.96±1.21
2	Thyme	Zanjan	Brown	10.31±1.06	5.99±0.03
3	Thyme	Takab	Brown	6.91±0.28	13.3±0.91
4	Thyme	Gardanekhan	Brown	5.77±0.61	20.05±2.30
5	Astragal	Divandare	Yellow	5.34±0.49	16.77±0.98
6	Thyme	Sabalan	Brown	5.01±0.17	30.97±0.65
7	Thyme	Hamedan	Brown	4.81±0.073	54.17±3.23
8	Thyme	Orazan	Brown	8.89±0.93	8.1±0.32
9	Astragal	Kamyaran	Yellow	3.90±0.089	62.72±0.45
10	Astragal	Gorgan	Yellow	3.66±0.059	49.28±3.21
11	Barberry	Birjand	Maroon	6.69±0.41	14.98±0.96
12	Sunflower	Golestan	yellow		

13	Cedar	Shoshtar	Saddle Brown	6.27±0.58	16.79±1.24
14	Astragal	Gandoman	Yellow	3.53±0.708	45.27±2.37
15	Jujube	Birjand	Maroon	4.31±0.21	54±1.65
16	honeydew	Hamedan	Dark brown	3.86±0.13	75.98±4.32
17	Green honey	Isfahan	Dark brown	4.13±0.16	41.15±0.36
18	Thyme	Youish	Brown	8.66±1.19	5.13±0.026
19	Coriander	Nahavand	Dark Gold	3.95±0.069	54.57±3.14
20	Ucalyptus	South of Iran	Dark yellow	4.21±0.052	67.16±5.99
21	Dill	Isfahan	Dark brown	4.84±0.047	18.19±2.65
22	Astragal	Shahrkord	Yellow	2.98±0.038	89.66±6.36
23	Alfafa	Ghom	yellow	4.45±1.03	46.96±4.82
24	Thyme	Takab	Brown	4.52±0.94	11.9±0.09
25	Cedar	Jiroft	Saddle Brown	4.92±0.16	11.81±1.01
26	Cotton	Eshtehard	yellow	3.47±0.20	71.97±4.87
27	Summer crops	Boinzahra	Dark brown	10.73±0.65	24.37±1.60
28	Astragal	Gardanekhan	Yellow	4.36±0.29	43.88±2.59
29	Astragal	Garab	Yellow	8.64±0.55	6.27±0.065
30	Ucalyptus	Ahvaz	Dark yellow	2.40±0.074	69.64±3.28
31	Astragal	Shahrkord	Yellow	2.98±0.021	71.83±3.79
32	Thyme	Sira	Brown	8.59±0.65	14.27±2.64
33	Astragal	Isfahan(south)	Yellow	2.33±0.011	48.95±4.28
34	Astragal	Sahand	Yellow	4.05±0.032	19.91±3.49

RSA, radical scavenger activity

Thirty-four honey samples of different floral origins were analysed in order to assess their antioxidant activity and find relationship between the antioxidant activity (DPPH) and the total antioxidant potential (FRAP). The results obtained showed (Table 2) that all the samples tested were antioxidantively active, however the total antioxidant potential and free radical scavenging activity varied greatly among the honey types.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay is the only one that directly estimates antioxidants or reductones in a sample, and is based on the ability of the analyte to reduce the Fe3+/Fe2+ couple [23]. It has been found that the total polyphenolic content and the Fe2+ content formed in the presence of the honey antioxidants are significantly correlated, Similar findings were reported by others [24, 25]. It has been stated that the antioxidant activity of reductione was based on the breaking of the free radical chain bydonating a hydrogen atom [26].

Ferric reducing antioxidant power (FRAP*) assay* Assessment of the FRAP method were interpolated in a calibration curve of $FeSO_4.7H_2O$. Results were expressed as mmol $FeSO_4/L$ ($R^2 = 0.9866$). Total antioxidant potential (FRAP) were varied from 2.335(mmol $FeSO_4L^{-1}$) to 10.737(mmol $FeSO_4L^{-1}$) in Iranian honey samples(Table 1). The highest antioxidant potential of honeys were found in Summer crops honey of Boinzahra 10.737(mmol $FeSO_4L^{-1}$) in Alborz province at north of Iran and honey Thyme of Zanjan with10.31(mmol $FeSO_4L^{-1}$) While the lowest antioxidant potential were observed in Astragal honey of south Isfahan in central Iran.

The results showed the dark honeys had higher FRAP value or higher antioxidant potential. The Summer crops, cedar and Thyme honeys with dark colors had higher FRAP value and Alfalfa , Cotton, Coriander and Astragal honeys with light color had lower FRAP value or antioxidant activity.

Radical scavenging activity (DPPH)

Radical scavenging activity measurement by DPPH method was one of the two methods used to determine the antioxidant activity of honey. This method is specific because lower absorbance value means higher antioxidant activity. The results of DPPH radical scavenging activity (RSA) and the antioxidant content of different honey samples are summarized in Table 1. The IC50 values ranged from 5.99 to 89.66.The highest DPPH RSAs were found in Thyme honey of Zanjan while the lowest was observed in a Astragal honey of Shahrkord.

Meda et al. (2005) had found the highest DPPH value in Vitellaria honey and also lowest was observed in a multifloral Honey [27], and 2.30% to 51.5 % observed Turkish honeys [28].

Wilczyńska (2010) reported the radical scavenging activity of honey varied from23.81percentage to 100% in the DPPH reaction system [29]. The results of DPPH radical scavenging activity showed that dark honeys tended to be highly active in the reaction with DPPH.The highest antioxidant capacity had dark honeys, while the lowest antioxidant capacity were found in pale honeys [29]. This trend was generally similar to the relationship found for some Slovenian, Burkina Faso and Italian honeys [24, 27, 25].

CONCLUSION

This study showed that the 34 samples of Iranian honey contained antioxidant capacity of good quality. The total phenolic content and antioxidant activity varied between honey types. Summer crops honey of Boinzahra had the highest total antioxidant potential (Frap) and Astragal honey of Garab had the most active radical scavenger activity of all samples. High correlation coefficient between FRAP and DPPH assay can demonstrate the high quality of honey samples and confirms our results. This explains that the purity of honey samples is a great representative for high antioxidant content.

REFERENCES

1. FAO. (1996).Value-added products from beekeeping.FOA Agricultural Services Bulletin.Rome, Italy: FAO.
2. Cherbuliez, T. (2001).The medicine from the bees. CD-ROM Apimondia.
3. Cherbuliez, T.& Domerego, R., Apitherapie, L. (2004). Medicine des abeilles. Editions Amyris.
4. The National Honey Board. (2003). Honey-Health and therapeutic qualities. 390 Lashley Street Longmont, www.nhb.org.
5. Giasson, H., Ischiropoulos, V.M.Y., Lee, J.Q. (2002). The relationship between oxidative/nitrative stress and pathological inclusions in Alzheimer's and Parkinson's diseases, *Free Radic. Biol. Med. 32*: 1264–1275.
6. Abdel-Hameed. (2009). Total phenolic contents and free radical scavenging activity of certain Egyptian Ficusspecies leaf samples, Food Chem 114 : 1271–1277.
7. Alzahrani, A.H., Boukraâ, L., Bellik, Y., Abdellah, F., Bakhotmah, B.A., Kolayli, S., Sahin, H. (2012). Evaluation of the Antioxidant Activity of Three Varieties of Honey from Different Botanical and Geographical Origins. Global Journal of Health Science 4, No. 6.
8. Antony, S.M., Han, I.Y., Rieck, J.R., Dawson. P.L. (2000). Antioxidative effect of Maillard reaction products formed from honey at different reaction times. Journal of Agricultural and Food Chemistry 48: 3985–3989.
9. Ferreres, F., Garcia-Vigera, C., Tomas-Lorente, F., Tomas-Barberan, F.A. (1993). A marker of the floral origin of citrus honey. Journal of the Science of Food and Agriculture 61: 121–123.
10. Amiot, M.J., Aubert, S., Gonnet, M., Tacchini, M. (1989). Les compose´sphe´noliques des miels: e´tudepre´liminairesurl_identificationet la quantification par families. Apidologie 20: 115–125.
11. Ferreres, F., Tomas-Barberan, F.A., Gil, M.I., Tomas- Lorente, F. (1991). An HPLC technique for flavonoid analysis in honey. Journal of the Science of Food and Agriculture 56: 49–56.
12. Snow, M.J.& Manley-Harris, M. (2004). On the nature of nonperoxide antibacterial activity in New Zealand manuka honey. Food Chemistry 84: 145–147.
13. Wu, H.C., Shiau, C.Y., Chen, H.M., Chiou, T.K. (2003). Antioxidant activities of carnosine, anserine, some free amino acids and their combination. Journal of Food and Drug Analysis 11: 148–153.
14. Gheldof, N., Wang, X.H., Engeseth, N.J.(2002). Identification and quantification of antioxidant components of honeys from various floral sources. Journal of Agricultural and Food Chemistry 50: 5870–5877.
15. Nagai, T., Sakai, M., Inoue, R., Inoue, H., Suzuki, N.(2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. Food Chemistry 75: 237–240.
16. Chen, L., Mehta, A., Berenbaum, M., Zangerl, A.R., Engeseth, N.J. (2000). Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. Journal of Agriculture and Food Chemistry 48: 4997–5000.
17. McKibben, J., Engeseth, N.J. (2002). Honey as a protective agent against lipid oxidation in ground turkey. Journal of Agricultural and Food Chemistry 50: 592–595.
18. Beretta, G., Granata, P., Ferrero, M. Orioli, M., Maffei Facino, R. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/ fluorimetric assays and chemometrics. AnalyticaChimicaActa 533: 185-191.
19. Frankel, S., Robinson, G.E., Berenbaum, M.R. (1998). Antioxidant capacity and correlated characteristics of 14 unifloral honeys. Journal of Apicultural Research 37: 27–31.
20. Schramm, D.D., Karim, M., Schrader, H.R., Holt, R.R., Cardetti, M., Carl, L., Keen, C.L.(2003). Honey with high levels of antioxidants can provide protection to healthy human subject. Journal of Agricultural and Food Chemistry 51: 1732–1735.
21. Benzie, I.F., Strain, J.J.(1996). The ferric reducing ability of plasma (FRAP) as a measure of Antioxidant power: the FRAP assay. Analytical Biochemistry 239: 70–76.
22. Velazquez, E., Tournier, H.A., Mordujovich de Buschiazzo, P., Saavedra, G., Schinella, G.R. (2003). Antioxidant activity of Paraguayan plant extracts. Fitoterapia 74: 91–97.
23. Lim, T.T., Tee, J.J.(2007). Antioxidant properties of several tropical fruits: a comparative study. *Food Chemistry 103*: 1003-1008.
24. Bertoncelj, J., Doberšek, U., Jamnik, M., Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey.*Food Chemistry* 105: 822-828.

25. Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M.P., Albertini, M.C., Piatti, E. (2006). Raw Millefiori honey is packed full of antioxidants. Food Chemistr 97: 217-222.
26. Gordon, M.F.(1990). The mechanism of antioxidant action in vitro. In B.J.F. Hudson (ed.), Food Antioxidants. Elsevier Applied Science, London 1-18.
27. Meda, A., Lamien, C.E., Romito, M.(2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity Food Chemistry 91: 571–577.
28. Silici, S., Sagdic, O., Ekici E. (2012). Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys .Food Chem 121: 238-243.
29. Wilczyńska A.(2010). Phenolic Content and Antioxidant Activity Of Different Types Of Polish Honey – A Short Report. polish journal of food and nutrition sciences 60: 309-313.

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