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 Boizahra 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

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, ascophor, 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] the 1,1-diphenyl-2-picrylhydrazyl.
(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.

**RESULTS AND DISCUSSION**

Determination of AO activities was carried out as described previously [21] with some minor modifications. Honey samples were dissolved in methanol at a concentration of 2.65 µg/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) and the absorbance then measured at 517 nm. The inhibition percentage of DPPH radical scavenging activity for each honey samples was calculated by the following formula, where \( A_c \) is absorbance of blank sample and \( A_s \) is absorbance of sample solution.

\[
\% \text{ Inhibition} = \left( \frac{A_c - A_s}{A_c} \right) \times 100
\]

% Inhibition = \( [(A_c - A_s) / A_c]\) × 100

**RESULTS AND DISCUSSION**

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

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

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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 reduction was based on the breaking of the free radical chain by donating 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\(_2\)O. Results were expressed as mmol FeSO\(_4\)/L \((R^2 = 0.9866)\). Total antioxidant potential (FRAP) were varied from 2.335(mmol FeSO\(_4\).L\(^{-1}\)) to 10.737(mmol FeSO\(_4\).L\(^{-1}\))  in Iranian honey samples(Table 1). The highest antioxidant potential of honeys were found in Summer crops honey of Boinzahra 10.737(mmol FeSO\(_4\).L\(^{-1}\)) in Alborz province at north of Iran and honey Thyme of Zanjan with10.31(mmol FeSO\(_4\).L\(^{-1}\)). 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 from 23.81% 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


**CITATION OF THIS ARTICLE**