Antioxidant properties and phenolic content of sulla (Hedysarum spp.) honeys from Southern Italy

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Summary
The geographical origin greatly influences the qualitative and nutraceutical characteristics of honey. In this study, a total of twenty-four sulla honeys from eight different geographical areas of Southern Italy have been examined for total phenolic content (Folin–Ciocalteu method), antioxidant activity (FRAP and DPPH assays), colour intensity (ABS450), and identification and quantification of phenolic acids (RP-HPLC/UV-VIS method). The total phenolic content ranged from 47.9 (Potentino honeys) to 248.3 mg GAE per kg honey (Penisola Sorrentina honeys). The antioxidant activity ranged from 47.06% (Basso Pollino honeys) to 88.25% (Penisola Sorrentina honeys), and from 98.26 lM Fe (II) (Potentino honeys) to 786.53 lM Fe (II) (Tarantino honeys) for DPPH and FRAP assays, respectively. Major phenolic acids identified in analysed samples were gallic, caffeic and ferulic acids. Correlations between the parameters analysed were statistically significant ($P < 0.05$). The results of the study showed that the parameters studied are greatly affected by the peculiarities of their production area.

Keywords
1,1-diphenyl-2-picrylhydrazyl, colour intensity, ferric reducing antioxidant power, geographical origin, high-performance liquid chromatographic analysis, phenolic acids, sulla honey, total polyphenol.

Introduction
Antioxidants are natural components of foods and are, also, widely used as ingredients in dietary supplements with diverse physiological role in the body. They are substances capable of slowing or preventing the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidising chain reactions and are involved in the scavenging of free radicals. There are a wide range of antioxidants found in nature, such as vitamins C and E, glutathione, thiol antioxidants, carotenoids, flavonoids, phenolic acids and other compounds. Many authors showed that many phenols are stronger antioxidants than vitamins C and E (Vinson et al., 2001; Oboh & Akindahunsi, 2004). Phenolic acids have received considerable attention due to their physiological functions, including antioxidant, antimutagenic and antitumor activities (Kono et al., 1995; Spencer et al., 2008). They are a very important group of secondary plant metabolites, which, in many cases, serve in plant defence mechanisms to counteract reactive oxygen species (ROS) (Al-Mamary et al., 2002; Havsteen, 2002). These substances can be subdivided into two major groups: hydroxybenzoic acid derivatives (such as gallic acid and protocatechuic acid) and hydroxycinnamic acid derivatives (such as $p$-coumaric, caffeic, ferulic acid) (Manach et al., 2004; Skerget et al., 2005). Cinnamic and benzoic acid derivatives are present in virtually all plant foods (i.e. fruit, vegetables and grains) and are physically dispersed throughout the plant in seeds, leaves, roots and stems (Robbins, 2003). Also, phenolic acids have been widely studied due to their contribution to honey colour, taste and flavour (Robbins, 2003; Manach et al., 2004). It is widely known that fruit, vegetables and grains contain a wide range of these components, which can be transferred to honey because most of them represent an important resource of nectar for bees grazing (Baltrusaityte et al., 2007). The phenolic composition of honey and so its antioxidant capacity varies widely in relation to its botanical and geographical origin (Al-Mamary et al., 2002; Ghedolf & Engeseth, 2002).

Environmental factors have a major effect on polyphenol content. These factors may be pedoclimatic (soil type, sun exposure, rainfall) or agronomic (culture in greenhouses or fields, biological culture,
hydroponic culture, fruit yield per tree, etc.) (Manach et al., 2004; de Andrés-de Prado et al., 2007). Many polyphenols, especially phenolic acids, are directly involved in plant response to abiotic and biotic stresses. Nutritional stress such as low iron levels can cause increased release of phenolic acids, presumably to help solubilise metals and thereby facilitate their uptake (Marschner, 1991). Thus, phenolic composition of honeys from same floral source is affected both seasonal climatic changes and different geographical origin (Castro-Vázquez et al., 2010).

Sulla (Hedysarum spp.) is a biennial or short-lived perennial, herbaceous, legume grown for grazing, hay and silage production. It is effective example of a multiple-use species exploited for environmental protection, landscape enhancement and honey production (Sulas et al., 1997). It is native to Northern Africa (Algeria, Morocco, Tunisia), south-western Europe, naturalised elsewhere in parts of Europe, New Zealand and Australia. It forms a major source of nectar for honey production in southern and central Italy. The colour of sulla honey is almost white, up to straw-coloured. The smell is very faint and floral, and the taste is sweet and slightly acidic. The Italian sulla honey boasts a long tradition; however, its composition and bioactive properties until now have not been studied more comprehensively.

The aim of our study was to identify and quantify the phenolic acids and to evaluate the antioxidant activity in sulla honey from eight different geographical areas of Southern Italy.

Materials and methods

Chemicals

All used chemicals and solvents were of analytical grade. Gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, benzoic acid, quercetin, HPLC-grade methanol, potassium phosphate monobasic for HPLC, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), glacial acetic acid, sodium acetate, hydrochloric acid, iron (II) sulphate heptahydrate, ferric chloride were purchased from Sigma-Aldrich (Milan, Italy). Folin–Ciocalteu’s reagent was purchased from Carlo Erba (Milan, Italy).

Samples

Twenty-four sulla honey samples were collected between April and June from individual bee-keepers during the 2012 harvest in eight geographical areas of Southern Italy (Fig. 1). The honey purity was carefully checked by pollen analysis carried out according to DIN 10760 (DIN, 2002; Von der Ohe et al., 2004). On the basis of this analysis (Table 1), the predominant pollen type was Hedysarum spp. (frequency >50%). The characteristics of the considered areas in this experiment are reported in Table 2. Honey samples were stored at 4 °C in the dark until analysed. The experiments were performed using freshly prepared 10% honey solutions in distilled water. A sugar analogue (80% sugar, w/v), serving as a blank, was prepared by dissolving 0.2 g of sucrose, 0.8 g of maltose, 4 g of fructose and 3 g of glucose in distilled water to make a solution of 10 mL final volume (White, 1979). All tests were performed in triplicate.

Determination of total phenolic content

Total phenolic content of honey samples were determined according Folin–Ciocalteu spectrophotometric method described by Perna et al. (2012).

High-performance liquid chromatographic–UV analysis of phenolic compounds

Sample preparation for high-performance liquid chromatographic (HPLC) analysis and identification and quantification of phenolic compounds was carried out as described by Perna et al. (2013a).
Assessment of antioxidant activity was performed using different in vitro methods: radical-scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP) assays, as reported by Perna et al. (2012).

Colour intensity: ABS$_{450}$

The colour intensity (AU) was determined by spectrophotometric measurement as described by Perna et al. (2013b).

Statistical analysis

Statistical analysis was performed using the general linear model (GLM) procedure of statistical analysis system (SAS, 1996), using a monofactorial model:

\[ y_{ik} = \mu + \alpha_i + \varepsilon_{ik} \]

where: \( \mu \) = average mean; \( \alpha_i \) = effect of geographical origin ($1,\ldots,8$); \( \varepsilon_{ik} \) = experimental error. Before setting the values, expressed in percentage terms, they were subjected to angular transformation. The Student’s $t$-test was used for all variables comparisons. A Pearson’s correlation test was conducted to determine the linear correlation among the variables. Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

Results and discussion

Total phenolic content of sulla honey

Total phenolic content (mg GAE per kg honey) of sulla honeys from different geographical areas was

<table>
<thead>
<tr>
<th>Area</th>
<th>Characterising Pollen</th>
<th>Frequency %</th>
<th>Others pollens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarantino</td>
<td>Hedysarum spp.</td>
<td>51-56</td>
<td>Lotus corniculatus, Onobrychis vicifolia, Rubus sp., Trifolium repens, Trifolium pratense, Cruciferae</td>
</tr>
<tr>
<td>Penisola Sorrentina</td>
<td>Hedysarum spp.</td>
<td>54-58</td>
<td>Citrus spp., Lotus corniculatus, Rhamnaceae, Cruciferae, Eucalyptus</td>
</tr>
<tr>
<td>Lecese</td>
<td>Hedysarum spp.</td>
<td>55-58</td>
<td>Trifolium pratense, Lotus corniculatus, Rubus sp., Vicia sp., Trifolium repens, Cruciferae</td>
</tr>
<tr>
<td>Basso Pollino</td>
<td>Hedysarum spp.</td>
<td>57-60</td>
<td>Trifolium repens, Trifolium pratense, Castanea sativa, Onobrychis vicifolia, Rubus sp.</td>
</tr>
<tr>
<td>Collina Materana</td>
<td>Hedysarum spp.</td>
<td>55-58</td>
<td>Trifolium pratense, Citrus spp., Eucalyptus, Rubus sp.; Cruciferae</td>
</tr>
<tr>
<td>Potentino</td>
<td>Hedysarum spp.</td>
<td>58-62</td>
<td>Castanea sativa, Lotus corniculatus, Cruciferae, Rubus sp., Trifolium pratense</td>
</tr>
<tr>
<td>Vulture Melfese</td>
<td>Hedysarum spp.</td>
<td>56-60</td>
<td>Castanea sativa, Rubus sp.; Trifolium repens, Trifolium pratense Eucalyptus, Echium vulgare</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Area</th>
<th>m above sea level</th>
<th>Pedological characteristics</th>
<th>Yearly Average Temperature ($^\circ$C)</th>
<th>Annual Rainfall (mm)</th>
<th>Density (Inhabitants km$^{-2}$)</th>
<th>Presence of industries with high environmental impacts</th>
<th>Agricultural activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarantino</td>
<td>130–480</td>
<td>Sandy-clayey soil</td>
<td>12.7–20.5</td>
<td>416.50</td>
<td>Medium</td>
<td>High</td>
<td>Intensive</td>
</tr>
<tr>
<td>Penisola Sorrentina</td>
<td>0–600</td>
<td>Marble-clayey soil</td>
<td>12.4–20.4</td>
<td>1078</td>
<td>High</td>
<td>Low</td>
<td>Intensive</td>
</tr>
<tr>
<td>Camasta – Dolomiti Lucane</td>
<td>700–1100</td>
<td>Clayey soil</td>
<td>4.0–24.0</td>
<td>737.44</td>
<td>Low</td>
<td>Low</td>
<td>Extensive</td>
</tr>
<tr>
<td>Lecese</td>
<td>57</td>
<td>Chalky soil</td>
<td>11.3–21.3</td>
<td>550–600</td>
<td>Medium</td>
<td>Low</td>
<td>Semi-intensive</td>
</tr>
<tr>
<td>Basso Pollino</td>
<td>200–1000</td>
<td>Chalky soil</td>
<td>12.9–21.3</td>
<td>504</td>
<td>Low</td>
<td>Semi-intensive</td>
<td>Semi-intensive</td>
</tr>
<tr>
<td>Collina Materana</td>
<td>20–770</td>
<td>Silty-clayey soil</td>
<td>10.2–20.0</td>
<td>500</td>
<td>Low</td>
<td>Semi-intensive</td>
<td>Semi-intensive</td>
</tr>
<tr>
<td>Potentino</td>
<td>400–1100</td>
<td>Clayey soil</td>
<td>7.6–15.5</td>
<td>613</td>
<td>Medium</td>
<td>High</td>
<td>Extensive</td>
</tr>
<tr>
<td>Vulture Melfese</td>
<td>350–730</td>
<td>Volcanic soil</td>
<td>9.4–18.7</td>
<td>800–1000</td>
<td>Low</td>
<td>High</td>
<td>Intensive</td>
</tr>
</tbody>
</table>

Density: high >600 inhabitants km$^{-2}$; medium: between 300 and 600 inhabitants km$^{-2}$; low <300 inhabitants km$^{-2}$.
investigated by the Folin–Ciocalteu assay (Table 3). Total phenolic content of the honey samples ranged from 47.9 (Potentino honeys) to 248.3 mg GAE per kg (Penisola Sorrentina honeys), with an overall mean of 118.7 mg GAE per kg ($P < 0.05$). The average polyphenol content was similar to that found in Italian honeys by other authors (Pichichero et al., 2009; Perna et al., 2012), while Socha et al. (2011), in Polish honeys, reported lower values. As can be observed in Table 3, the phenolic content was different depending on the sampling area. Penisola Sorrentina honeys showed the highest total phenol content (248.3 mg GAE per kg; $P < 0.05$) if compared with the other studied honeys, exceeding by more than two times the average (118.7 mg GAE per kg). The lowest values were obtained from Potentino, Vulture Melfese and Leccese honeys (47.9, 48.6 and 52.4 mg GAE per kg, respectively). Geographical origin of honey plays a role in total phenolic content as was reported by Silici et al. (2010), for rhododendron honey. The differences observed can be related to various factors, such as soil composition, temperature, humidity, altitude, possible land contamination, industry mining, emission of automobile exhaust gases that affect systematically or occasionally the plant’s physiological state, thus influencing phenolic biosynthesis. In particular, Tarantino, Potentino and Vulture Melfese areas are characterised by the presence of factories that have a high environmental impact, while Penisola Sorrentina area, a well known tourist area, is characterised by a high population density and high movement of vehicles, especially in the spring–summer period. Camastra-Dolomiti Lucane area is mainly agricultural–pastoral, with a low population density (30 inhabitants km$^{-2}$), and it is included in the Natural Park of Dolomiti Lucane, representing one of the main green lungs of Southern Italy but located at about 25 km from Viggiano oil centre (Basilicata region), considered the greatest of continental Europe, with a high environmental impact. Also, biotic and abiotic stresses caused by environmental factors are able to trigger changes in the plant’s metabolism. These changes may affect the polyphenol biosynthesis, especially phenolic acids, which represent the evolutionary response to plants adaptation to different environmental characteristics (Al-Mamary et al., 2002; Cheynier, 2005; Muñoz et al., 2007). In support of this, in our previous work (Perna et al., 2012), a positive correlation between heavy metal and total phenol contents was reported.

### Table 3 Total phenolic content, DPPH and FRAP values of sulla honey samples from different geographical origin

<table>
<thead>
<tr>
<th>Area</th>
<th>Phenolic content (mg GAE per kg honey)</th>
<th>DPPH (I%)</th>
<th>FRAP [µmol Fe(II)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarantino</td>
<td>138.7 ± 19.9$^{a,d}$</td>
<td>86.48 ± 6.68$^a$</td>
<td>786.53 ± 91.28$^a$</td>
</tr>
<tr>
<td>Penisola Sorrentina</td>
<td>248.3 ± 31.1$^b$</td>
<td>88.25 ± 9.85$^b$</td>
<td>454.50 ± 55.44$^b$</td>
</tr>
<tr>
<td>Camastra-Dolomiti Lucane</td>
<td>164.9 ± 24.4$^e$</td>
<td>81.75 ± 4.61$^e$</td>
<td>166.32 ± 38.25$^{c,d}$</td>
</tr>
<tr>
<td>Leccese</td>
<td>52.4 ± 20.3$^{c,e}$</td>
<td>57.92 ± 3.68$^{c,e}$</td>
<td>115.7 ± 25.95$^{c,e}$</td>
</tr>
<tr>
<td>Basso Pollino</td>
<td>114.6 ± 21.6$^{a,e}$</td>
<td>47.06 ± 8.60$^{a,e}$</td>
<td>132.74 ± 26.30$^{a,c,e}$</td>
</tr>
<tr>
<td>Collina Materana</td>
<td>79.6 ± 22.3$^{a,c,e}$</td>
<td>57.28 ± 5.17$^{a,c,e}$</td>
<td>123.51 ± 25.43$^{a,c,d}$</td>
</tr>
<tr>
<td>Potentino</td>
<td>47.9 ± 25.6$^{d}$</td>
<td>51.47 ± 4.05$^{d}$</td>
<td>98.26 ± 28.61$^{d}$</td>
</tr>
<tr>
<td>Vulture Melfese</td>
<td>48.6 ± 13.2$^{c}$</td>
<td>56.63 ± 7.09$^{c}$</td>
<td>198.93 ± 42.44$^{c,d}$</td>
</tr>
<tr>
<td>Total</td>
<td>118.7 ± 70.1</td>
<td>65.85 ± 16.90</td>
<td>259.56 ± 234.35</td>
</tr>
</tbody>
</table>

Mean values from three repetition ± standard deviations.  
Means in the same column with different letters are significantly different according to the Student’s t-test ($P < 0.05$).
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Oddo et al. (2008) reported that gallic acid was often identified in the natural honeys. Contrary, gallic acid was not found in sulla honey from Lazio (Italy) by Pichichero et al. (2009). This demonstrated that phenolic content was highly dependent on the geographical source. The gallic acid content ranged between 6.68 (Penisola Sorrentina honeys) and 89.53 mg kg\(^{-1}\) honey (Tarantino honeys), showing a mean value of 40.07 mg kg\(^{-1}\) honey. Chlorogenic acid is determined in many sorts of honey and is considered as an informative part of honey characteristic phenolic profile (Yao et al., 2003, 2004). The values ranged between 0.32 (Collina Materana honeys) and 10.33 mg kg\(^{-1}\) honey (Tarantino honeys; \(P < 0.05\)), with an average value of 3.25 mg kg\(^{-1}\) honey, which represented 5.6% of total phenolic acids. Several authors (Ghedolf et al., 2002; Truchado et al., 2008) showed that hydroxycinnamic acids, such as caffeic acid, \(p\)-coumaric acid and ferulic acid, derived from propolis that the bee would directly incorporate into honey. In plants, \(p\)-coumaric acid is an intermediate metabolite in the synthesis of more complex phenolic compounds (Grace & Logan, 2000). \(p\)-coumaric acid represented 5.0% of total phenolic acids. Tomás-Barberá et al. (2001), in European honeys, found the lower level of \(p\)-coumaric acid, while Baltrusietyte et al. (2007) found high level of \(p\)-coumaric acid in natural honeys and in the honeys produced with additives of plant extracts especially birch extract. The mean content of \(p\)-coumaric acid (2.87 mg kg\(^{-1}\) honey) was significantly higher than that reported by other authors (Dimitrova et al., 2007; Pichichero et al., 2009). The values ranged from 1.24 (Collina Materana honeys) to 6.40 mg kg\(^{-1}\) honey (Tarantino honeys; \(P < 0.05\)). The content of ferulic acid in tested samples varied from 1.24 (Basso Pollino honeys) to 18.08 mg kg\(^{-1}\) honey (Tarantino honeys), with a mean value of 3.94 mg kg\(^{-1}\) honey which represented 6.8% of the total phenolic acids. The ferulic content was higher than that found in Romanian honeys (Marghitas et al., 2010), while it resulted similar than that found in Australian honeys (Yao et al., 2004). Among hydroxycinnamic acids, the most abundant is caffeic acid, representing 11.8% from total amount. The concentration for caffeic acid ranged from 0.92 (Potentino honeys) to 22.25 mg kg\(^{-1}\) honey (Tarantino honeys; \(P < 0.05\)), with a mean value of 6.81 mg kg\(^{-1}\) honey. The caffeic acid content was higher than that found in Italian sulla honeys by Pichichero et al. (2009). Benzoic acid is present in much smaller amounts which represented only 1.4% from the total amount of identified phenol acids. The amounts of benzoic acid ranged between 0.27 (Camastra-Dolomiti Lucane honeys) and 1.34 mg kg\(^{-1}\) honey (Collina Materana honeys).

Antioxidant capacity

In evaluating the antioxidant activity of honey samples, the DPPH and FRAP assays were used (Table 3). The antioxidant activity was measured using different methods because of the lack of a widely accepted standardised method. DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay (Bozin et al., 2006). The principle of the assay is based on the colour change in the DPPH solution from purple to yellow as the radical is quenched by the antioxidant (Karagözler et al., 2008). FRAP assay is considered as a useful indicator of the antioxidant status to counteract the oxidative damage due to ROS (Küçük et al., 2007). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.
The average values were 65.85% and 259.56 µM Fe(II) for DPPH and FRAP assays, respectively. Although it is difficult to make direct comparisons between our DPPH values and available literature data because the reaction conditions employed by different authors are different, we can say that our results are in line with those presented in Malaysian honeys by Khalil et al. (2011). Our reported FRAP values were similar than those found in Malaysian honeys by Moniruzzaman et al. (2013). The values varied from 47.06% (Basso Pollino honeys) to 88.25% (Penisola Sorrentina honeys) in the DPPH assay, and from 98.26 µM Fe(II) (Potentino honeys) to 786.53 µM Fe(II) (Tarantino honeys) in the FRAP assay. Penisola Sorrentina and Tarantino honeys showed an antioxidant activity, measured by DPPH assay, higher than that found in the honeys from the other considered areas (I% = 88.25% and 86.48%, respectively). The trend of the antioxidant activity, evaluated by FRAP assay, confirmed the results obtained by DPPH assay, in particular for Tarantino and Penisola Sorrentina honeys (786.53 and 454.50 µM Fe(II), respectively; P < 0.05). The differences among the studied honeys were due to the variation in their content in biologically active compounds. Ghedolf et al. (2002) reported that the antioxidant activity is the result of the overall action of biologically active components that may act synergistically. Many studies have demonstrated that, both in vitro and in vivo, the antioxidant activity of honey is due to the large amount of phenolics present (Ghedolf et al., 2002; Vela et al., 2007; Estevinho et al., 2008; Ferreira et al., 2008). The obtained results in this work demonstrated that the variations in antioxidant activity are a function of different locations.

Colour intensity

The colour of honey, beside flavour and aroma, is one of characteristics that serve to define the quality. The ABS450 parameter is related to the presence of pigments, such as carotenoids and phenolic compounds that have absorption maxima at 450 nm (Furr, 2004; Mendiola et al., 2008). As shown in Fig. 5, there were significant differences among the honeys from different geographical areas (P < 0.05).

The wide range of observed honey colours is due to (i) a different presence of pigments with antioxidant activity (Abu-Tarboush et al., 1993), (ii) a different concentration of Maillard reaction products (Antony et al., 2000), and (iii) a different minerals concentration that is related to the production area (González-Miret et al., 2005). The ABS450 parameter of studied honeys from the different areas presented the highest value in Tarantino honeys (1.82 AU; P < 0.05), followed by Penisola...
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Sorrentina and Camasta-Dolomiti Lucane honeys (0.82 and 0.37 AU, respectively; \( P < 0.05 \)), while the other honeys do not show any significant differences. Furthermore, it has been observed that the colour intensity increases with increases in the phenolic contents of honey. ABS\(_{450}\) values were in a similar range as those reported by other authors (Beretta et al., 2005; Mendiola et al., 2008).

**Correlation among the parameters**

Correlations between the parameters analysed are showed in Table 4. The \( r \) value obtained from FRAP and DPPH assays were slightly lower (\( r = 0.75; \ P < 0.001 \)) than that reported by Bertonecelj et al. (2007) (\( r = 0.89 \)). The antioxidant activity assessed by DPPH and FRAP assays was positively correlated with phenolic content (\( r = 0.82 \) and \( r = 0.54 \), respectively; \( P < 0.001 \)), confirming that these compounds contribute to antioxidant activity, as observed by other authors (Ghedolf et al., 2002; Vela et al., 2007; Estevinho et al., 2008; Ferreira et al., 2008). Beretta et al. (2005) showed values of \( r \) much higher than those found in this study (\( r = 0.91 \)). The low value of correlation confirms that phenolic compounds are not solely responsible of honey’s colour.

In addition, the contribution given by individual phenolic acids to the antioxidant activities of honeys was estimated. No linear correlation between individual phenolic compounds and antioxidant activity in reaction with DPPH was observed, except for ferulic acid (\( r = 0.45; \ P < 0.001 \)). The linear correlation values between chlorogenic, \( p \)-coumaric and ferulic acids and FRAP assay were observed (\( r > 0.50; \ P < 0.05 \)). Similar results were found by Rekika et al. (2005) in strawberry. They reported that it was probably due to synergism among these compounds. These findings showed that the individual phenolic acids have a higher reducing capacity than radical-scavenging ability.

**Conclusions**

Antioxidant activity, phenolic content and colour of honey are greatly affected by the peculiarities of their production area. Therefore, the characteristics of honey from different geographical origin are mainly due to the interactive effects. These effects, determined by dynamic balances over time, ordinarily occur among plant, soil, thermohygrometric and environmental conditions and are specific for each area. The consumer, as end-user of honey, is get involved in this dynamic balance.

**References**


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**Table 4** Correlation matrix of investigated parameters (Pearson correlation coefficients)

<table>
<thead>
<tr>
<th>Phenolic content</th>
<th>DPPH</th>
<th>FRAP</th>
<th>ABS(_{450})</th>
</tr>
</thead>
<tbody>
<tr>
<td>gallic acid</td>
<td>0.82***</td>
<td>0.75***</td>
<td>0.98***</td>
</tr>
<tr>
<td>chlorogenic acid</td>
<td>0.54***</td>
<td>0.77***</td>
<td>0.73***</td>
</tr>
<tr>
<td>caffeic acid</td>
<td>0.82***</td>
<td>0.77***</td>
<td>0.73***</td>
</tr>
<tr>
<td>( p )-coumaric acid</td>
<td>0.82***</td>
<td>0.77***</td>
<td>0.73***</td>
</tr>
<tr>
<td>ferulic acid</td>
<td>0.82***</td>
<td>0.77***</td>
<td>0.73***</td>
</tr>
</tbody>
</table>

*** \( P < 0.001; \ * P < 0.05; \ n.s., \) not significant.
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