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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 (ABS_{450}), 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 μM Fe (II) (Potentino honeys) to 786.53 μM 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 (Baltrŭsaityte *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,

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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-Várquez *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 the Mediterranean region and cultivated in other 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).

Legend	
Area	
1	Tarantino
2	Penisola Sorrentina
3	Camastra-Dolomiti Lucane
4	Leccese
5	Basso Pollino
6	Collina Materana
7	Potentino
8	Vulture Melfese

Figure 1 Map of Italy showing the sampling sites of sulla honey.

Table 1 Pollen analysis of sulla honeys

Area	Characterising Pollen	Frequency %	Others pollens
Tarantino	*Hedysarum* spp.	51–56	*Lotus corniculatus*, *Onobrychis viciifolia*, *Rubus* sp., *Trifolium repens*, *Trifolium pratense*, *Cruciferae*
Penisola Sorrentina	*Hedysarum* spp.	54–58	*Citrus* spp., *Lotus corniculatus*, *Rhamnaceae*, *Cruciferae*, *Eucalyptus*
Camastra – Dolomiti Lucane	*Hedysarum* spp.	58–62	*Trifolium pratense*, *Cruciferae*, *Castanea sativa*, *Liliaceae*, *Rubus* sp.
Leccese	*Hedysarum* spp.	55–58	*Trifolium pratense*, *Lotus corniculatus*, *Rubus* sp., *Vicia* sp., *Trifolium repens*, *Cruciferae*
Basso Pollino	*Hedysarum* spp.	57–60	*Trifolium repens*, *Trifolium pratense*, *Castanea sativa*, *Onobrychis viciifolia*, *Rubus* sp.
Collina Materana	*Hedysarum* spp.	55–58	*Trifolium pratense*, *Citrus* spp., *Eucalyptus*, *Rubus* sp.; *Cruciferae*
Potentino	*Hedysarum* spp.	58–62	*Castanea sativa*, *Lotus corniculatus*, *Cruciferae*, *Rubus* sp., *Trifolium pratense*
Vulture Melfese	*Hedysarum* spp.	56–60	*Castanea sativa*, *Rubus* sp.; *Trifolium repens, Trifolium pratense Eucalyptus, Echium vulgare*

Table 2 Pedological, altimetric, climatic and economic characteristics of the considered areas

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

Density: high >600 inhabitants km^{-2}; medium: between 300 and 600 inhabitants km^{-2}; low <300 inhabitants km^{-2}.

Antioxidant activity

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: μ = average mean; α_i = effect of geographical origin (1,. . .,8); ε_{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

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 a our previous work (Perna *et al*., 2012), a positive correlation between heavy metal and total phenol contents was reported.

High-performance liquid chromatographic analysis of honey phenolic compounds

A high-performance liquid chromatographic method with UV-VIS detector had been developed for simultaneous quantification of gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), benzoic acid (peak 4), *p*-coumaric acid (peak 5) and ferulic acid (peak 6) (Fig. 2). In general, these phenol acids were detected in all analysed honey samples. The concentrations of individual phenolic compounds are given in Figs 3 and 4. The observation was that there are high differences among quantities of phenolic compounds in the samples originating from different geographical areas and not among the profiles of these compounds. Overall, the order of mean contents of phenol acids was as follows: gallic acid (40.01 mg kg^{-1} honey) > caffeic acid (6.81 mg kg^{-1} honey) > ferulic acid (3.94 mg kg^{-1} honey) > chlorogenic acid (3.25 mg kg^{-1} honey) > *p*-coumaric acid (2.87 mg kg^{-1} honey) > benzoic acid (0.78 mg kg^{-1} honey) (Fig. 3). The results clearly showed that the dominating compound is gallic acid, representing 69.4% from total amount. Our results agree with those reported by Yaoa *et al*. (2005) who found that gallic acid was the most abundant phenolic compound identified in Australian honeys and could be used as its floral marker. Aljadi and Yusoff (2003) and

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

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

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 ($P < 0.05$).

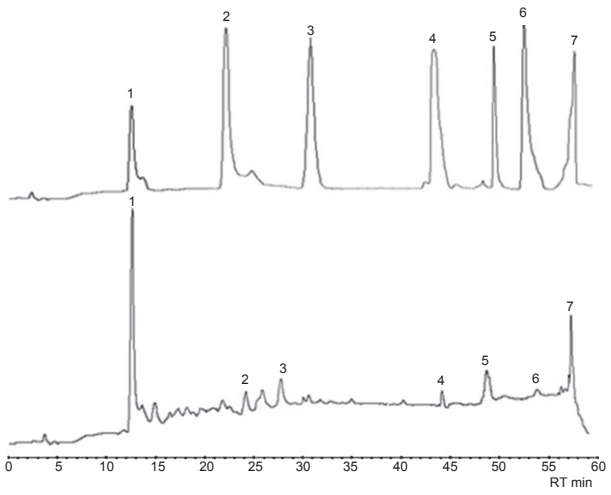

Figure 2 High-performance liquid chromatographic chromatograms (detected at 280 nm) of sulla honey and standard mixture of phenolic acids. Peaks: 1, gallic acid; 2, chlorogenic acid; 3, caffeic acid; 4, benzoic acid; 5, *p*-coumaric acid; 6, ferulic acid; 7, internal standard.

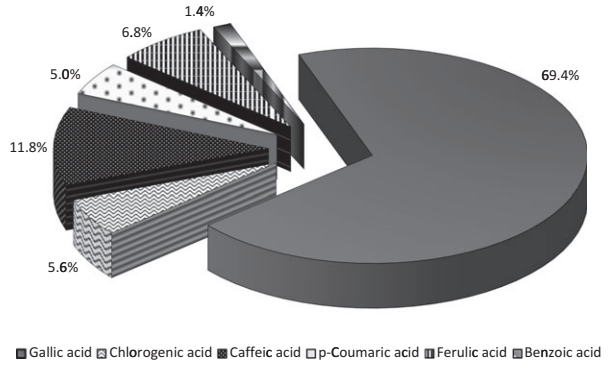

Figure 3 Percentage amount of each phenolic acid based on total.

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-Barberán *et al.* (2001), in European honeys, found the lower level of *p*-coumaric acid, while Baltrŭsaityte *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.

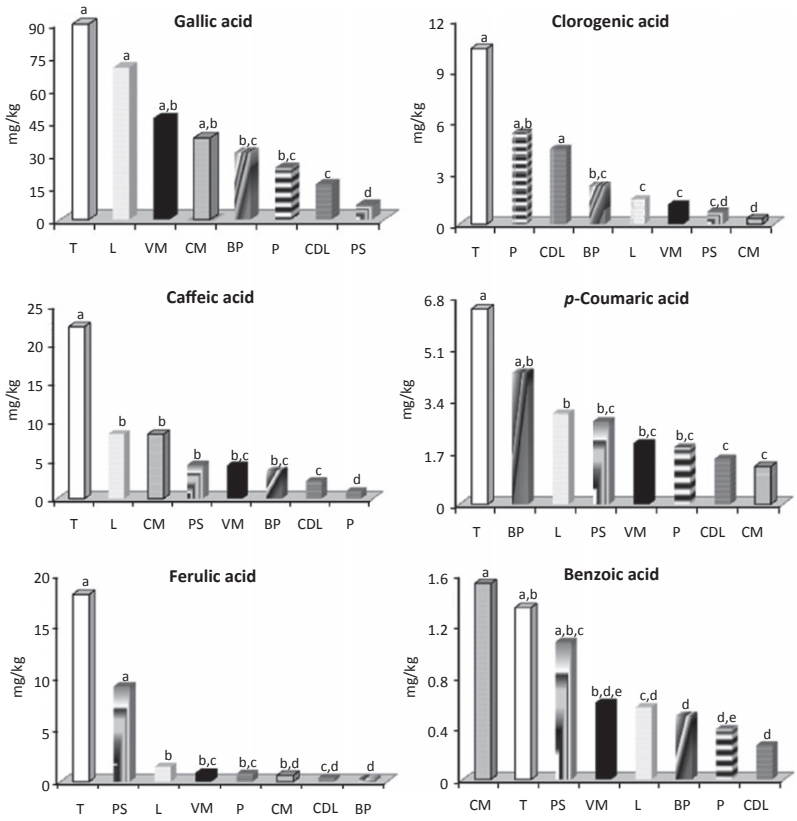

Figure 4 Phenolic profiles (mg kg^{-1} honey) in sulla honey from different geographical areas. [a,b,c,d,e]Significant statistical differences according to Student's *t*-test ($P < 0.05$). T, Tarantino; PS, Penisola Sorrentina; CDL, Camastra-Dolomiti Lucane; L, Leccese; BP, Basso Pollino; CM, Collina Materana; P, Potentino; VM, Vulture Melfese.

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 Pollimo 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 ABS_{450} 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 ABS_{450} parameter of studied honeys from the different areas presented the highest value in Tarantino honeys (1.82 AU; $P < 0.05$), followed by Penisola

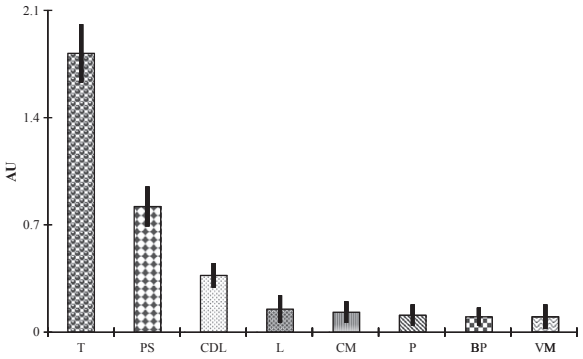

Figure 5 Colour intensity of sulla honeys from different geographical origin. [a,b,c,d]Significant statistical differences according to Student's *t*-test ($P < 0.05$)

Sorrentina and Camastra-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 Bertoncelj *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: 0.918 for phenolic content measured by DPPH assay and 0.885 for phenolic content measured by FRAP assay. Similar to our findings, Khalil *et al.* (2011) reported strong correlation between the antioxidant capacity, according to DPPH assay, and total phenolic content ($r = 0.83$). The linear correlation between the total phenolic content and DPPH assay suggested that phenolic compounds were the strongest contributing factor to the radical-scavenging activity of these honeys, compared with FRAP assay. The correlation values between ABS_{450} and FRAP and ABS_{450} and DPPH were 0.98 and 0.77, respectively ($P < 0.001$). Thus, the colour pigments may have a role in the antioxidant activity of the honey. Almost similar values were obtained between ABS_{450} and FRAP values ($r = 0.96$) for Algerian honeys (Khalil *et al.*, 2012). Khalil *et al.* (2011) found that correlation between DPPH value and ABS_{450} for Malaysian honeys was 0.85, while Khalil *et al.* (2012) found it stronger ($r = 0.96$) for Algerian honeys. However, the correlation between ABS_{450} and total phenolic content ($r = 0.53$) was lower even if statistically significant ($P < 0.001$). Bertoncelj *et al.* (2007) 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.

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

	Phenolic content	DPPH	FRAP	ABS_{450}
DPPH	0.82***			
FRAP	0.54***	0.75***		
ABS_{450}	0.53***	0.77***	0.98***	
gallic acid	$-0.38^{n.s.}$	$-0.058^{n.s.}$	$0.32^{n.s.}$	$0.35^{n.s.}$
chlorogenic acid	$0.0036^{n.s.}$	$0.27^{n.s.}$	0.51*	0.59***
caffeic acid	$0.0219^{n.s.}$	$0.32^{n.s.}$	0.70***	0.73***
p-coumaric acid	$0.073^{n.s.}$	$0.11^{n.s.}$	0.52***	0.53***
ferulic acid	$0.31^{n.s.}$	0.45***	0.68***	0.68***
benzoic acid	$0.062^{n.s.}$	$0.11^{n.s.}$	$0.29^{n.s.}$	$0.28^{n.s.}$

***$P < 0.001$; *$P < 0.05$; n.s., not significant.

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.

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