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Antioxidant Properties, Polyphenol Content and Colorimetric Characteristics of Different Floral Origin Honeys from Different Areas of Southern Italy

Annamaria Perna, Amalia Simonetti, Immacolata Intaglietta and Emilio Gambacorta

School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Potenza, Viale dell'Ateneo Lucano 10 –85100, Italy

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Abstract: Ninety honey samples of five different floral origins (chestnut, eucalyptus, citrus, multifloral and sulla) from nine areas of southern Italy, were screened to evaluate the polyphenol content by spectrphotometric methods, the antioxidant activity by ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) diamonium salt, FRAP (ferric reducing antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays, and the colorimetric characteristics by spectrophotometric and CIE (Commission Internationale de l'Eclairage) L*a*b* methods. Moreover, the correlations between the analysed parameters were studied. Overall, the results showed that all the honey samples presented high total phenolic and flavonoid contents (12.23 mg gallic acid equivalent/100g honey and 8.16 mg quercetin equivalent/100g honey, respectively), and a high antioxidant activity (59.17%, 66.50% and 349.11 μM Fe (II) for ABTS, DPPH and FRAP assays, respectively), but those results differ widely according to the honey types, suggesting that honey composition has been influenced by its floral and geographical origin. In particular, the darkest honeys, such as chestnut honey (L* = 59.94; 1.26 AU), showed the highest polyphenol content and the highest antioxidant activity. Honeys from the areas with major anthropogenic activities and high population density presented the highest antioxidant activity. Correlations between the analysed parameters were statistically significant ($P < 0.05$), suggesting that the polyphenol content is correlated with the antioxidant activity and colour of honey.

Key words: Honey, total phenolic, total flavonoid, ABTS, DPPH, FRAP, colorimetric characteristics.

1. Introduction

Honey is considered to be an excellent natural product which presents a complex composition consisting of water, a high concentration of sugars, minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids and enzymes. Honey is one of the products for which the link between the area of origin and the qualitative characteristics is extremely strong and its double vegetable and animal nature is at the basis of its peculiarities and variability. Honey production in southern Italy in 2009 was around 2600 t/year, which means about 22.8% of the total national production [1]. Southern Italy honeys represent a wide and diversified typology, more than 30 unifloral honeys together with plenty of multifloral ones, a result of the production obtained in different areas having different climatic characteristics and a high variability of the botanical species. Honey composition depends on several factors, such as floral and geographical origin [2-4]. Honey has excellent antioxidant properties useful in reducing the risk of cardiovascular diseases, cancer, cataract and several inflammatory processes [5]. Several authors have associated these properties to phenolic acids and flavonoids content [6-8]. These components are products of secondary plant metabolism and are

Corrisponding author: Annamaria Perna, full researcher, research fields: physical-chemical analysis of the animal origin products. E-mail: anna.perna@unibas.it.

characteristic for each vegetable species. They are characterized by an aromatic ring with one or more hydroxyl substituents and, in many cases, serve in plant defense mechanisms to counteract reactive oxigen species [9-12]. Polyphenols may act in various processes, such as free radical-scavenging, hydrogen donation, single oxygen quenching, metal ion chelation, substrate for superoxide and hydroxyl radicals [13]. The polyphenol content in the plant and, therefore in honey, depends on genetic, environmental and technological factors [14], and it is also correlated with honey colour [15, 16]. This latter ranges from very pale yellow to shaded black [17], and it depends on several factors, such as pollen type, minerals and polyphenols content [18]. Many authors reported that the dark honeys have higher polyphenol content, and, consequently, a higher antioxidant activity [6, 7]. The aim of our study was to evaluate the total phenolic and flavonoid contents, the colorimetric characteristics and the antioxidant activity of different floral origin honeys from different areas of southern Italy.

2. Materials and Methods

2.1 Chemicals

All used chemicals and solvents were of analytical grade. DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-s-triazine), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) diamonium salt, gallic acid, quercetin, HCl, $FeSO_4 \cdot 7H_2O$, $FeCl_3$, NaOH, $AlCl_3$, $K_2S_2O_8$, were purchased from Sigma-Aldrich (Milan, Italy). Folin-Ciocalteu's reagent was purchased from Carlo Erba (Milan, Italy).

2.2 Samples

Ninety honey samples were collected directly from beekeepers during the 2009 harvest in nine geographical areas in Southern Italy. The honey purity was carefully checked by pollen analysis carried out according to DIN 10760 [19, 20]. Honey samples were classified into five categories: chestnut (predominant pollen (PP): *Castanea sativa*; frequency (F): 75%-90%), sulla (PP: *Hedysarum* spp.; F > 50%), citrus (PP: *Citrus* spp.; F: 65%-76%), eucalyptus (PP: *Eucalyptus* spp.; F: 82%-93%) and multifloral honey. Multifloral honey resulted to be composed of a mixture of pollen, these come from different plant species that have synchronized flowering. The characteristics of the considered areas and the details of honey samples used in this experiment are reported in Table 1. Honey samples were stored in the dark at 4 °C until the analysis. The experiments were performed by using freshly prepared 10% honey

Table 1 Characteristics of the geographical areas and presence of honey samples for each considered area.

Geographical area	m above sea level	Pedological characteristics	Density (Inhabitants/km^2)	Presence of industry to high environmental impact	Agricultural activity	Chestnut	Eucalyptus	Multifloral	Citrus	Sulla
Tarantino	130-480	Sandy-clayey soil	Medium	High	Semi-intensive	x	x	x	x	x
Penisola Sorrentina	0-600	Marble-clayey soil	High	Low	Intensive	x	x	x	x	x
Camastra-Dolomiti Lucane	700-1100	Clayey soil	Low	Low	Extensive	x		x		x
Leccese	57	Chalky soil	Medium	Low	Intensive		x	x	x	x
Basso Pollino	200-1000	Chalky soil	Low	Low	Intensive	x	x	x	x	x
Collina Materana	20-770	Silty-clayey soil	Low	Low	Intensive	x	x	x	x	x
Potentino	400-1100	Clayey soil	Medium	High	Extensive	x		x		x
Vulture-Melfese	350-730	Volcanic soil	Low	High	Intensive	x	x	x		x
Cilento	450-650	Chalky soil	Low	Low	Intensive	x	x	x	x	x

Density: high > 600 Inhabitants/km^2; medium: between 300 Inhabitants/km^2 to 600 Inhabitants/km^2; low < 300 Inhabitants/km^2.

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 [21]. All tests were performed in triplicate.

2.3 Determination of Total Phenolic and Flavonoid Contents

The total phenolic content of honeys was estimated according to the Folin-Ciocalteau method as modified by Beretta et al. [7]. Gallic acid (0-200 mg/L) was used as standard to derive the calibration curve and the results were expressed as mg of gallic acid equivalent (GAE)/100g honey. Total flavonoid content was determined by using the Dowd method as adapted by Arvouet-Grand et al. [22]. Quercetin (0-200 mg/L) was used as standard to derive the calibration curve and the results were expressed as mg of quercetin equivalent (QE)/100 g honey.

2.4 ABTS Assay

The antioxidant activity of honey samples in the reaction with the ABTS cation radical was determined according to Re et al. [23] method with a slight modification. $ABTS^{\bullet+}$ was produced by reacting 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS) with potassium persulfate ($K_2S_2O_8$). Stock solution (50 mL) of ABTS (2 mM) was prepared in phosphate-buffered saline (PBS), pH 7.4. $ABTS^{\bullet+}$ was produced by reacting 50 mL of stock solution with 200 μL of 70 mM $K_2S_2O_8$. The mixture was left standing in the dark at room temperature for 12-16 h before use. For the evaluation of antioxidant activity, the $ABTS^{\bullet+}$ solution was diluted with PBS to obtain an absorbance of 0.700 ± 0.020 at 734 nm. Honey solutions (100 μL) were mixed with 2 mL of $ABTS^{\bullet+}$ solution in a cuvette, and the decrease in the absorbance was measured after 10 min. The blank reagent was prepared by adding artificial honey instead of the sample. The percentage decrease in the absorbance at 734 nm was calculated by the formula:

$$I = [(A_B - A_A)/A_B] \times 100 \quad (1)$$

where, I is the $ABTS^{\bullet+}$ inhibition, %; A_B is the absorbancy of a blank sample (t = 0 min); A_A is the absorbancy of a tested honey solution at the end of the reaction (t = 10 min).

2.5 DPPH Assay

DPPH radical scavenging method of honeys was determined according to the procedure described by Beretta et al. [7], with some modifications. Honey samples were dissolved in distilled water at a concentration of 30-600 mg/mL and 100 μL of each solution was mixed with 1.9 mL of 130 μM $DPPH^{\bullet}$ dissolved in methanol and 1 mL of 0.1 M acetate buffer (pH 5.5). The mixture was shaken on a vortex, then incubated for 60 min at 37 °C in a water bath in the dark. The absorbance of the remaining $DPPH^{\bullet}$ was determined at 517 nm against a blank. The blank was honey at the same concentration as described earlier containing all reagents except $DPPH^{\bullet}$. The radical scavenging activity was expressed as a percent of inhibition of DPPH radical and calculated from the same equation as for ABTS.

2.6 FRAP Assay

The procedure described by Bertoncelj et al. [24] was used with some modifications. The FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer (pH 3.6), 1 volume of 10 mM TPTZ in 40 mM HCl and 1 volume of 20 mM $FeCl_3$. It was prepared daily and warmed to 37 °C before use. Honey samples (5 g) were diluted with 50 mL of 0.25 M phosphate buffer (pH 7.2) and aliquots of 200 μL of honeys samples solution were mixed with 1.8 mL of FRAP reagent. The absorbance of each mixture was measured at 593 nm against a blank after incubation at 37 °C for 15 min. Aqueous standard solutions of $FeSO_4$*$7H_2O$ (100-1000 μM) were used for the

calibration curve, and the results were expressed as the FRAP value (μM Fe(II)) of the honey solution.

2.7 Colour Analysis

Colour characteristics were assessed by the CIE L*a*b* method where lightness L* and two colour coordinates, a* and b*, were defined by means of a MINOLTA CM 2002 spectrophotometer. Honey samples were heated to 50 °C to dissolve sugar crystal and then placed in a plastic container 7 cm in diameter and covered with a plastic plate. The measured layer was 1 cm thick. L*, a* and b* parameters were measured against a white background and were directly obtained from the apparatus. In addition colour intensity was determined by spectrophotometric measurement as described by Beretta et al. [7]. Briefly, the honey samples were diluted to 50% (w/v) with warm water (45-50 °C), sonicated for 5 min and filtered to eliminate large particles, and the net absorbance was defined as the difference between the absorbance at 450nm and at 720 nm. The results were expressed as AU.

2.8 Statistical Analysis

Statistical analysis was performed by using the general linear model (GLM) procedure of statistical analysis system [25], by using a two-factorial model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha*\beta)_{ij} + \varepsilon_{ijk} \quad (2)$$

where, μ is the average value; α_i is the effect of botanical origin (1,…,5) ; β_j is the effect of geographical origin (1,….,9); $(\alpha*\beta)_{ij}$ is the interaction of I order, i (1,..,5); j (1, 2,…,9); ε_{ijk} is the 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.

3. Results and Discussion

3.1 Total Phenolic and Flavonoid Contents

Total phenolic and flavonoid contents of different floral origin honeys are reported in Table 2. The values ranged from 5.49 mg QE/100g honey to 14.16 mg QE/100g honey for flavonoid content, and from 10.65 GAE/100g honey to 15.05 mg GAE/100g honey for total phenolic content. The average polyphenol contents are in close agreement

Table 2 Total phenolic and flavonoid contents, ABTS, FRAP and DPPH values and colorimetric characteristics of honey samples from different floral origin.

Parameter	Type of honey					
	Chestnut	Eucalyptus	Multifloral	Citrus	Sulla	Total
	(n = 18)	(n = 18)	(n = 18)	(n = 18)	(n = 18)	(n = 90)
Phenolic content (mg GAE/100g honey)	15.05 ± 4.14^a	11.51 ± 3.05^b	11.79 ± 3.34^b	12.15 ± 3.48^b	10.65 ± 6.23^b	12.23 ± 4.35
Flavonoid content (mg QE/100g honey)	14.16 ± 7.53^a	5.98 ± 2.15^b	8.94 ± 3.89^c	5.49 ± 2.77^b	6.22 ± 4.84^b	8.16 ± 5.36
ABTS (*I*%)	60.83 ± 2.28^a	58.62 ± 1.88^b	58.99 ± 1.28^b	59.01 ± 1.43^b	58.42 ± 2.18^b	59.17 ± 1.69
DPPH (*I*%)	78.89 ± 9.87^a	71.93 ± 8.02^b	62.26 ± 8.94^c	54.87 ± 8.31^d	64.55 ± 16.95^{bc}	66.50 ± 10.22
FRAP (μM Fe(II))	894.34 ± 315.32^a	242.32 ±121.07^b	208.97 ± 114.27^{bc}	159.74 ± 111.34^c	240.07 ± 240.29^{bc}	349.11 ± 184.26
L*	59.94 ± 8.26^a	68.52 ± 6.39^b	66.59 ± 6.19^{bc}	67.00 ± 7.59^b	61.88 ± 17.71^{ac}	64.79 ± 10.49
a*	16.52 ± 2.79^a	15.68 ± 2.09^{ab}	14.63 ± 1.83^{bc}	14.01 ± 2.07^{cd}	12.89 ± 2.69^d	14.75 ± 2.60
b*	38.16 ± 8.94^{a,c}	45.30 ± 12.12^b	42.67 ± 11.53^{a,b}	40.83 ± 13.22^{abc}	35.61 ± 13.49^c	40.52 ± 12.18
ABS_{450} (AU)	1.26 ± 0.43^a	0.47 ± 0.27^b	0.40 ± 0.28^{bc}	0.31 ± 0.23^c	0.41 ± 0.57^{bc}	0.57 ± 0.51

Mean values from three repetition ± standard deviations.
Means in the same row with different letters are significantly different according to the Student's t-test (P < 0.05).

with the results found in Italian honeys by other authors [26, 27], while Socha et al. [28], in Polish honeys, reported lower values. The polyphenol content of honey samples decreased in the order: chestnut > multifloral (8.94 mg QE/100g honey) > sulla (6.22 mg QE/100g honey) > eucalyptus (5.98 mg QE/100g honey) > citrus for total flavonoid content, and in the order: chestnut > citrus (12.15 mg GAE/100g honey) > multifloral (11.79 mg GAE/100g honey) > eucalyptus (11.51 mg GAE/100g honey) > sulla for total phenolic content. Total phenolic and flavonoid content was highest in chestnut honey (15.05 mg GAE/100g honey and 14.16 mg QE/100g honey, respectively; $P < 0.05$). The lowest content in total phenolic was found in sulla honey (10.65 mg GAE/100g honey), while citrus honey showed the lowest content in total flavonoid (5.49 mg QE/100g honey). The values found in studied samples match the results reported by other authors for chestnut and sulla honey [7, 24, 26, 27], while Pichichero et al. [26], in orange honey, reported higher values. The polyphenol content of the honeys from 9 considered areas is reported in Table 3. The considered areas present different pedological characteristics, density and agricultural activities, furthermore they are characterized by a high presence of wild flowers and botanical species at all the altitudes. In particular, Tarantino, Potentino and Vulture-Melfese areas are also characterized by the presence of factories that have a high environmental impact, while Penisola Sorrentina area is characterized by a high population density. Total phenolic content of the honeys from the considered areas ranged from 9.19 mg GAE/100g honey (Vulture-Melfese honeys) to 16.14 mg GAE/100g honey (Penisola Sorrentina honeys; $P < 0.05$). Cilento and Tarantino honeys showed the highest total flavonoid content (14.24 mg QE/100g and 11.65 mg QE/100g, respectively; $P < 0.05$), while the lowest value was found in Camastra-Dolomiti Lucane honeys (4.27 mg QE/100g honey). The significant variation among the analysed honey samples was likely due to the different floral and geographical origin [4, 7, 16, 24]. In fact, different plants contain different phenolic compounds and each plant has its own characteristic phenolic pattern [29]. Polyphenols, finally, represent the evolutionary response to plants adaptation to different environmental characteristics [4, 15].

3.2 Antioxidant Activity

The antioxidant activity of phenolic compounds lies

Table 3 Total phenolic and flavonoid contents, ABTS, FRAP and DPPH values of honeys from different areas of southern Italy.

Area	Parameter				
	Phenolic content (mg GAE/100g honey)	Flavonoid content (mg QE/100g honey)	ABTS (I%)	DPPH (I%)	FRAP value (μM Fe(II))
Tarantino	9.94 ± 3.19^{ad}	11.65 ± 7.11^{ae}	59.65 ± 2.22^{acd}	73.03 ± 14.96^{a}	695.64 ± 428.31^{a}
Penisola Sorrentina	16.14 ± 5.94^{b}	5.27 ± 3.42^{bc}	58.83 ± 1.35^{abc}	$69.32 \pm 20.63^{,cd}$	426.69 ± 157.37^{b}
Camastra-Dolomiti Lucane	12.98 ± 3.39^{c}	4.27 ± 2.30^{b}	58.48 ± 1.61^{ab}	71.27 ± 11.85^{ac}	216.57 ± 81.73^{c}
Leccese	11.19 ± 4.50^{acd}	8.77 ± 6.47^{ac}	58.18 ± 1.28^{b}	60.03 ± 6.64^{b}	279.14 ± 300.46^{cd}
Basso Pollino	13.57 ± 3.73^{bc}	8.19 ± 7.35^{cd}	58.55 ± 1.77^{ab}	64.10 ± 19.31^{cb}	323.93 ± 351.57^{de}
Collina Materana	12.62 ± 3.75^{ac}	5.47 ± 5.53^{bd}	58.77 ± 2.61^{cd}	62.39 ± 11.74^{bd}	271.73 ± 308.07^{cd}
Potentino	9.74 ± 4.61^{d}	7.82 ± 3.06^{cd}	58.03 ± 1.44^{b}	$63.10 \pm 9.46^{b,d}$	267.15 ± 330.77^{cd}
Vulture-Melfese	9.19 ± 3.36^{d}	$5.65 \pm 3.57^{bc,}$	60.15 ± 1.80^{d}	68.45 ± 11.42^{acd}	375.06 ± 404.81^{be}
Cilento	13.15 ± 5.02^{c}	14.24 ± 6.40^{e}	59.55 ± 1.59^{ad}	65.65 ± 9.30^{ab}	267.19 ± 342.90^{cd}

Mean values from three repetition ± standard deviations.

Means in the same row with column letters are significantly different according to the Student's t-test ($P < 0.05$).

mainly in their chemical structure and is manifested by different mechanisms [30]. Several methods are used to evaluate antioxidant activity of a substance. However, in the case of honey, there is not an officially-accepted method. As shown by Beretta et al. [7], to fully evaluate the antioxidant capacity of honey it is necessary to use different types of assays. In this study, three different methods were used: the FRAP assay, which uses reductants in a redox-linked colorimetric method employing an easily reduced oxidant in stoichiometric excess; the DPPH assay, used to detect the ability of water-soluble compounds to act as free radicals scavengers; and the ABTS assay, which highlights the activity of both hydrophilic and lipophilic antioxidants. Overall, the results showed that all the tested samples exhibited antioxidant activity (Tables 2, 3). The average values were 59.17% and 66.50 % for ABTS and DPPH assays, respectively, and 349.11 μM Fe (II) for FRAP assay. These results were higher than those reported by other authors [24, 31], suggesting a higher nutraceutical quality of the studied honeys. In honeys with different floral origin (Table 2), the values ranged from 60.83% (chestnut honey) to 58.42% (sulla honey) in the ABTS assay, from 78.89% (chestnut honey) to 54.87% (citrus honey) in the DPPH assay and from 894.34 μM Fe(II) (chestnut honey) to 159.74 μM Fe(II) (citrus honey) in the FRAP assay. Chestnut honey showed the highest antioxidant activity, in all the assays ($P < 0.05$). These results were similar to those found by Perna et al. [30], while FRAP value was higher than that reported by other author [7, 24]. The antioxidant activity showed a high and significant ($P < 0.05$) variability among the honeys from the considered areas (Table 3). Tarantino, Camastra-Dolomiti Lucane, and Penisola Sorrentina honeys showed an antioxidant activity, measured by DPPH assay, higher than that found in the honeys from the other considered areas (I% = 73.03%, 71.27% and 69.32%, 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 (695.64 and 426.69 μM Fe (II), respectively; $P < 0.05$). ABTS values showed a low variability among the honeys of the different considered areas.

3.3 Colorimetric Characteristics

The honey colour is a physicochemical parameter which plays a crucial role in choosing the product. The colorimetric characteristics of different floral origin honeys are reported in Table 2. The results, measured by CIE L*a*b* method, showed a high and consistent ($P < 0.05$) variability among the different types of honey. In particular, chestnut and sulla honeys were the darkest, with very similar L* values (59.94 and 61.88, respectively). A statistically remarkable difference existed between these two samples and the other studied honeys ($P < 0.05$). The L* value increased further in multifloral (66.59), citrus (67.00), and eucalyptus (68.52) honeys. These values were higher than those reported by other authors [18, 24]. The a* (red-green) and b* (yellow-blue) parameters of the honey may be interpreted as a reliable index of the richness in pigments such as carotenoids, xanthophylls and anthocyans of the species of origin [2, 15, 32]. These values varied from 12.89 (sulla honey) to 16.52 (chestnut honey) for a* parameter, and from 35.61 (sulla honey) to 45.30 (eucalyptus honey) for b* parameter. The b*, except for chestnut and multifloral honeys, and a* values were in a similar range as those reported by other authors [18, 24]. The colour intensity of a 50% honey solution (w/v) is reported in Table 2. Net absorbance varied between 0.31 AU (citrus honey) and 1.26 AU (chestnut honey; $P < 0.05$). This variability could be due to different pigments concentration, as well as flavonoids, that have absorption maxima at 450 nm [33, 34]. Moreover, the contamination during processing and stocking of honey, and the biochemism during

ripening can influence the honey colour [7]. Net absorbances of different honey samples resulted higher than those reported by other authors [7, 34, 35]. The colorimetric characteristics were also influenced by origin area (Table 4). The brightest were Basso Pollino honeys (L* = 68.98), while Camastra-Dolomiti Lucane honeys showed the lowest average value of L* (60.53; $P < 0.05$). The parameters a* and b* varied from 13.34 (Camastra-Dolomiti Lucane honeys) to 17.16 (Penisola Sorrentina honeys; $P < 0.05$), and from 35.06 (Potentino honeys) to 53.54 (Camastra-Dolomiti Lucane honeys; $P < 0.05$), respectively. The ABS_{450} parameter of studied honeys from the different areas presented the highest value in Tarantino honeys (1.23 AU; $P < 0.05$), followed by Penisola Sorrentina honeys (0.81 AU; $P < 0.05$), while the other honeys do not show any significant differences (Table 4). The wide range of observed honey colours be due to (a) a different presence of pigments with antioxidant activity [36]; (b) a different concentration of Maillard reaction products [32]; (c) a different minerals concentration that is related to the production area [18].

Table 4 Colorimetric characteristics of honeys from different areas of southern Italy.

Area	Parameter			
	L*	a*	b*	ABS_{450} (AU)
Tarantino	64.37 ± 14.24^{ab}	15.75 ± 2.52^{ac}	37.88 ± 12.97^{acd}	1.23 ± 0.54^{a}
Penisola Sorrentina	63.57 ± 10.34^{ab}	17.16 ± 3.35^{a}	42.14 ± 13.47^{ad}	0.81 ± 0.25^{b}
Camastra-Dolomiti Lucane	60.53 ± 19.53^{a}	13.34 ± 2.85^{b}	53.54 ± 23.22^{b}	0.48 ± 0.14^{c}
Leccese	67.54 ± 7.36^{bc}	15.46 ± 1.93^{c}	42.09 ± 7.79^{ad}	0.52 ± 0.63^{c}
Basso Pollino	68.98 ± 6.36^{b}	14.71 ± 1.54^{cb}	40.15 ± 7.80^{acd}	0.37 ± 0.36^{c}
Collina Materana	61.71 ± 9.77^{ac}	13.43 ± 2.27^{b}	35.81 ± 10.40^{ac}	0.48 ± 0.48^{c}
Potentino	67.61 ± 7.29^{bc}	13.55 ± 2.07^{b}	35.06 ± 4.97^{c}	0.37 ± 0.47^{c}
Vulture-Melfese	65.26 ± 6.57^{ab}	15.70 ± 2.35^{ac}	42.34 ± 4.50^{d}	0.40 ± 0.39^{c}
Cilento	63.53 ± 6.21^{ab}	13.62 ± 1.98^{b}	35.63 ± 3.83^{c}	0.49 ± 0.61^{c}

Mean values from three repetition ± standard deviations.
Means in the same row with column letters are significantly different according to the Student's t-test ($P < 0.05$).

Table 5 Correlation matrix (Pearson correlation coefficients) among the considered parameters.

	Phenolic content	Flavonoid content	ABTS	DPPH	FRAP
Flavonoid content	0.37^{**}				
ABTS	0.58^{***}	0.72^{***}			
DPPH	0.53^{***}	0.45^{***}	0.71^{***}		
FRAP	0.42^{***}	0.76^{***}	0.64^{***}	0.73^{***}	
ABS_{450}	0.49^{***}	0.66^{***}	0.58^{***}	0.63^{***}	0.91^{***}
L*	-0.12^{ns}	0.01^{ns}	-0.28^{**}	-0.15^{ns}	-0.18^{ns}
a*	0.39^{***}	0.22^{*}	0.17^{ns}	0.39^{***}	0.45^{***}
b*	0.06^{ns}	-0.09^{ns}	-0.22^{*}	0.04^{ns}	-0.07^{ns}

$^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ns not significant.

3.4 Correlations

In this study, the correlation between the considered parameters were analysed (Table 5). A positive and statistically significant correlation ($P < 0.01$) was found between total phenolic and flavonoid contents ($r = 0.36$). This result is similar to that reported by other authors [27, 37], while Alvarez-Suarez et al. [38], showed a higher correlation coefficient. The low correlation coefficient was likely caused by the methods used to determine total phenolic and flavonoids [39]. A positive correlation was found between total flavonoid content and ABS_{450} value ($r = 0.66$; $P < 0.001$), while a lower correlation was found

between total phenolic content and ABS_{450} value (r = 0.49; P < 0.001), suggesting a greater influence of flavonoids on the color intensity. The obtained data showed a positive and significant correlation (P < 0.001) between ABS_{450} value and antioxidant assays, with $r > 0.58$. In particular, the highest correlation was observed between ABS_{450} and FRAP values (r = 0.91), indicating that honey color intensity may be treated as an indicator of its antioxidant capacity. These results are in agreement with those reported by other authors [2, 7, 40, 41]. The correlation coefficients between polyphenol content and L* value were negative and not significant, suggesting that lighter honeys have higher L* values because of their smaller content of polyphenols. As expected, negative correlations were found between L* parameter and FRAP, DPPH, ABTS values (r = -0.18, -0.15 and -0.28, respectively). A positive and statistically significant correlation was found between the antioxidant assays, confirming what reported by other authors [7, 24, 42]. Moreover, the positive and statistically significant correlations were found between flavonoid content and FRAP, DPPH, ABTS values (r = 0.76, 0.45 and 0.71, respectively), indicating that the reducing power of honey may be due to flavonoid content because of their ability to reduce Fe^{3+} to Fe^{2+}. Finally, the positive correlations found between total phenolic content and antioxidant assays confirm the relationship between polyphenol concentrations and relative antioxidant potential of the honey [7, 24].

4. Conclusions

Honey is affected by the peculiarities of its production area. Therefore its nutritional and nutraceutical characteristics are mainly due to the type of flora looted by bees which varies from one area to the other. To our knowledge, no studies have reported on the polyphenol content and antioxidant activity of different floral honeys from different areas of Southern Italy. Our study demonstrated that floral and geographical origin markedly influenced the total phenolic and flavonoid contents, which are closely related to the antioxidant activity of honey. As a consequence the identification of the botanical and geographical origin of honey could be an useful instrument for product differentiation in order to guarantee a better qualitative characterization and the traceability of the product itself.

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