IJFANS
International Journal of Food And Nutritional Sciences

olume 3, Issue 6, Oct-Dec 2014, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.3, Iss.6, Oct-Dec 2014

Research Paper *Open Access*

EFFECT OF GEOGRAPHICAL PROVENANCE ON THE PHYSICO-CHEMICAL, ANTIOXIDANT CHARACTERISTICS AND SENSORY EVALUATION OF CHESTNUT HONEYS

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Received on: 12th October, 2014 **Accepted on: 20th December, 2014**

ABSTRACT

In this study we investigated the effect of geographical provenance on the physico-chemical properties, total phenols (Folin-Ciocalteau method), total flavonoids (aluminum chloride method), vitamin C content (RP-HPLC method), and antioxidant activity (FRAP and ABTS assays), evaluating also the consumers' acceptability of fifty chestnut honey (*Castanea sativa*) samples from five different geographical areas of the Basilicata region (Southern Italy). A statistically significant effect of geographic origin on the all studied parameters was observed. The results showed that polyphenol content ranged from 11.28 (Camastra-Dolomiti lucane honeys) to 15.33 mg gallic acid equivalent per 100 g honey (Vulture Melfese honeys), and from 5.38 (Camastra-Dolomiti lucane honeys) to 19.94 mg quercetin equivalent per 100 g honey (Basso Pollino honeys), for total phenolic and flavonoid contents, respectively. The antioxidant activity ranged from 58.86 (Camastra Dolomiti lucane honeys) to 63.59% (Collina Materana honeys) in the ABTS assay, and from 281.04 (Camastra Dolomiti lucane honeys) to 1129.16 µM Fe (II) (Vulture Melfese honeys) in the FRAP assay. Honeys from the areas with major anthropogenic activities and high population density presented the highest antioxidant activity. Consumer preference was significantly ($P < 0.05$) affected by geographical origin of chestnut honeys. The results indicate that chemical, physical or biological properties of honey are greatly influenced by the geographic origin.

Key words: Chestnut honey, geographical origin, physic-chemical traits, antioxidant activity, consumers' acceptability

INTRODUCTION

Honey is a natural sweet food, well known for thousands of years for its high nutritive value and healing properties. In southern Italy, especially in Basilicata region, thanks to geographical and climatic conditions that provide a suitable environment for beekeeping, honey production has been well developed, representing an important agricultural activity. Honey contains many different substances, mainly sugars such as fructose, glucose and sucrose (65-75% of total soluble solids) in addition to various organic and inorganic acids, enzymes, vitamins, hormones, flavonoids, proteins, amino acids and elements. Honey could be considered as a product 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. In fact, honeys of the same floral source can show different physicochemical, antioxidant and sensory characteristics that are due to a different geographical origin (Anklam, 1998; Castro-Várquez *et al*., 2010). Honey can be understood as a "terroir product" whose characteristics depend by the environmental factors such as, climate, soil, landscape, vegetation, etc. and by the interactions between them. Chestnut honey is considered both one of the most delicious and a high quality product. Because of its properties and for good flavor, chestnut honey is one of the most important in the range of honeys produced domestically, although there are many variations that depend on the place of origin because of the climate, soil type and then the variety of chestnut present. Chestnut honey is one of the major products of Italy, where the chestnut is found in abundance, in particular in Basilicata region. Basilicata region is a small country mostly mountainous, being covered by the Lucan-Apennine chain. Highlights include the Lucanian dolimites, also it has two short coastlines, one on the Tyrrhenian Sea and another on the Ionian Sea. Basilicata region is the eleventh place for

the production of honey in Italy. Chestnut honey has a strong aromatic taste and a slightly bitter after taste. Rich in pollen, mineral salts and tannin, with a high proportion of fructose that resists crystallization and a relatively low acidity. Dark in color, ranging from yellowish brown to almost black, sometimes with amber hues. Chestnut honey, both for the dark color which it presents and for the strong aroma and bitter, it generally appreciated by those who like a less sweet honey. It can stimulate blood circulation and is recommended in cases of anaemia. The properties of honey are mainly determined by its sensorial, chemical, physical and microbiological characteristics. Many authors have suggested that quality-control methods, in conjunction with multivariate statistical analysis can be employed for the botanical and geographical determination of honey (Al-Mamary *et al*., 2002; Ghedolf *et al*., 2002; Gheldof and Engeseth, 2002). The parameters physico-chemical of honey are important for the certification process that determines honey quality as specified by the EC Directive 2001/110 (EU, 2001). The major criteria of interest are moisture content, electrical conductivity, ash content, reducing and non-reducing sugars, free acidity, diastase activity and hydroxymethylfurfural (HMF) content. The aim of this paper was to study the changes induced by geographical provenance area to the chemical-physical and antioxidant properties and sensory evaluation of chestnut honeys (*Castanea sativa*) from five different geographical areas of the Basilicata region (Southern Italy). Also, to evaluate consumer acceptability a preference test was effectuated.

MATERIALS AND METHODS

CHEMICALS

All used chemicals and solvents were of analytical grade, TPTZ (2,4,6-tripyridyl-s-triazine), ABTS 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt, hydrochloric acid, iron (II) sulphate heptahydrate, ferric chloride, glacial acetic acid, sodium acetate, sodium hydroxide, aluminium chloride, potassium persulfate, L-ascorbic acid, HPLC-grade methanol, potassium phosphate monobasic for HPLC, gallic acid, quercetin, HMF standard (97% purity), phenolphthalein, were purchased from Sigma-Aldrich (Milan, Italy). Folin–Ciocalteu's reagent and all the reagents for the spectrophotometric method were purchased from Carlo Erba (Milan, Italy). kit Phadebas Amylase Test was purchased from Pharmacia Diagnostics (Sweden).

SAMPLES

A total number of 50 chestnut honey samples were collected between June and July from individual beekeepers, members of the Consorzio Regionale di Tutela e Valorizzazione del Miele Lucano, over two years, providing two sets of 25 samples, collected in five geographical areas of Basilicata region (Southern Italy) (5 samples for each area and for each year): Camastra Dolomiti lucane (CDL; n=10); Basso Pollino (BP; n=10); Vulture Melfese (VM; n=10); Potentino (P; n=10); Collina Materana (CM; n=10) (Figure 1). The first set was collected during the 2012 harvest while the second was collected during the 2013 harvest. Sampling sites are large areas with different pedology characteristics, density, and productive activities (Table 1) and they are characterized by the high presence of wild flowers and botanical species in all altitudes. 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 2), the predominant pollen type was *Castanea sativa* (frequency 75-90 %). Honey samples were stored at 4°C in the dark and both the physico-chemical and antioxidant characteristics and sensory evaluation were carried immediately after collection. 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 preparedby 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.

PHYSICOCHEMICAL ANALYSES

The moisture content was determined by an Abbe-type refractometer reading at 20°C, according to the relationship between honey water content and refractive index (IHC, 2002; Bogdanov, 2002).

The soluble solids were determined by an Abbe-type refractometer reading at 20°C (IHC, 2002). The homogenized sample was put in a flask and placed in a water bath at 50°C until all the sugar crystals were dissolved. The resulting solution was cooled to room temperature, stirred and immediately covered evenly on the surface of the refractometer prism and the refractive index was recorded. Each sample was measured three times and the average value taken. After each reading the refractometer prism was cleaned using distilled water. Soluble sugars (% Brix) were obtained from the refractive index of the honey by making reference to the standard table.

The pH of honey samples were performed potentiometricaly a 20 °C using a pH meter (model PHM 92, Radiometer, Copenhagen, Denmark), in a solution containing 10 g of honey in 75 mL of distilled water.

For ash determination, five gram of each honey sample was separately weighed out into a porcelain crucible previously weighed. Organic matter was charred by igniting the sample on a hot plate in the fume cupboard. The crucible were then placed in the in the muffle furnace and maintained at 600°C for 6 h. They were then cooled in a desiccator and weighed immediately (AOAC, 1990).

Honey electrical conductivity was measured at 20°C with a Crison Basic 30 conductimeter. Results were expressed in milliSiemens per centimeter (mS/cm) (AOAC, 1990).

The free, lactonic, and total acidity were determined by the titrimetric method (AOAC, 1990). Ten grams of honey were then dissolved in 75 mL of distilled water, and alcoholic solution of phenolphthalein added. The addition

of 0.05 N NaOH, was stopped at pH 8,50 (free acidity), immediately, a volume of 10 mL of 0.05 N NaOH was added and without delay back titrated with 0.05 M HCl from 10 mL to pH 8.30 (lactonic acid). Total acidity was obtained by adding free plus lactonic acidities. Results were expressed as milliequivalent of acid per kg of honey (meq/kg).

The 5-hydroxymethylfurfural (HMF) determination of honeys was estimated according to the method of White (1979). Five grams of honey were dissolved in 25 mL of water. 0.5 mL of Carrez solution I and 0.5 mL of Carrez II was added to the sample and mixed well. The sample was brought to a final volume of 50 mL with deionized water. The sample was filtered and the first 10 mL of filtrate was discarded. Aliquots of 5 mL were put in two test tubes; to one tube was added 5 mL of distilled water (sample solution); to the second was added 5 mL of sodium bisulphite solution 0.2% (reference solution). The absorbance of the solutions at 284 and 336 nm was determined using a UV-Vis spectrophotometer 1204 (Schimadzu, Tokyo, Japan). The HMF was quantified by using the proposed formula for the method reported by White (1979). Results were expressed as mg per kg of honey.

The diastase activity of honey samples was determined based on the method described in the International Honey Commission (IHC, 2002) by using the kit Phadebas Amylase Test. An insoluble blue dyed cross-linked type of starch is used as the substrate. The absorbance of the solutions was determined using an UV-Vis spectrophotometer 1204 (Schimadzu, Tokyo, Japan) at 620 nm. The diastase activity is calculated as diastase number in Schade scale (DN). DN expresses units of diastase activity (Gothe unit). Diastase activity was obtained by using the following equation:

$DN = 35.2 \times A_{620} - 0.46$

DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS

Total phenolic and flavonoid content of honey samples were determined according Folin-Ciocalteu and Dowd spectrophotometric method, respectively, as described by Perna *et al*. (2012).

ANTIOXIDANT ACTIVITY

Assessment of antioxidant activity was performed by using different in vitro methods: radical scavenging activity (ABTS) and Ferric reducing antioxidant power (FRAP) assays, as reported by Perna *et al*. (2012).

COLOUR ANALYSIS

Colour characteristics and the colour intensity (AU) were determined by the CIE L*a*b* method and spectrophotometric measurement, respectively, as described by Perna *et al*. (2013a).

HPLC-UV ANALYSIS OF VITAMIN C

Sample preparation for HPLC analysis and identification and quantification of Vitamin C was carried out as described by Perna *et al*. (2013b).

SENSORY ANALYSIS

An affective method of preference was used to evaluate consumer acceptability. The test consisted of 410 untrained consumers who had been selected based on their regular consumption of commercial honey as well as their sex and age, attempting to represent the distribution of the population as closely as possible. In particular, were selected 213 females and 197 males between the ages of 21 and 60. The test was conducted on ten days with one session per day carried out between 11:00 and 13:00. Each consumer participated in one session and tasted the five honey samples. Twenty g of each honey sample were presented in random order, at room temperature, to each consumer in 40-mL glass vials sealed with a twist-off cap coded with 3-digit numbers. The design was balanced for order and carry over effects. Consumers were asked to evaluate the samples, visually (appearance, and color) and then organoleptically (odor, and taste), finally expressing a judgment on overall acceptability. The judgments were expressed individually, assigning a numerical value, on a hedonic scale, between 1 (dislike extremely) and 9 (like extremely) (Peryam and Pilgrim, 1957). The consumers were isolated in individual booths to reduce collaboration, and oligomineral water and unsalted crackers were provided for the consumers mouth-rinsing between samples. All assessments were carried out in a sensory laboratory equipped according to UNI-ISO 8589 recommendations (International Organization for Standardization, 1988).

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,…,5); 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. Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

RESULTS AND DISCUSSION

PHYSICOCHEMICAL ANALYSIS

A total number of fifty chestnut honey samples (twenty-five collected during 2012 and twenty-five collected during 2013) were sampled directly from hives located in different areas of Basilicata region (Italy) (Figure 1 and Table 1)**.** These areas differ according to the differences in geographical position, climatic and environmental factors and composition of soil, showing a different climate ecological-vegetation and great biodiversity. These factors have a direct influence on natural selection and diversity of wild-growth honey plants

adapted to the conditions of that territory. The physicochemical parameters of honey samples, grouped regarding origin in five geographical areas of Basilicata region were summarized in Table 3. A statistically significant effect of geographic origin on the physicochemical parameters of chestnut honey from five different areas in one-way ANOVA was confirmed for all analysed parameters ($P < 0.05$). Our results are in agreement with other investigations, which reported that the geographical origin of honey influence the physicochemical parameters (Osman *et al*., 2007; Khalil *et al*., 2011), and on the volatile composition and sensory characteristics (Castro-Vázquez *et al*., 2010).

Table 1: Pedological, altimetric 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.				
CDL	700-1100	Clayey soil	4.0	737.44	Low	Low	Extensive	24.0
BP	200-1000	Chalky soil	12.9	504	Low	Low	Semi-intensive	21.3
CM	20-770	Silty-clayey soil	10.2	500	Low	Low	Semi-intensive	20.0
P	400-1100	Clayey soil	7.6	613	Medium	High	Extensive	15.5
VM	350-730	Volcanic soil	9.4	800-1000	Low	High	Intensive	18.7

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

Figure 1: Map of Italy showing the sampling sites of Chestnut honey

HMF is the most commonly parameter used to determine the honey freshness (Oddo *et al.*, 1999; Bogdanov and Martin, 2002). Bogdanov *et al.* (1999) reported that in fresh honeys there is practically no hydroxymethylfurfural (HMF), but it increases upon storage, depending on the pH of honey and on the storage temperature. As with HMF, diastase activity can be used as indicative of good quality of the honeys. According to EU honey legislation (Council Directive 2001/110/EC), the maximum HMF content is 40 mg/kg and the minimum value for diastase activity is set to 8 on Gothe's scale. The our values ranged between 1.10 (Basso Pollino honeys) and 3.30 mg/kg (Camastra Dolomiti lucane honeys) for HMF content, and between 4.83 (Collina Materana honeys) and 7.52 (Vulture Melfese honeys) for diastase number. The results of the study on HMF content and diastase activity showed very low variability and statistical significance, so the results were omitted. However, these low values indicate the high freshness of honey samples.

The moisture content of honey is highly important factor, contributing to its stability against fermentation and granulation during storage (Singh and Bath, 1997), also, it influences the physical properties of honey as well as sensory parameter. The moisture content of honey depends by climatic conditions, season of the year, and degree of maturity of any given honey sample (White, 1975). The average moisture content (%) in the investigated samples ranged from 16.90% (Collina Materana honeys) to 17.73% (Tarantino honeys; $P < 0.05$) (Table 2), which are within the allowed parameters (< 20%) according to the international regulations of quality (Codex Alimentarius, 2001). These values indicate optimum harvesting and a good degree of maturity of product. In our samples, the values were slightly less than those found in Sicilian chestnut honeys (Fallico *et al*., 2004) and Spanish chestnut honeys (Escuredo *et al*., 2013). In particular, Potentino, Camastra-Dolomiti Lucane and Basso Pollino showed the highest moisture content compared to other areas ($P < 0.05$).

The value of soluble solids (expressed as °Brix) is directly related to sugar content and may be a reliable index of adulteration. The our honey samples presented °Brix without significant differences, with values above the 80 % (Table 3), which are similar to those found in others geographical locations (Habib *et al*., 2014; Silva *et al*., 2009).

Table 2: Pollen analysis of chestnut honey

Area	Characterizing Pollen	Frequency %	Others pollens
CDL	*Castanea sativa*	78-82	*Trifolium pratense, Cruciferae, Trifolium repens, Liliaceae, Rubus sp.; Hedysarum spp.; Echium vulgare, Trigonella*
BP	*Castanea sativa*	77-84	*Trifolium repens, Trifolium pratense, Onobrychis viciifolia, Rubus sp,, Quercus sp.*
CM	*Castanea sativa*	85-90	*Trifolium pratense, Citrus spp. Eucalyptus, Rubus sp., Cruciferae, Hedysarum spp., Trifolium repens*
P	*Castanea sativa*	80-88	*Hedysarum spp, Lotus corniculatus, Rosmarinus officinalis, Rubus sp., Trifolium pratense, Robinia, Hedera helix,*
VM	*Castanea sativa*	81-88	*Eucalyptus sp., Rubus sp., Rhamnaceae; Trifolium repens, Trifolium pratense; Liliacee; Robinia; Hedera helix*

Free acidity represents the organic acids content in honey, mainly gluconic acid, pyruvic acid, malic acid, and citric acid, in equilibrium with their corresponding lactones or internal esters, and to inorganic ions, such as phosphate, sulphate and chloride (Terrab *et al*., 2004). Bogdanov *et al*. (2008) reported that the acidity of the honey is responsible of the flavor and of its stability against microbial spoilage. The mean values of free acidity of all honey samples were within the required limits (below 40 meq/kg), indicating the absence of undesirable fermentations. Lactonic acidity ranged from 10.16 to 19.93 meq/kg, total acidity varied between 33.74 and 41.52 meq/kg in agreement with data reported from other geographical locations (Terrab *et al.*, 2002; Terrab *et al.*, 2004). Basso Pollino honeys showed the highest acidity ($P < 0.05$). The results demonstrated that the variation of acidity could be attributed to their different geographical locations. In support of this, Castro-Vázquez *et al*. (2010) reported that the geographic origin of chestnut honey influenced the volatile composition and sensory impression.

The pH is a parameter related to microorganism growth and honey stability (Feás *et al*., 2010). The lowest pH value was found in Potentino honeys (4.29), while it resulted significantly the highest in Vulture Melfese honeys (5.21; $P < 0.05$). The pH limit is not described in EU Directive 2001/101/EC, however, honey pH should be low to avoid microbiological contamination. Overall, the pH values for studied honey samples were within the recommended limits (pH 3.4 to 6.1) for fresh honey (Chiş and Purcărea, 2011), and they were similar to previous reports on the pH of European chestnut honeys (Küçük *et al*., 2007; Zappala *et al*., 2005; Devillers *et al*., 2004; Fallico *et al*., 2004; Marini *et al*., 2004).

Ash content is a reflection of the total inorganic minerals that are present in the sample and it is used for the determination of the botanical origin. The ash values ranged between 0.38 (Collina Materana honeys) and 0.96% (Vulture Melfese honeys), and the results found fell within the range of 0.02-1.03 %, as well as was reported by Anonn (2003). The values we obtained for ash content in the studied honey samples (Table 3) were consistent with those reported in Croatian chestnut honeys by Šarić *et al*. (2008). The high variability for ash content detected in studied honeys could be due to soil and clime characteristics, as suggested by Acquarone *et al*. (2007). Honey normally has low ash content and it depends on the material collected by the bees during foraging on the flora (Abu-Tarboush *et al*., 1993). Electrical conductivity is a parameter used in routine honey quality control and for determination of honey's botanical origin or, more specifically, for differentiation between nectar honey and honeydew (ICH, 2002). The honey is closely related to the concentration of mineral salts, organic acids, some complex sugars, polyphenols, and proteins (Terrab *et al*., 2003). The electrical conductivity (mS/cm) values in honey samples varied in the range of 0.33-0.82. As apparent from Table 3, the highest electrical conductivity value was measured in Vulture Melfese honeys, while the lowest value was measured in Collina Materana honeys. A significant ($P < 0.05$) variation in electrical conductivity between honey samples was observed, while Kropf *et al*. (2010) no significant variation in electrical conductivity between chestnut honey samples have found. The variability among samples, as could be expected, could be linked to the different compositions of the soils and their vegetation.

TOTAL PHENOLIC AND FLAVONOID CONTENTS AND COLORIMETRIC CHARACTERISTICS

Total phenolic and flavonoid contents of chestnut honeys from different geographical areas are reported in Table 4. Total phenolic (mg GAE per 100 g honey) and total flavonoid (mg QE per 100 g honey) showed a high and significant ($P < 0.05$) variability among the honeys from different geographical areas. The values ranged from 11.28 to 15.33 mg GAE per 100 g honey for total phenolic content, and from 5.38 to 19.94 mg QE per 100 g honey for total flavonoid content. Our results are comparable with those reported by other authors for the same type of honey (Beretta *et al*., 2005; Perna *et al*., 2013a; Pichichero *et al*., 2009). Camastra dolomite lucane honeys showed the lowest polyphenols content (11.28 mg GAE per 100 g honey for total phenolic and 5.38 mg QE per 100 g honey for total flavonoid content); ($P < 0.05$) if compared with the other studied honeys. Muñoz *et al*. (2007) have hypothesized that the significant variation among the analysed honey samples was likely due to different geographical origin, and this hypothesis was confirmed by Silici *et al*. (2010), in rhododendron honey. The

differences observed can be related to various factors, such as soil composition, temperature, humidity, altitude that affect the plant's physiological state, and thus the phenolic biosynthesis. Many authors reported that the concentration and composition of phenolics in foods vary with species, variety, season and a wide range of environmental and management factors such as climate, soil conditions, canopy management and weather (Mazza *et al*., 1999). Then, they represent the evolutionary response to plants adaptation to different environmental characteristics (Al-Mamary *et al*., 2002; Cheynier, 2005; Muňoz *et al*., 2007). In support of this, Perna *et al*. (2012) reported a positive correlation between heavy metal and total phenol contents. Many studies have demonstrated that, both in vitro and in vivo, the polyphenol are responsible of antioxidant activity of honey (Ghedolf *et al*., 2002; Dharmalingam and Nazni 2013; Vela *et al*., 2007) and, their concentration is due to most of all to agroclimatic factors (Robbins, 2003). Also, phenolic acids contribute to honey colour, taste and flavour (Robbins, 2003; Manach *et al*., 2004). The honey colour is a physicochemical parameter which plays a crucial role in choosing the product. The colorimetric characteristics of chestnut honeys from different geographical areas are reported in Table 4. The results, measured by CIE L*a*b* method, showed a high and consistent ($P < 0.05$) variability among the chestnut honeys by different production areas. In particular, Camastra Dolomiti lucane and Collina Materana honeys showed the lowest value of L* (53.52 and 53.59, respectively), while the brightest were Basso Pollino and Potentino honeys (L* = 62.37 and 60.32, respectively). These values were higher than those reported by other authors (Bertoncelj *et al*., 2007; Gonzalez- Miret *et al*., 2005). 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 (Frankel *et al*., 1998; Al-Mamary *et al*., 2002). These values varied from 13.68 (Camastra-Dolomiti Lucane honeys) to 17.95 (Vulture Melfese honeys) for a* parameter, and from 31.47 (Collina Materana honeys) to 41.92 (Camastra-Dolomiti Lucane honeys) for b* parameter. The colour intensity of a 50% honey solution (w/v) is reported in Table 4. Net absorbance varied between 0.56 AU (Camastra-Dolomiti Lucane honeys) and 1.34 AU (Collina Materana honeys; $P < 0.05$). This variability could be due to different pigments concentration, as well as flavonoids, that have absorption maxima at 450 nm (Furr, 2004; Mendiola *et al*., 2008). Net absorbances of chestnut honey samples resulted higher than those reported by other authors (Beretta *et al*., 3005; Bertoncelj *et al*., 2007). 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 honey color intensity may be treated as an indicator of its antioxidant activity, as demonstrated by several authors, which showed a positive and significant correlation between ABS_{450} and antioxidant assay (Taormina *et al*., 2001; Beretta *et al*., 2005 Blasa *et al*., 2006; Perna *et al*., 2013a).

Table 3: Physicochemical properties of chestnut honeys samples from five geographical areas

Parameter	CDL		BP		CM		P		VM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Moisture (%)	17.73^{a}	0.48	17.54^{a}	0.46	16.90^{b}	0.56	17.75^{a}	0.49	17.00^{b}	0.25
Brix (%)	81.08^{a,b}	0.63	81.01^{a}	0.53	81.56^{b,c}	0.57	80.93^{a}	0.45	81.68^{c}	0.27
Free acidity (meq/kg)	26.11^{a}	1.98	24.30^{b}	2.15	22.75^{b}	2.26	23.00^{b}	2.11	23.99^{a,b}	4.00
Lactonic acidity (meq/kg)	10.16^{a}	3.35	19.93^{b}	2.3	10.78^{a,c}	2.75	12.98^{c}	2.7	10.37^{a}	1.54
Total acidity (meq/kg)	37.49^{a}	4.87	41.52^{b}	3.18	33.74^{a}	4.42	35.70^{a}	4.52	34.03^{a}	5.24
pH	4.59^{a}	0.18	4.39^{b}	0.21	4.30^{b}	0.16	4.29^{b}	0.15	5.21^{c}	0.14
Ash (%)	0.39^{a}	0.21	0.41^{a}	0.19	0.38^{a}	0.21	0.73^{b}	0.22	0.96^{c}	0.22
Electrical conductivity (mS/cm)	0.33^{a}	0.14	0.42^{a}	0.13	0.40^{a}	0.16	0.57^{b}	0.14	0.82^{c}	0.15

Mean values from three repetition ± standard deviations.
^{a,b,c} Means in the same row with different letters are significantly different according to the Student's t-test ($P < 0.05$).
CDL, Camastra-Dolomiti Lucane; BP, Basso Pollino; CM, Collina Materana; P, Potentino; VM, Vulture Melfese.

Table 4:Total phenolic and flavonoid content, and colorimetric characteristics of chestnut honey samples from different geographical origin.

Parameter	CDL		BP		CM		P		VM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Phenolic content (mg GAE per 100 g honey)	11.28[a]	3.14	14.71[b]	2.87	14.01[b]	2.94	15.33[b]	3.26	13.56[b]	2.02
Flavonoid content (mg QE per 100 g honey)	5.38[a]	3.72	19.94[b]	3.77	14.88[c]	3.93	11.61[d]	3.77	8.66[d]	4.80
ABS 450	0.56[a]	0.21	1.01[b]	0.19	1.34[c]	0.21	1.23[c,d]	0.20	1.07[b,d]	0.21
L*	53.52[a]	6.97	62.37[b]	4.35	53.59[a]	6.41	60.32[b,c]	5.93	58.00[a,c]	5.02
a*	14.61[a,b]	1.89	15.39[a]	1.25	13.68[b]	1.72	15.68[a]	1.33	17.95[c]	1.55
b*	41.92[a]	7.31	35.83[b]c	5.06	31.47[b]	7.18	33.66[b]	5.44	38.48[a,c]	4.04

Mean values from three repetition ± standard deviations.
[a,b,c,d] Means in the same row with different letters are significantly different according to the Student's t-test ($P < 0.05$).
CDL, Camastra-Dolomiti Lucane; BP, Basso Pollino; CM, Collina Materana; P, Potentino; VM, Vulture Melfese.

VITAMIN C CONTENT

The vitamin C content of the studied honeys is reported in Figure 2. The results showed that Vulture Melfese honeys presented the highest vitamin C content (5.11 mg per kg honey; $P < 0.05$), while Basso Polino honeys the lowest value (2.37 mg per kg honey). These values were similar to those found in chestnut honeys by Leon-Ruiz *et al.* (2013). However, Kesić *et al.* (2009) reported higher values in dark honeys (263 mg per 100 g), even if the used methodology (titrimetric method) is not as reliable as the HPLC method used here. The variation in vitamin C content in honeys from different locations could be due to geographical and environmental conditions such as rain, temperature and soil. Also, other factors such as temperature, relative humidity, oxidative stress, exposure of sun as well as pollutants are considered the main responsible of the variation in vitamin C content (Dewanto *et al*., 2002).

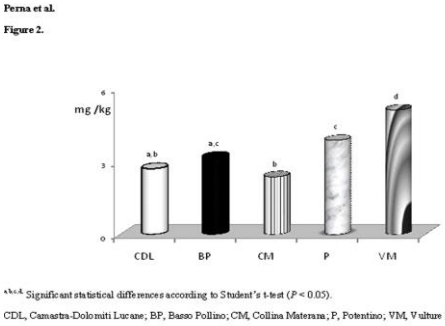


Figure 2: Vitamin C content (mg L-ascorbic acid per kg honey) of chestnut honey from different geographical areas.

ANTIOXIDANT CAPACITY

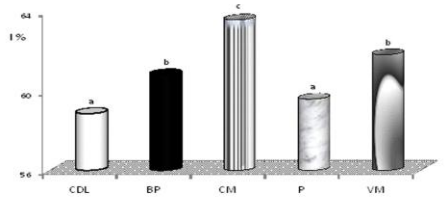
In evaluating the antioxidant activity of honey samples, the ABTS and FRAP assays were used (Figure 3 and Figure 4). Moon and Shinamoto (2009) suggested that for evaluating the antioxidant activity must be used at least two different test systems, since no single method exists to test the antioxidant capacity. Many authors have demonstrated that the antioxidant activity depends on the extraction method as well as on the type of the reactive species in the reaction mixture assay (Heimler et al., 2006; Parente *et al*., 2013). Based on antioxidant tests, chestnut honey from different geographical areas have been shown to exhibit varying degrees of antioxidant capacity. The average values were 60.94% and 822.25 □M Fe(II) for ABTS and FRAP assays, respectively. There were significant differences among the FRAP and ABTS values for chestnut honeys from different locations, suggesting that they have different antioxidant potentials. These results were similar to those found by Perna *et al*. (2012), while FRAP value considerably higher than that found in chestnut honey (388.60 μM Fe (II)) from Slovenia (Beretta *et al*., 2005). As shown in Figure 2 and 3, the values varied from 58.86 (Camastra Dolomiti lucane honeys) to 63.59% (Collina Materana honeys) in the ABTS assay, and from 281.04 (Camastra Dolomiti lucane honeys) to 1129,16 □ M Fe (II) (Vulture Melfese honeys) in the FRAP assay. In particular, Camastra-Dolomiti Lucane and Potentino honeys showed an antioxidant activity, measured by ABTS assay, lower than that found in honeys from other considered areas (I%= 58.86 and 59.59; respectively). Collina Materana honeys showed the highest radical scavenging activity (63.59%). The trend of the antioxidant activity, evaluated by FRAP assay, confirmed the results obtained by ABTS assay, in particular for Camastra-Dolomiti Lucane and Potentino honeys (281.04 and 881.20μM Fe (II), respectively; $P < 0.05$). Vulture Melfese honeys showed the highest FRAP values (1129.16 μM Fe (II)) compared to other samples. The obtained results in this work demonstrated that the variations in antioxidant activity are a function of different locations, which differ according to geographic and climate factors and soil composition. These factors greatly influence the content in

biologically active compounds in honey, such as organic acids, polyphenols, vitamin C, peptides, enzymes, Maillard reaction products, and possibly other minor components, which may act synergistically and increase the antioxidant action (Ghedolf *et al.*, 2002). In support of this, a positive significant linear correlation has been reported between the antioxidant activity and total phenolic content of the honey (Al-Mamary *et al*., 2002; Nazni and Dharmalingam, 2013; Beretta *et al*., 2005; Vela *et al*., 2007; Al *et al*., 2009).

CONSUMERS' ACCEPTABILITY

To investigate the degree of acceptance of the chestnut honey from five geographical areas, a preference test was carried out: overall acceptability, appearance, color, odor, and taste descriptors were assessed and the results (mean scores and standard deviation values) are shown in Table 5. All evaluated attributes, except odour, were significantly ($P < 0.05$) affected by geographical origin. In general, the honeys showed a good overall acceptability score (range 6.11-6.85). Camastra-Dolomiti Lucane, Basso Pollino and Potentino honeys showed a higher overall acceptability score than Collina Materana and Vulture Melfese honeys ($P < 0.05$). The colour is one of the characteristics that most influences the choice of the consumers (Krell, 1996; Belay *et al*., 2014). The average score observed for the color (4.90) is resulted be low. This could be explained by habit of consumers to use commercial honey of light amber color. Camastra-Dolomiti Lucane honeys showed the highest score for appearance, colour, and taste, while Collina Materana and Vulture Melfese honeys the lowest score for these attributes ($P < 0.05$). Many authors have reported that a higher minerals, polyphenols, and vitamins C content confer a stronger colour and taste in honey (Bogdanov *et al*., 2004; Montenegro, 2005).

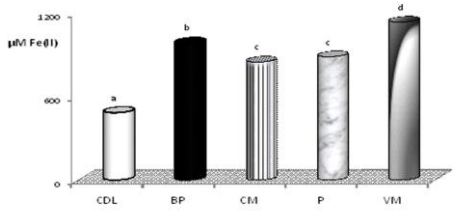
Perna et al.
Figure 3



a,b,c,d Significant statistical differences according to Student's t-test ($P < 0.05$).
CDL, Camastra-Dolomiti Lucane; BP, Basso Pollino; CM, Collina Materana; P, Potentino; VM, Vulture Melfese.

Figure 3: Radical scavenging activity (ABTS) of chestnut honey from different geographical areas.

Perna et al.
Figure 4



a,b,c,d Significant statistical differences according to Student's t-test ($P < 0.05$).
CDL, Camastra-Dolomiti Lucane; BP, Basso Pollino; CM, Collina Materana; P, Potentino; VM, Vulture Melfese.

Figure 4: Ferric reducing antioxidant power (FRAP) of chestnut honey from different geographical areas.

Table 5: Sensory evaluation of chestnut honey samples from different geographical origin

Descriptor	CDL		BP		CM		P		VM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Overall acceptability	6.85[a]	1.01	6.73[a]	0,59	6.22[b]	0.68	6.75[a]	0,99	6.11[b]	1.03
Appearance	6.83[a]	0.86	5.76[b]	0.91	5.11[c]	0.21	5.73[b]	0.89	5.07[c]	0.57
Colour	5.98[a]	1.23	5.07[b]	0.76	4.48[c]	0.71	5.03[b]	1.01	4.33[c]	1.22
Odour	6.35[a]	1.21	6.31[a]	1.02	6.47[a]	1.35	6.21[a]	0.99	6.45[a]	1.07
Taste	6.71[a]	0.81	6.01[b,c]	0.69	5.87[c]	0.55	6.17[b]	0.73	5.71[c]	0.84

Each attribute was evaluated on a hedonic scale from 1 (dislike extremely) to 9 (like extremely).
[a,b,c,d] Means in the same row with different letters are significantly different according to the Student's t-test ($P < 0.05$).
CDL, Camastra-Dolomiti Lucane; BP, Basso Pollino; CM, Collina Materana; P, Potentino; VM, Vulture Melfese.

CONCLUSIONS

This study summarizes for first time, the state of knowledge on the characteristics of chestnut honey produced in the Basilicata region and the factors which should be used to define honey quality. The chemical, physical or biological properties of honey are greatly influenced by the geographic origin. The characteristics of honey from different geographical origin are mainly due to the interactive effects among plant, soil, thermohygrometric and environmental conditions and are specific for each area. These results represent a understanding key for the characterization of honey through physico-chemical and sensory analysis, that are intimately related to the its nutritional and nutraceutical qualities. 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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