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Qualitative characteristics of four Sicilian monofloral honeys from *Apis mellifera* ssp. sicula

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PAPER

Abstract

Four monofloral honeys, obtained from the Sicilian black bee by foraging on thistle, sulla, chestnut and eucalyptus, were studied. Results showed that the phenolic composition of chestnut honey was the highest (316 mg gallic acid equivalent GAE/kg), while that of sulla honey was the lowest (122 mg GAE/kg). Data confirmed a correlation between the total phenol content and colour intensity in chestnut honey, which was the darkest of the four samples. Sulla honey showed the highest antioxidant activity, while eucalyptus honey had the highest mineral content (K, Ca, Mg, and Na). Thistle honey showed the most intense floral and fruity aromas, as well as an intense yellow colour. Principal component analysis showed the potential to discriminate different honeys in three different quadrants.

Keywords: colour; honey; sensory analysis; Sicilian black bee

Introduction

The composition of honey varies due to differences in botanical, geographical and entomological origins, and is also influenced by the seasonal, production and storage conditions (Wang *et al.,* 2022). The European Union (EU, 2014) has imposed strict labelling rules on honey, requiring that the botanical and geographical origins of honey be correctly labelled before sale (Thrasyvoulou *et al.*, 2018). Based on their botanical origins, honey products can be classified as monofloral or multifloral. The commercial value of monofloral honey is much higher than that of multifloral honey due to its unique aroma and taste, which are in greater demand by consumers (Marcazzan *et al*., 2014). To confirm the typicality of honey, it is necessary to identify its botanical origin, generally through melissopalynological analysis. Honey can also be classified according to its geographical origin, considering that, in addition to the influence of the flower species, the physico-chemical and sensory characteristics of honey may vary according to the subspecies of bees (Silva *et al.*, 2016). The Sicilian black bee (*Apis mellifera* ssp. sicula) is an African subspecies of *A. mellifera* that has adapted to the warm lands of the Mediterranean, including Sicily (Italy) (Mannina *et al.*, 2015). It differs from the more common *A. mellifera* ssp. ligustica in the colour and size of its wings, as well as in the fact that it is more resistant to high temperatures, allowing it to tolerate temperatures above 40°C, whereas other subspecies of bees do not produce honey under such extreme conditions (Attanzio *et al.,* 2016). *A. mellifera* ssp. sicula also possesses considerable immunological resistance, enabling it not to succumb to varroasis nor virosis (Franck *et al.*, 2000). *A. mellifera* ssp. sicula has a pronounced pollination capacity that ensures the continuity of many plant species, including some that are in danger of extinction. This subspecies risked extinction in the 1970s, when beekeepers imported the *Ligustica* subspecies into Sicily. Following this massive introduction, a group of entomologists and beekeepers recovered the

genetically pure Sicilian subspecies by transferring some old hives to the island of Ustica (PA), where the selected bees were bred without risk of contamination. A reintegration plan was then launched in western Sicily by the Slow Food Presidium founded in 2008.

The plan included fertilization stations for pure reproduction of *A. mellifera* ssp. sicula. Purity was periodically checked through genetic screening (Attanzio *et al*., 2016).

The physico-chemical and sensorial properties of honey produced by *A. mellifera* ssp. sicula has thus been of scientific interest. The aim of this study was to evaluate, by means of sensory and physico-chemical analysis, the differences between four monofloral honeys obtained by *A. mellifera* ssp. sicula.

Materials and Methods

Honey samples

Honey samples of thistle (*Silybum marianum* L.), sulla (*Hedysarum coronarium* L.), chestnut (*Castanea sativa* Mill.) and eucalyptus (*Eucalyptus globulus* Labill.) were collected from Sicily from the 2019 production by Nettare di Sicilia, Caltavuturo (PA). Nettare di Sicilia is a company situated inside the Madonie Park and part of the Slow Food Presidium. The various monofloral honeys were produced by moving the bees to the most suitable environments in Sicily with the specific botanical species. Precisely, the honeys analysed were chestnut honey, produced in the Nebrodi Nature Park in the province of Messina at an altitude of over 1000 m above sea level; sulla honey, produced in a hilly area in the Madonie Park at around 700 m above sea level; eucalyptus honey, produced in the province of Agrigento in a fairly arid area with small woods; and thistle honey, produced in the province of Palermo at sea level where this plant grows wild. All samples were classified by melissopalynological analysis (Soares *et al.*, 2017), whereby the pollen grains of the different botanical species were distinguishable by microscopic observation. Three different samples of honey from different hives were analysed for each of the four botanical species, all processed in the same way. Honey samples were kept away from sunlight at room temperature before analysis.

Physico-chemical parameters

The moisture content of each honey sample was determined from its refractive index using a digital refractometer (NR 101 Spain) thermostated at 20°C and regularly calibrated with distilled water (Bogdanov, 2009). The pH was assessed by the Crison GLP 21 pH meter. The ash content was determined according to official methods (AOAC, 1999): about 5 g of honey was placed in a combustion pot preheated in the dark with a gas flame to prevent the honey from foaming. Then, the sample was incinerated (burned) at a high temperature (550°C) in a burning muffle furnace for 5 h. After cooling to room temperature, the ash obtained was weighed. The total polyphenol content was determined spectrophotometrically using a Folin–Ciocalteu method as reported by Singleton *et al.* (1999), with some modification. The results were expressed as gallic acid equivalents (GAE) per 100/g honey. Colour measurements were performed using a Konica Minolta chroma meter CR-C2500 (Konica Minolta Sensing Singapore Pte Ltd, Singapore). Results were recorded as a^*, b^*, and L^* values, where a^* is an index of redness (+) or green (–), b^* an index of yellow (+) or blue (–), and L^* indicates brightness on a scale of 0–100 (Adiletta *et al.*, 2020). The overall colour difference (ΔE), *chroma* (C) and *hue* angle (H°) were also calculated, where *chroma* indicates the dullness or vividness and *hue* angle indicates how an object's colour is perceived by the human eye (red, orange, green or blue). The samples were placed in an optical glass cell for measurement. Glucose and fructose were determined by the HPLC system using an Agilent 1100 chromatograph with a refractive index detector (Agilent, Santa Clara, USA) equipped with a Eurokat, 300 × 8 mm, 10 µm column (Knauer, Berlin, Germany). The mobile phase was a water solution with a flow rate of 1 mL/min and a column temperature of 80°C. The results were expressed as "mg glucose/g honey."

The antioxidant activity of honey was evaluated using the DDPH radical scavenging activity (Larrauri *et al.*, 1998) and expressed as µmol Trolox equivalents (TE)/g honey. All measurements were repeated three times. All results represent the average of three measurements per sample.

Mineral content

The mineral content of each honey sample was determined according to the procedure of Chudzinska *et al.* (2011), with some modifications. Two grams of each sample were dispersed in 5 mL HNO_3 (65 %) and 1 mL H_2O and then digested in a microwave digestion system (MARS 6, CEM, Matthews, NC, USA) by increasing the temperature up to 210°C. At the end of the procedure, after appropriate dilutions with bi-distilled H_2O, samples were analysed by inductively coupled plasma spectroscopy (iCAP 6200 DUO, Thermo Scientific, Waltham, MA, USA), and Ca, Fe, K, Mg, Na, Cu, Mn and Zn content were determined. Each sample was analysed in triplicate, and the reported results are the average of the three measurements.

Sensory analysis

The different honeys were judged by a trained panel of nine tasters (7 males and 2 females, aged between 24 and 48 years), consisting of technical experts. Twenty grams of honey was weighed into 200 cc transparent glasses, sealed with foil and kept at 20°C for 2 h before tasting. Samples were presented to each taster in random order. A descriptive sensory profile test based on quantitative descriptive analysis was used for the evaluation. Based on the frequency of citation (>60%), 17 descriptors were identified: three visual (yellow intensity, amber intensity and crystallisation), nine olfactory (ripe fruit, herbaceous, floral, caramel, liquorice, beeswax, hay, medicinal and off-flavour), three gustatory (sweet, sour and bitter), one taste persistence and one overall liking. Each of the descriptors was measured on a structured intensity scale of 1–9, with 1 denoting absence and 9 denoting maximum perception. Because all types of honey were suitable for trade, the Council of Ethics exempted the authors to ask for a formal ethical approval. The panelists did, however, give verbal informed consent prior to participation.

Statistical analysis

Analysis of variance (ANOVA) and Tukey's honest significant difference test at a 5% level were used to compare analytical differences between samples. Principal component analysis (PCA) was performed to reduce the multidimensionality of the dataset, generating new principal components that accounted for most of the total variation. All statistical analyses were done using the SPSS software package, Version 20.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Physico-chemical traits

The moisture content did not differ significantly ($P < 0.05$) among the honey samples, as this parameter was controlled during processing (Table 1). Moisture in each analysed honey sample was around 14–15%, which is the optimal level, ensuring stability and spoilage resistance against yeast fermentation (Bacandritsos *et al.*, 2006) while prolonging shelf life and limiting granulation (Singh and Kuar Bath, 1997). The four monovarietal honey samples were all acidic, with pH values ranging between 2.96 (sulla honey) and 5.21 (chestnut honey). All of these values fall within the standard limit (pH 3.40–6.10) (Codex Alimentarius, 2001), ensuring the freshness of the honey samples (Table 1). The low pH of honey is related to the fermentation of the sugars, which results in two important characteristics of honey: flavour and stability against microbial spoilage (Bogdanov, 2009). The ash content of honey is often used to determine its botanical origin (floral, blend or honeydew). Ash concentrations in the honey samples ranged from 0.10 (sulla honey) to 0.79% (chestnut honey), which are all values within the limits allowed for floral honeys (0.60%) except for chestnut honey, in which ashes were present in a higher percentage, showing that it belongs to the dark honeys (Oddo *et al.*, 1995). The high ash content could explain the high pH value of the chestnut honey samples, being that ash depends on the constituents of the flora type, geographical area, physiology of the plants and soil type on which the plants from which the bees collect nectar grow.

The total phenol content (Figure 1) was highest in chestnut honey (316.3 mg GAE/Kg), followed by eucalyptus (193.5 mg GAE/Kg), thistle and sulla honey; these results are in agreement with those reported by Preti and Tarola (2022). The phenolic content of monofloral honeys of *A. mellifera* ssp. sicula varied according to the botanical origin of the plants from which the nectar was collected (Al-Mamary *et al.*, 2002; Amiot *et al.*, 1989). In Sicilian environments characterised by a warm climate and a high level of exposure to sunlight, the plants may contain many more total phenols than the same plant varieties grown in colder environments (Spayd *et al.*, 2002). Phenolic compounds are responsible for the colour and taste characteristics of honey and for multiple biological

Table 1. Physico-chemical traits of different monofloroal honeys.

Parameters	Thistle	Sulla	Chestnut	Eucalypt
Water %	85.5 ± 0.501^{a}	85.1 ± 0.47^{a}	84.90 ± 0.50^{a}	85.5 ± 0.48^{a}
Ash (g/100 g)	0.32 ± 0.02^{b}	0.10 ± 0.01^{a}	0.79 ± 0.06^{c}	0.10 ± 0.01^{a}
pH	3.26 ± 0.04^{b}	2.96 ± 0.04 ^{a}	5.21 ± 0.04^{c}	3.30 ± 0.04^{b}
Sucrose (mg/g)	62.16 ± 4.51^{b}	61.79 ± 5.63^{b}	53.60 ± 6.87^{a}	57.14 ± 7.85^{a}
Glucose (mg/g)	287.31 ± 16.22^{b}	287.03 ± 17.51^{b}	232.10 ± 19.58^{a}	312.36 ± 28.11^{c}
Fructose (mg/g)	293.87 ± 22.76^{a}	392.52 ± 34.26^{b}	356.81 ± 41.85^{b}	391.23 ± 49.27^{b}
DPPH (μmol TE/100g)	16.57 ± 0.05^{a}	17.19 ± 0.01^{b}	16.27 ± 0.04^{a}	17.01 ± 0.03^{a,b}

Mean ± SD (n = 3) (different letters in the same row indicate significant differences for $P \leq 0.05$, ANOVA, Tukey's test).

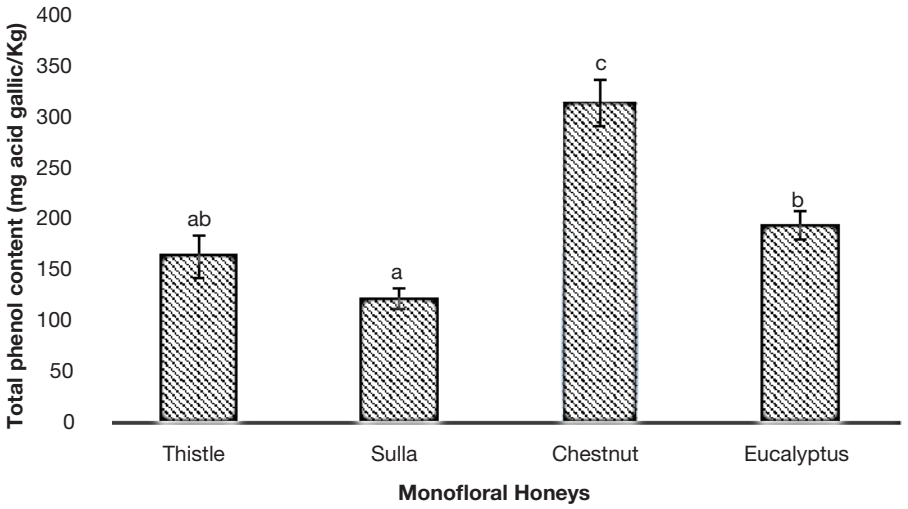

Figure 1. Total phenol content (mg gallic acid equivalent/kg) in different monofloral honeys. Different letters indicate significant differences for P ≤ 0.05, ANOVA, Tukey's test.

properties, such as antioxidant, antibacterial and radical-scavenging activities.

Results reported by Karabagias *et al.* (2014) and Preti and Tarola (2022) confirmed the correlation between the total phenol content and colour intensity, with darker honeys having a higher phenolic content and antioxidant capacity. In this regard, it should be noted that the average value of the parameter *a** (index of red) was about 9.3 for chestnut honey (Rodríguez-Flores *et al.*, 2019), compared to values below 1.0 for the other three samples (Figure 2). The value of *b** (yellow index) was also highest for chestnut honey, while sulla honey showed the lowest value. The *L** (lightness) value was highest for chestnut honey, but lowest for the sulla sample. The colour saturation (C) was highest for chestnut honey and was lowest for the sulla sample. For the parameter indicating colour hue, no significant variations were found in the three samples of thistle, sulla and eucalyptus, while chestnut had a slightly lower value than these three. Sucrose was highest in thistle and sulla honey, and lowest in the chestnut sample. Glucose was present in concentrations of 232–312 mg/g of honey in chestnut and eucalyptus, while fructose was present in concentrations of 293–393 mg/g of honey in thistle and sulla. The antioxidant activity of honey measured by DPPH protocol showed values between 16.27 and 17.19 μmol TE/100g, with sulla honey showing the highest activity.

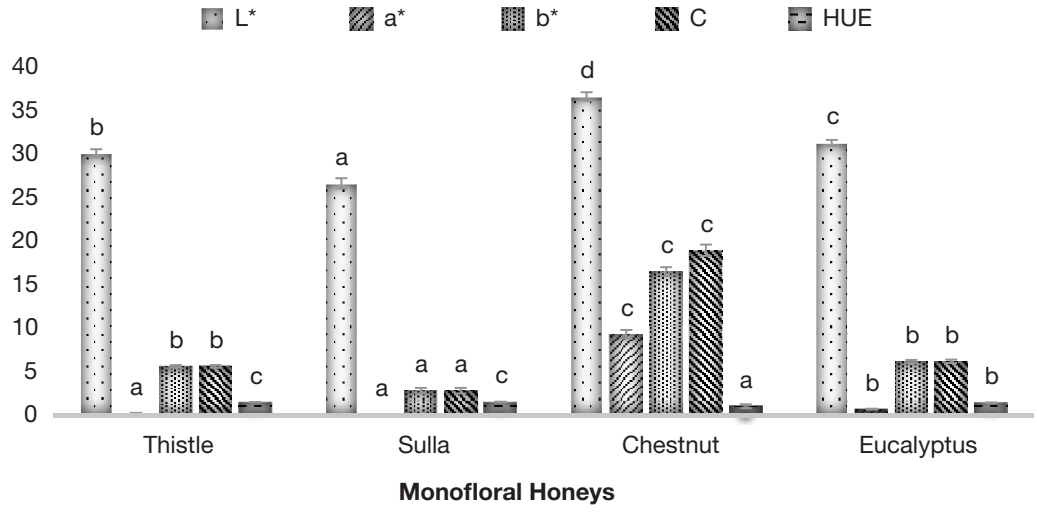

Figure 2. Colour traits of different monofloral honeys. Different letters indicate significant differences for P ≤ 0.05, ANOVA, Tukey's test.

Table 2. Mineral content of different monofloroal honeys.

Sample	Ca (mg/kg)	Fe (mg/kg)	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Sulla	80.36 ± 4.56^{a}	0.28 ± 0.02^{a}	422.38 ± 23.24^{a}	8.47 ± 1.02^{a}	5.50 ± 0.95^{a}	0.10 ± 0.02^{a}	0.50 ± 0.10^{a}	0.50 ± 0.08^{a}
Eucalyptus	113.90 ± 8.49^{b}	1.47 ± 0.09^{c}	884.55 ± 52.57^{c}	14.46 ± 1.12^{b}	84.10 ± 10.66^{b}	0.20 ± 0.07^{b}	1.00 ± 0.09^{b}	0.80 ± 0.13^{b}
Thistle	90.19 ± 7.34^{a}	0.27 ± 0.04^{a}	527.93 ± 34.36^{b}	9.50 ± 1.04^{a}	6.30 ± 1.29^{a}	0.10 ± 0.04^{a}	0.40 ± 0.03^{a}	0.60 ± 0.09^{a}
Chestnut	120.38 ± 10.12^{c}	0.83 ± 0.11^{b}	518.08 ± 42.31^{b}	31.28 ± 3.12^{c}	7.47 ± 0.84^{a}	0.30 ± 0.06^{b}	2.00 ± 0.24^{c}	0.90 ± 0.16^{b}

Mean ± SD (n = 3) (different letters in the same row indicate significant differences for $P \leq 0.05$, ANOVA, Tukey's test).

Mineral content

Chemical evaluation of the most common minerals present in honey samples was performed.

According to the literature (Alves *et al.*, 2013; Bontempo *et al.*, 2017; Lobos *et al.*, 2022), for all types of honey, the most abundant elements were, in decreasing order of concentration, K, Ca, Mg and Na (Table 2). Eucalyptus honey contained the highest amount of potassium (884.55 mg/kg), which was significantly different ($P < 0.05$) from thistle (527.93 mg/kg), chestnut (518.08 mg/kg) and sulla (422.38 mg/kg) samples.

Ca and Mg were the most abundant macroelements in eucalyptus and chestnut honey, with respective values of 113.90 mg/kg and 14.46 mg/kg for eucalyptus and 120.38 mg/kg and 31.28 mg/kg for chestnut. Moreover, eucalyptus honey was also very rich in Na. Sulla and thistle samples showed no significant ($P < 0.05$) differences in the content of all analysed macro- and microelements except for K, which was higher in sulla honey.

All the other elements, such as Fe, Cu, Mn and Zn, were present in traces in all samples.

Sensory analysis

Honey from different floral sources may have distinct aromas and flavours due to differences in volatile composition, which in turn may depend on the geographical origins (Manyi-Loh *et al.*, 2011). Sensory analysis of the sulla honey showed a high yellow intensity, with an advanced state of crystallisation (Figure 3). For the sense of smell, this honey registered the highest floral value, while ripe fruitiness, beeswax and hay were less marked, owing to the presence of norisoprenoids

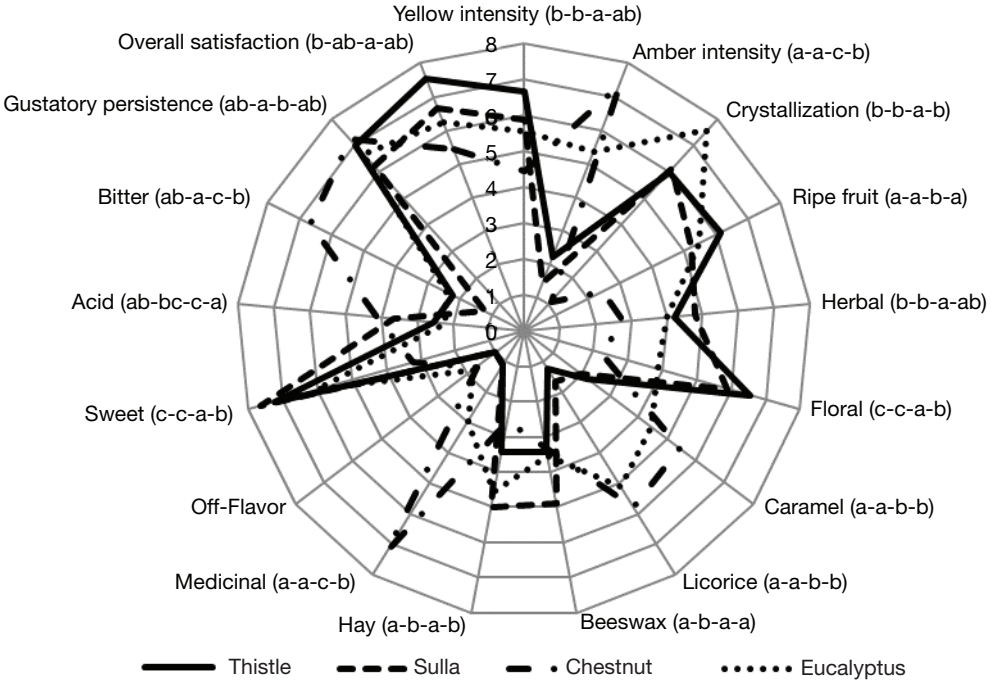

Figure 3. Sensory analysis of different monofloral honeys. Different letters indicate significant differences for $P \leq 0.05$, ANOVA, Tukey's test.

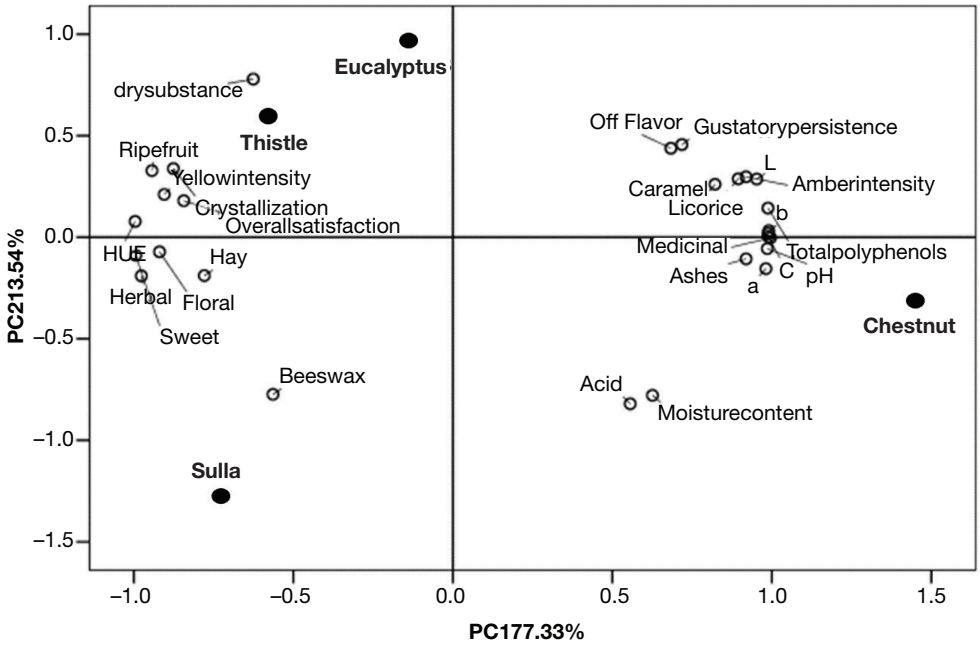

Figure 4. Principal component analysis of the physico-chemical and sensory traits in different monofloral honeys.

(Jerković *et al.*, 2010). The eucalyptus honey was characterised by a good amber intensity, which predominated the yellow intensity, and showed the highest level of crystallisation. On the nose, notes of ripe fruit, caramel, liquorice and hay prevailed (Castro-Várquez *et al.*, 2009), while on the palate, sweetness overcame acidity and bitterness. Chestnut honey had a significantly higher amber intensity than all the other samples, a low yellow intensity, and no crystallisation. The aroma also differed from those of the other honeys: the descriptors with the highest values were caramel, liquorice and medicinal. In terms of taste, bitterness, due to the high concentration of phenols, was the most distinctive and pronounced note, while acidity and sweetness were less noticeable (Marcazzan *et al.*, 2014). The thistle honey had the highest yellow intensity, and the ripe fruitiness and floral smell prevailed in the olfactory analysis. The taste profile showed higher levels of sweetness with no acidity and bitterness.

Principal component analysis

The first two principal components explained 77.33 and 13.54% of the total variance, respectively (Figure 4). PCA showed the potential to discriminate different honeys in three different quadrants: the second quadrant showed thistle and eucalyptus honey, the third quadrant showed sulla honey, and the fourth quadrant showed chestnut honey. The PCA bi-plot showed a positive correlation between pH, a*, polyphenols and ash in chestnut honey. The thistle sample was characterised by the following sensory parameters: yellow intensity, crystallization, and ripe fruit odour.

Conclusion

The analysed Sicilian honeys, produced using the same techniques and obtained from black bees, showed different sensory and quality profiles. These differences reflect the botanical species and environmental characteristics in which the bees developed. The phenolic composition of honey, which is important from taste and health points of view, was the highest in chestnut honey and lowest in sulla honey. Ca and Mg were the most abundant macronutrients in chestnut and eucalyptus honey, with the latter also containing the highest amount of potassium. The sensory analysis, which considered 17 descriptors (visual, olfactory, taste and persistence), showed that the overall liking was higher for thistle honey, followed by sulla, eucalyptus and finally chestnut.

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