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FULL ARTICLE

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Physicochemical characterization and antioxidant activity of honey with *Eragrostis* spp. pollen predominance

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Abstract

This study aimed to characterize the pollen and physicochemical profiles, and to evaluate the antioxidant capacity of six samples of Moroccan honey. The pollen grains of *Eragrostis* spp. were predominant in all samples (from a minimum of 46.58 ± 1.59% to 72.72 ± 2.43% as a maximum), exceeding the minimum (generally 45%) required for the monoflorality nomination of a honey sample, and suggestion a case of newly reported honey. The analyzed samples had low diastase activity and hydroxymethylfurfural (HMF) content. Potassium was the dominant element in practically all samples, except the sample with the highest *Eragrostis* spp. pollen percentage, which showed sodium predominance. The honey color ranged from water white to light amber, and positively correlated the concentrations of phenols, proline and the capacity for scavenging free radicals. However, great variability observed in all the analyzed parameters, and could not be explained by the variability of the *Eragrostis* spp. pollen percentage, which suggests a hypothesis of low concentrated nectar.

Practical applications

The melissopalynological profile of honey is an important parameter for determining the type of honey (multifloral, monofloral, blossom, honeydew) and its relationship to the botanical and geographical provenance. There is generally a relationship between the pollen profile of honey and the biological properties. The physicochemical characterization, antioxidant activity and melissopalynological profile of *Eragrostis* honey was reported for the first time in this study. These data intend to contribute for the introduction of a new type of honey labeled as *Eragrostis* honey. At the same time, the evaluation of the antioxidant activity may also trigger the search of other biological properties with medicinal attributes for the new *Eragrostis* ssp. honey.

KEYWORDS

food quality, melanoidins, natural antioxidants

1 | INTRODUCTION

Beekeeping in Morocco is one of the most important economical activities, being the current annual honey production about 2,500–3,500 tons. According to the estimations of the Ministry of Agriculture, Rural Development and Maritime Fisheries, which is making major efforts to modernize the sector and assist beekeepers, honeys' production may reach 16,000 tones by 2020 (Moujanni, Essamadi, & Terrab, 2017). Even so, despite the great potentiality of this sector, it is feared that honey's production may stagnate due to difficulties concerning repopulating colonies and the lack of scientific studies that characterize the product and ensure its good quality, safety and allow assessing their biological activities and, therefore, expanding the range of applications.

Even though the legislation regarding honey production and trading in Morocco is scarce, the European legislation establishes, since 1995, that the labeling of the floral origin of honey is essential to protect consumers and marketers [Council Directive EEC/74/409 amended by the Proposal COM/95/0722 (1996)]. It is, therefore, important to develop scientific tools to assess the floral origins of honey and/or the presence of any adulteration in samples. Indeed, melissopalynology provides information on the melliferous plants yet there is no correlation between a pollen count and the quantity of the delivered nectar. This gives rise to the problematic of the minimum pollen required for the monoflorality nomination. In the case of *Lavandula* spp., as an example of a strong nectar delivering plant, the presence of 15% of its pollen in a honey sample is sufficient to obtain the lavender monoflorality nomination. In contrast *Castanea* spp. needs to be represented by more than 90% of total pollen content to provide the monoflorality of a honey sample (Gomes, Dias, Moreira, Rodrigues, & Estevinho, 2010). Due to these limitations other characteristics, like the physicochemical parameters, are also essential to ascertain the botanical origin of honey and are widely used in routine honey analysis (Elamine et al., 2017).

The above-mentioned interest came, mainly, from the rich chemical composition of honey (carbohydrates, proteins, enzymes free amino acids, essential minerals, vitamins, and polyphenols), which makes it an important source of bioactive compounds responsible for several biological activities including antioxidant, anti-inflammatory, antimicrobial, anti-ulcer, antihyperlipidemic, antidiabetic, and anticancer properties (Alvarez-Suarez, Giampieri, & Battino, 2013).

These beneficial properties, particularly antioxidant, are due to the presence of a great variety of compounds like phenolic acids (ellagic, caffeic, *p*-coumaric and ferulic acids), ascorbic acid, flavonoids (apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin, and hesperetin), tocopherols, enzymes (catalase, superoxide dismutase), reduced glutathione, amino acids and carotenoids (Almeida-Muradian, Stramm, & Estevinho, 2014; Rao et al., 2016).

It is important to consider that honey's physicochemical characteristics are strongly related to the botanical source of the nectar, which determines the content of secondary metabolites (El-Sohaimy, Masry, & Shehata, 2015; Silva et al., 2013), but also to the climatic conditions of the locations where the beehives are placed.

Therefore, the present work aimed at the characterization of a Moroccan honey with predominance of *Eragrostis* spp. pollen, regarding its physicochemical characteristics and antioxidant activity. The results showed high variability among the analyzed samples, with low contribution of *Eragrostis* spp. pollen presence.

2 | MATERIAL AND METHODS

2.1 | Reagents and solvents

NaOH, Na_2CO_3, sodium phosphate were purchased from Pronalab, Madalena, Portugal; HCl was purchased from Fisher Scientific UK Ltd.; Loughborough, UK; KCl was purchased from BHD Prolabo; Leuven, Belgium; acetic acid, ascorbic acid, Folin–Ciocalteu, fuchsin, KOH, methanol, 2-propanol were purchased from Merck KGaA; Darmstadt, Germany; 2,2′-azino-bis(3- ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferrozine, $FeSO_4$, gallic acid, NBT, ninhydrine, PMS, proline were purchased from Acros Organics; NJ; $AlCl_3$ and anhydride acetic were purchased from Panreac Quimica, Montcada i Reixac; Barcelona, Spain; catechin was purchased from Fluka; glucose, fructose, sucrose, were purchased from Sigma Aldrich; H_2SO_4, sodium acetate, starch, potassium iodide, I_2, sodium nitroprusside (SNP), were purchased from Riedel-de-Haen; Germany; acetic acid was purchased from CHEM-Lab, Belgium.

2.2 | Sampling

The six honey samples ($n = 6$) harvested in 2013 were directly purchased from Moroccan beekeepers of the Tiznit region, in the southern Moroccan region of Souss-Massa. The samples were centrifuged on arrival at the laboratory, wrapped in aluminum for light protection, and stored at 4°C until analysis, which was about one month later.

2.3 | Melissopalynological analysis

The honey samples pollen qualitative and quantitative spectrum analysis was carried out according to the International Commission for Bee Botany (ICBB), as previously described (Louveaux, Maurizio, & Vorwohl, 1978) and using an optic microscope (Leitz Messtechnik GmbH; Wetzlar, Germany). Reference standards obtained from Portugal honey flora were used for grain identification, and the classes were determined as follows: dominant pollen (> 45% of a specific pollen type), secondary pollen (16–45%), important minor pollen (3–15%), and minor pollen (< 3%).

2.4 | Physicochemical analysis

Free acidity, pH, lactone acidity, ash content, water content, electrical conductivity, proline, diastase activity, HMF were performed according to the harmonized method (Bogdanov, 2002).

2.5 | Estimation of honey color, and melanoidins content

The determination of the honey color was carried out by measuring the absorbance at 635 nm (A_{635}) of an aqueous solution containing

10 g of honey and 20 mL of water, using a UV/Vis Shimadzu (Tokyo, Japan) spectrophotometer (Naab, Tamame, & Caccavari, 2008). The absorbance values were converted on to the Pfund Scale through the equation: mm Pfund $= -38.70 + 371.39 \times A_{635}$. Additionally, color was determined by spectrophotometry by calculating net absorbance (A_{560}–A_{720}).

The determination of honey melanoidin content was carried out based on the browning index (net absorbance $= A_{450}$–A_{720}) (Brudzynski & Miotto, 2011). Spectrophotometric measurements were performed in a 1 cm quartz cell and results were expressed as absorbance units (AU).

2.6 | Sugars content

Fructose, glucose and sucrose contents were evaluated according to the method described by Anjos, Campos, Ruiz, and Antunes (2015).

2.7 | Polyphenols content

The total phenol content was determined using the Folin–Ciocalteau method, with some modifications (Singleton & Rossi, 1965) and the results were expressed as mg gallic acid equivalents (GAE)/100 g. In brief, 5 g of honey were treated with 10 mL of distilled water, mixed and centrifuged. Five hundred microliters of this solution was mixed with 2.5 mL Folin–Ciocalteau reagent (0.2 N) for 5 min and then 2 mL of a Na_2CO_3 solution added (75 g/L). All samples were incubated at room temperature in the dark for 2 hr and their absorbance was read at 760 nm. The blank solution contained water instead of honey. To obtain the calibration curve, a stock solution of gallic acid (1 mg/mL) was prepared for further dilutions (8–250 μg/mL). As reducing sugars can interfere in this reaction, an artificial sugar solution was made according to the sugar profile of each sample, and used to remove the interference (Elamine et al., 2017).

2.8 | Flavonoids content

The flavonoid content (FC) of the different honey samples was quantified using the aluminum chloride assay through colorimetric method (Samatha, Shyamsundarachary, Srinivas, & Swang, 2012). Possible interaction of sample color with the reaction reagents was avoided using a blank with water (substituting the other reaction components) and the sample as described by Sancho et al. (2016). The FC was expressed in mg of catechin equivalents (CE) per 100 g of honey.

2.9 | Minerals content

To analyze the mineral content, samples (5 g) were subjected to calcination (550°C). After cooling, the residue was placed in a desiccator for further analysis. Nitric acid (5 mL) was added to the ash and the mixture was stirred on a heating plate to almost complete dryness. Then, nitric acid (10 mL) was added and the mixture was brought up to 25 mL with distilled water. Afterwards, the mineral content was determined by flame atomic absorption (Ca, Mg, Mn, Zn, Cu, and Fe) or emission (Na, K) spectrometry (air-acetylene) using a novAA 350 (Analytik Jena, Germany). Results were expressed as milligrams of mineral content per kilogram of honey.

2.10 | Antioxidant activity

This antioxidant activity was studied using four methodologies since the use of more than one method has been recommended, as such at least two methods must be performed (Sakanaka & Ishihara, 2008). All measurements described in this section were performed in triplicate. The determination of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity, and ferrous ions chelating effect were carried out as reported previously (Miguel, Nunes, Dandlen, Cavaco, & Antunes, 2010). As for the determination of DPPH and superoxide anion scavenging activities, protocols were detailed by Brand-Williams, Cuvelier, and Berset (1995), and Soares (1996), respectively.

2.11 | Statistical analysis

Statistical analysis was carried out by ANOVA through the SPSS 22.0 program (SPSS Inc., Chicago, IL) and using the Tukey post hoc test at $p < .05$. Correlations between phenol, flavonoid, proline, melanoidins contents, color, and antioxidant activity were achieved by Pearson correlation coefficient (r) at a significance level of 99% ($p < .01$). Principal component analyses (PCA) were performed with Minitab 17.1.0. (Minitab Inc., State College, PA, USA).

3 | RESULTS AND DISCUSSION

3.1 | Melissopalynological analysis

The melissopalynological analysis is currently the only technique allowing for the direct assessment of the botanical and geographic origin of honey (El-Sohaimy et al., 2015).

The percentages of the pollen types identified in the six Moroccan honeys are presented in Table 1. The results show that in all samples, pollen grains of *Eragrostis* spp. predominated, ranging from 46.58 ± 1.59%, in sample S4, to 79.67 ± 1.82%, in sample S6, exceeding the minimal pollen percentage (45%) required to declare honey monofloral-ity, and suggesting that the analyzed sample are *Eragrostis* spp. monofloral honey. The last claim needs to be further studied due to the exceptions reported by some authors: chestnut honey needs 90% of *Castanea* spp. pollen to be monofloral, and lavender honey needs only 15% of *Lavandula* spp. pollen (Gomes et al., 2010). *Cardus* spp. and *Olea* spp. are other pollen grains also detected, being the secondary pollens in sample S4 (22.39 ± 0.99% and 16.76 ± 1.45%, respectively) and only an important minor pollen in sample S6 (9.05 ± 1.57% and 7.85 ± 0.13%, respectively) (Table 1). The secondary pollen grains in samples S1, S2 and S3 were *Cardus* spp. (17.85 ± 0.93%), *Phoenix* spp. (22.19 ± 2.28%), and *Thymus* spp. (19.28 ± 1.65%), respectively.

3.2 | Physicochemical characteristics

The values of pH ranged from 3.57, in S3, to 3.76, in S2 (Table 2), which were inferior to those reported by some authors for Moroccan

TABLE 1 Sample code, places of collection, year of production, and the most predominant pollen of six *Eragrostis* spp. honey samples from Morocco

Sample code	*Eragrostis* spp. (PP)	Type of pollen grain (%+*SD*)		
		SP	IMP	MP
S1	67.46 ± 1.82	*Cardus* spp. (17.85 ± 0.93)	*Phoenix* spp. (9.77 ± 0.56) + Others (4.93 ± 0.34)	–
S2	67.97 ± 1.84	*Phoenix* spp. (22.19 ± 2.28)	*Thymus* spp. (7.07 ± 0.56)	*Olea* spp. (2.78 ± 0.11)
S3	67.55 ± 2.64	*Thymus* spp. (19.28 ± 1.65)	*Cardus* spp. (6.46 ± 0.49) + *Phoenix* spp. (6.71 ± 0.49)	–
S4	46.58 ± 1.59	*Cardus* spp. (22.39 ± 0.99) + *Olea* spp. 16.76 ± 1.45)	*Thymus* spp. (8.06 ± 0.28) + Others (6.22 ± 0.85)	–
S5	72.72 ± 2.43	–	*Cardus* spp. (14.97 ± 0.75) + *Phoenix* spp. (7.34 ± 0.93) + *Thymus* spp. (4.97 ± 0.75)	–
S6	79.67 ± 1.82	–	*Cardus* spp. (9.05 ± 1.57) + *Thymus* spp. (7.85 ± 0.13) + Others (3.44 ± 0.37)	–

Abbreviations (*SD* = standard deviation; PP = predominant pollen (>45%); SP = secondary pollen (16–45%); IMP = important minor pollen (3–15%); MP = minor pollen (<3%); – = absent).

honeys from diverse floral origin (Bettar et al., in press; Diez, Andres, & Terrab, 2004; Malika, Mohamed, & Chakib, 2005) or within the pH range reported by Terrab, Vega-Pérez, Díez, and Heredia (2002). Free acidity ranged from 8.25 mEq/kg (S5) to 28.65 mEq/kg (S3). These free acidity values were within the European limits (<50 mEq/kg) (EU Council, 2002). Such low values are important to prevent undesirable fermentation. As for the lactone acidity, it ranged from 7.00 mEq/kg (S2) to 15.33 mEq/kg (S5). Total acidity ranged from 20.80 mEq/kg (S6) to 38.65 mEq/kg (S3).

Samples S4 and S5 had the highest (0.22%) and lowest (0.02%) ash content, respectively (Table 2). Those results correlated, as reported (Feás, Pires, Estevinho, Iglesias, & Araújo, 2010), the electrical conductivity parameter, and S4 had the highest value (792.33 μS/cm), while sample S5 had the lowest value (201.57 μS/cm). The values of ash and conductivity were within the range established by the European Community (below 0.6% and 800 μS/cm, respectively [EU Council, 2002]). The relative low ash content is like citrus honey (Aazza, Lyoussi, Antunes, & Miguel, 2014; Felsner et al., 2004), although great variability had been observed among samples. Despite being in the same floral origin, the variability can be attributed to the different geographical conditions (soil and edafoclimatic characteristics) where hives are located (Almeida-Muradian et al., 2014).

Only sample S1 had the diastase activity (12.42 Shade units/g) higher than the minimum value required by the European legislation (8 Schade units/g), or 3 Schade units/g in the case of citrus honey (EU Council, 2002). In this last case, the level of HMF should not exceed 15 mg/kg, as a maximal tolerable value, whereas in the remaining types of honey, the maximal allowed content of HMF is 40 mg/kg (Codex Alimentarius, 2001; EU Council, 2002). In almost all the analyzed samples, HMF content was even below the 15 mg/kg, and only S2 and S6 had values of 25.94 mg/kg and 21.47 mg/kg, respectively, but still below the generally recommended value (40 mg/kg) (Table 2).

Low diastase activities found in the samples may indicate inadequate storage conditions or processing (relative high temperatures), because the enzyme is susceptible to heat. Nevertheless diastase activities are also dependent on the geographic and floral origins of honey samples (Fallico, Arena, Verzera, & Zappala, 2006). Keeping in mind that in case of citrus honey, low diastase values are accepted, coming to a conclusion on whether the low value measured in the analyzed samples is a characterizing feature of this honey, or if it is a result of inadequate processing cannot be done. In fact, regarding the low HMF values seen in the samples, it can be suggested that the freshness and the adequate storage conditions of the studied samples, strengthen the first hypothesis, as reported for Citrus honey.

The moisture or water content may vary from 15 to 21% depending on several factors, such as the botanical origin of the honey, the level of maturity reached in the hive, processing techniques and storage conditions (da Silva, Gauche, Gonzaga, & Costa, 2016); however, high values may permit the growth of molds on the surface of the honey (Mendes, Proença, Ferreira, & Ferreira, 1998). Therefore, the established legislation fixed the suitable moisture at values below 20% (EU Council, 2002). In this work, the moisture was close to the permitted in the four samples, and exceeded that limit in two samples (S4 and S5) (Table 3). Higher percentages of moisture had already been reported for Moroccan honeys (Aazza et al., 2014; Diez et al., 2004; Terrab et al., 2002) of diverse floral origins.

Proline is the most abundant amino acid in honey, representing a total of 50–85% amino acids, and has been used as a criterion of honey ripeness and sugar adulteration with values not lower than 200 mg/kg (Hermosín, Chicón, & Cabezudo, 2003; Manzanares, García, Galdón, Rodríguez, & Romero, 2014). In the analyzed samples, the proline content varied greatly (Table 3) from a minimum of 251.46 mg/kg in S5 to maximum of 924.98 mg/kg in S3, respecting so the recommended limit, and being also within the previously reported values for other botanical origin Moroccan honey (Aazza, Lyoussi, Antunes, & Miguel, 2013, 2014; Hermosín et al., 2003).

Honey color is an indicator of the presence of polyphenols, terpenes and carotenoids (Naab et al., 2008; Wilczyńska, 2014). In addition, the color and the electrical conductivity also give an indication of the botanical origin of honey (Anjos et al., 2015b). In honey samples of

TABLE 2 Some physicochemical parameters of six *Eragrostis* spp. honey samples from Morocco

Samples	Moisture %	pH	Ash (%)	Electrical conductivity (µS/cm)	Free acidity (mEq/kg)	Lactonic acidity (mEq/kg)	Total acidity (mEq/kg)	Diastase activity (Shade units/g)	HMF (mg/kg)
S1	18.53 ± 0.23cd	3.68 ± 0.03b	0.20 ± 0.01ab	510.00 ± 2.00b	21.25 ± 0.95c	15.00 ± 5.68a	36.25 ± 4.78ab	12.42 ± 0.66a	5.77 ± 0.30e
S2	19.80 ± 0.12d	3.76 ± 0.01a	0.20 ± 0.08ab	416.33 ± 1.15d	14.05 ± 0.83d	7.00 ± 4.33b	21.05 ± 3.51c	6.48 ± 0.32cd	25.93 ± 0.68a
S3	19.67 ± 0.23cd	3.57 ± 0.01e	0.18 ± 0.01ab	477.00 ± 1.00c	28.65 ± 0.52a	10.00 ± 1.73ab	38.65 ± 1.21a	7.86 ± 0.51b	2.20 ± 0.17f
S4	20.40 ± 0.12e	3.61 ± 0.01cd	0.22 ± 0.01a	792.33 ± 1.53a	24.30 ± 0.79b	9.00 ± 2.29ab	33.30 ± 1.51b	6.91 ± 0.21bc	11.77 ± 0.27d
S5	20.27 ± 0.11e	3.58 ± 0.02de	0.02 ± 0.01c	201.57 ± 0.64e	8.25 ± 0.45f	15.33 ± 4.58a	23.58 ± 4.75c	5.60 ± 0.69d	14.13 ± 0.40c
S6	19.13 ± 0.11c	3.62 ± 0.02c	0.11 ± 0.03b	508.67 ± 2.52b	11.30 ± 0.31e	9.50 ± 1.50ab	20.80 ± 1.60c	5.77 ± 0.17cd	21.47 ± 0.43b
Mean ± *SD*	19.12 ± 1.14	3.64 ± 0.07	0.15 ± 0.08	484.32 ± 190.13	17.97 ± 7.99	10.33 ± 3.31	28.308 ± 8.69	7.51 ± 2.54	13.54 ± 9.04
Min–Max	17.27–20.53	3.57–3.76	0.02–0.22	201.57–792.33	8.25–28.65	7.00–15.33	21.05–38.65	5.60–12.42	2.20–25.93

Values in the same column followed by the same letter are not significant different ($p < .05$) by the Tukey's multiple range test.
Abbreviations (Means ± *SD* = Means ± standard deviation; Min-Max = Minimal–maximal).

TABLE 3 Some physicochemical parameters of six *Eragrostis* spp. honey samples from Morocco

Samples	Proline (mg/kg)	Melanoidins	Phenols (mg GAE/100 g)	Flavonoids (mg QE/100 g)	Color Pfund scale (mm)	ABTS	DPPH	Superoxide	Chelating
S1	631.46 ± 33.44b	0.52 ± 0.00b	283.68 ± 6.02b	36.57 ± 2.29c	10.56 ± 4.90c Extra white	20.08 ± 0.24b	38.80 ± 1.96a	17.48 ± 0.25d	46.95 ± 0.66e
S2	379.52 ± 10.74c	0.34 ± 0.01d	223.48 ± 6.57c	29.81 ± 2.06c	2.65 ± 1.24c Water white	16.97 ± 1.13c	26.54 ± 3.59b	19.34 ± 0.22c	53.21 ± 1.60d
S3	924.98 ± 6.56a	1.12 ± 0.02a	495.51 ± 14.19a	76.93 ± 2.81a	71.27 ± 6.13a Light amber	9.20 ± 0.16d	8.60 ± 2.24c	13.76 ± 0.29e	43.93 ± 0.33f
S4	337.80 ± 20.33d	0.37 ± 0.02c	175.01 ± 7.25d	44.38 ± 1.55b	24.86 ± 4.77b White	20.75 ± 0.12b	33.49 ± 0.61ab	19.30 ± 0.61c	110.91 ± 1.72a
S5	251.46 ± 4.39e	0.09 ± 0.00f	73.58 ± 1.26f	7.54 ± 0.71c	1.03 ± 0.06c Water white	–	–	20.56 ± 0.12b	72.67 ± 1.26c
S6	384.90 ± 14.54c	0.21 ± 0.00e	143.76 ± 12.72e	28.49 ± 0.70b	1.89 ± 1.00c Water white	30.20 ± 0.64a	31.35 ± 4.86ab	21.93 ± 0.28a	91.40 ± 1.43b
Mean ± *SD*	485.02 ± 249.95	0.44 ± 0.36	232.50 ± 147.18	37.29 ± 22.98	18.71 ± 27.28	19.44 ± 7.57	27.76 ±	18.73 ± 2.85	69.84 ± 26.95
Min–Max	251.46–924.98	0.09–1.12	73.58–495.51	7.54–76.94	1.03–71.27	9.20–30.20	8.60–38.80	13.76–21.93	43.96–110.91

Values in the same column followed by the same letter are not significant different ($p < .05$) by the Tukey's multiple range test; – = without activity in the concentrations assayed.
Abbreviations (Mean ± *SD* = mean ± standard deviation; Min–Max = Minimal-maximal).

TABLE 4 Sugar content (g/100 g) in six *Eragrostis* spp. honey samples from Morocco

Samples	Fructose	Glucose	Sucrose	Fructose + glucose	Fructose/glucose
S1	37.8 ± 0.1a	30.7 ± 0.1ab	< 0.2	68.5 ± 0.1a	1.2 ± 0.0b
S2	36.3 ± 0.4b	36.2 ± 0.1d	< 0.2	72.5 ± 0.5ab	1.0 ± 0.0a
S3	37.0 ± 0.3ab	30.0 ± 0.1a	< 0.2	67.0 ± 0.5b	1.2 ± 0.0bc
S4	38.5 ± 0.4cd	31.6 ± 0.0bc	< 0.2	70.0 ± 0.4bc	1.2 ± 0.0b
S5	38.5 ± 0.2cd	30.5 ± 0.2a	< 0.2	69.0 ± 0.4cd	1.3 ± 0.0c
S6	39.2 ± 0.3d	31.8 ± 0.5c	< 0.2	71.9 ± 0.8d	1.2 ± 0.0bc
Mean ± *SD*	37.9 ± 1.1	31.8 ± 2.2	–	69.7 ± 1.9	1.2 ± 0.1
Min–Max	36.0–39.4	29.9–36.3	–	66.7–72.9	1.0–1.3

Values in the same column followed by the same letter are not significant different ($p < .05$) by the Tukey's multiple range test.
Abbreviations (Mean ± *SD* = Means ± standard deviation; Min–Max = Minimal–maximal).

the present work, some variability in color, determined at A_{635}, was observed: from extra water white (1.03, 1.89, and 2.65 mm Pfund) in S5, S6, and S2 samples, respectively, to light amber (71.27 mm Pfund) in S3 (Table 3).

Total polyphenol content varied from 73.58 mg GAE/kg (S5) to 495.51 mg GAE/kg (S3) in honey samples. Flavonoid content ranged from 7.54 mg CE/kg (S5) to 76.94 mg CE/kg (S3) (Table 3). The values of phenols and flavonoids in these honey samples were within the range found for other monofloral Moroccan honeys Aazza et al. (2014), with the exception of sample S5 that had significantly lower amounts, although close to those found by the same authors for Portuguese honeys (Aazza et al., 2013). Several studies have detected that polyphenol amounts in honey depend on its floral (Escuredo, Míguez, Fernández-González, & Seijo, 2013; Küçük et al., 2007) and its geographical origins (Rodríguez, Mendoza, Iturriga, & Castaño-Tostado, 2012; Rosa et al., 2011).

The same variability among the analyzed samples was observed also in melanoidin content, and the values ranged from 0.09 for sample S5 and 1.12 for sample S3 (Table 3). Melanoidins, Maillard reaction products, are multicomponent polymers consisting of protein–polyphenol–oligosaccharide complexes and phenols, which can be measured at two wavelengths (A_{560}–A_{720}). They possess several biological properties, such as antioxidant activity (Brudzynski & Miotto, 2011; Moussa, Saad, & Noureddine, 2012).

The total fructose and glucose content present in the honey samples ranged from 67.0% in S3 to 72.5% in S2 (Table 4). These results are in accordance with those reported by other authors for honey samples from Morocco with different floral origin (Aazza et al., 2014) or from other regions (Aazza et al., 2013; Anjos, Campos, et al., 2015; Shin & Ustunol, 2005). In addition, and according to the results obtained, all the samples had the minimal concentrations required for blossom honeys (>60%) (Codex Alimentarius, 2001).

All the samples have higher amounts of fructose than glucose, however, one sample (S2) had practically the same amount of both. Codex Alimentarius (2001) established 5% as maximal limit for sucrose in honey, which was the case of the analyzed samples, with values below 0.2%.

Table 5 depicts the concentration of some minerals present in honey samples from Morocco. The total mineral content was always <1% (0.3–0.8%), with potassium present in higher amounts in practically all samples with the exception of sample S6, in which Na prevailed (Table 5). The percentages of potassium ranged from 49.34 mg/kg, in S6, to 607.24 mg/kg, in S1. Calcium and sodium were quantitatively the second most representative minerals in the samples. Sample S4 showed the highest concentrations of calcium and sodium (188.02 and 252.25 mg/kg, respectively). Magnesium was the fourth most abundant, with S4 presenting the highest concentration (30.32 mg/kg). The most elevated amounts of iron were found in samples S1 (6.92 mg/kg) and S5 (5.38 mg/kg). Zinc and copper, and particularly manganese were in lower amounts in all the samples when compared to the remaining mineral reported earlier. All the data is within the values generally reported for honey samples (Solayman et al., 2016 and references therein). Usually the variability in the individual amounts of minerals in the analyzed honey samples can be attributed to differences in floral type and soil composition (Solayman et al., 2016).

However, in this work the variability seen in almost all the analyzed parameters cannot be explained by the variability of the *Eragostis* spp. pollen ratios, suggesting its small nectar production or the nectar in itself is diluted nectar. This feature may question the monoflorality of those samples, and suggest the need of more pollen percentage to have the monofloral label.

3.3 | Antioxidant activity

The antioxidant activities of the honey samples were measured using five different methods: three assays assessed the capacity for scavenging free radicals (DPPH, ABTS, and superoxide), one assay assessed the capacity for chelating metal ions, and lastly one assay that determined the capacity of samples for reducing Fe(III) to Fe(II), in the test known as reducing power. The results are shown in Table 3 and Figure 1.

Sample 3 (S3) always had lower IC_{50} values than the remaining samples, independent on the method used, which indicates that it has the best capacity for scavenging free radicals and chelating metal ions. The capacity for reducing Fe(III) to Fe(II) is also better for S3 (Figure 1). In contrast, S5 was unable to scavenge ABTS and DPPH free radicals, at least at the concentrations assayed.

TABLE 5 Mineral content (mg/kg) in six *Eragrostis* spp. honeys from Morocco

Samples	Ca	Na	Mg	K	Fe	Zn	Cu	Mn	Sum
S1	59.05 ± 1.08^{de}	64.41 ± 0.47^{c}	13.80 ± 0.55^{d}	607.27 ± 1.25^{a}	6.92 ± 0.06^{a}	1.50 ± 0.02^{b}	1.80 ± 0.02^{a}	0.35 ± 0.00^{c}	755.11 ± 208.87^{d}
S2	67.00 ± 1.04^{d}	37.67 ± 0.13^{e}	13.44 ± 1.11^{d}	327.62 ± 0.82^{c}	3.41 ± 0.09^{e}	0.69 ± 0.00^{e}	0.87 ± 0.00^{e}	0.33 ± 0.05^{d}	451.03 ± 112.13^{e}
S3	103.21 ± 1.30^{c}	42.60 ± 0.23^{d}	27.39 ± 2.21^{b}	266.75 ± 0.65^{d}	2.23 ± 0.04^{f}	2.21 ± 0.02^{a}	1.70 ± 0.08^{b}	0.92 ± 0.06^{a}	447.30 ± 92.10^{c}
S4	188.02 ± 6.80^{a}	252.25 ± 0.59^{a}	30.32 ± 0.60^{a}	331.84 ± 1.28^{b}	3.93 ± 0.05^{c}	1.16 ± 0.02^{d}	1.34 ± 0.06^{c}	0.91 ± 0.06^{a}	809.76 ± 135.26^{a}
S5	57.13 ± 1.90^{e}	24.23 ± 0.12^{f}	8.05 ± 0.28^{e}	251.95 ± 2.05^{e}	5.38 ± 0.05^{b}	0.49 ± 0.00^{f}	0.94 ± 0.07^{d}	0.27 ± 0.02^{e}	348.44 ± 86.39^{f}
S6	115.48 ± 5.38^{b}	148.74 ± 0.982^{b}	21.57 ± 0.46^{c}	49.34 ± 0.98^{f}	3.61 ± 0.07^{d}	1.38 ± 0.02^{c}	0.62 ± 0.05^{f}	0.57 ± 0.00^{b}	341.31 ± 58.33^{b}
Mean ± *SD*	98.32 ± 50.16	94.98 ± 88.99	21.57 ± 8.75	305.79 ± 180.13	4.25 ± 1.66	1.29 ± 0.72	1.21 ± 0.48	0.56 ± 0.29	525.49 ± 205.16
Min–Max	57.13–188.02	24.23–252.25	8.05–30.32	49.34–607.27	2.23–6.92	0.49–2.21	0.62–1.80	0.27–0.92	341.31–809.76

Values in the same column followed by the same letter are not significant different ($p < .05$) by the Tukey's multiple range test.
Abbreviations (Means ± *SD* = Mean ± standard deviation; Min–Max = Minimal–maximal).

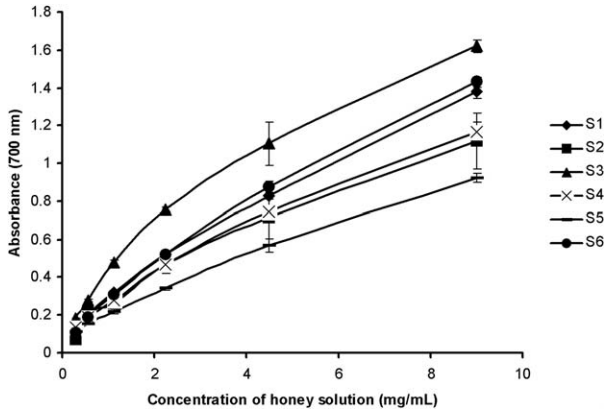

FIGURE 1 The reducing power of honey samples

In general, the antioxidant activity of honey samples depends on the honey floral origin, due to the differences in the content of polyphenolics and enzyme activities (Alvarez-Suarez et al., 2010, 2013; Sousa et al., 2016). However, differences in the activities and the polyphenol content were already reported by Aazza et al. (2014) for the several monofloral thyme honeys and monofloral jujube honeys from Morocco. The authors attributed such results to the different geographic origins from where samples had been collected. In the present work, the geographic origin is the same; nevertheless, differences were detected in the antioxidant activity as well as in other parameters already discussed above. Such results indicate that even in the same region, honeybees of diverse beekeepers have different plants available to visit. Melissopalynological analysis gave us this information (Table 1). Furthermore, the variability observed in the grain pollen of *Eragrostis* spp. does not explain the variability seen in the antioxidant activity results, suggesting the hypothesis that this honey type needs more *Eragrostis* spp. pollen percentage to be labeled as monofloral honey.

A negative correlation between total polyphenols, proline amounts, and IC_{50} values (Table 6) determined when analyzing the capacity for scavenging free radicals, demonstrates the importance of these compounds on the found activity. The correlation between polyphenols and antioxidant activity was previously reported (Aazza et al., 2013, 2014; Alvarez-Suarez et al., 2010). The negative correlation between IC_{50} values and proline content may reveal an important role of this amino acid on the capacity for scavenging free radicals. Antioxidant activity of some amino acids (histidine, taurine, glycine, alanine, proline) has been reported (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005; Wu, Shiau, Chen, & Chiou, 2003).

A positive correlation between color and antioxidant activity and a negative correlation between melanoidin and antioxidant activity (Table 6) were also observed in the present work and also previously reported (Aazza et al., 2014), in Morrocan honeys of different floral origins.

An overview made by some authors (Maurya, Kushwaha, Singh, & Singh, 2014) on antioxidant activity of honeys from different flora and geographical origins showed a great variability in the abilities for scavenging DPPH and ABTS free radicals. The capacity for scavenging ABTS free radicals by Portuguese honeys, independent on the floral origin, was superior to those of the present work (Aazza et al., 2013).

TABLE 6 Pearson correlation coefficients

	Proline	Melanoidins	Phenols	ABTS	Chelating	DPPH	Superoxide	Color (Pfund)
Proline	1							
Melanoidins	0.959[a]	1						
Phenols	0.969[a]	0.985[a]	1					
ABTS	−0.717[a]	−0.854[a]	−0.856[a]	1				
Chelating	−0.648[a]	−0.541[b]	−0.625[a]	0.633[b]	1			
DPPH	−0.658[a]	−0.779[a]	−0.769[a]	0.710[a]	-	1		
Superoxide	−0.915[a]	−0.954[a]	−0.936[a]	0.907[a]	0.633[a]	0.653[a]	1	
Color (Pfund)	0.834[a]	0.931[a]	0.861[a]	−0.767[a]	-	−0.789[a]	−0.876[a]	1

[a]Correlation is significant at the $p < .01$.
[b]Correlation is significant at the $p < .05$.
- = not significant.

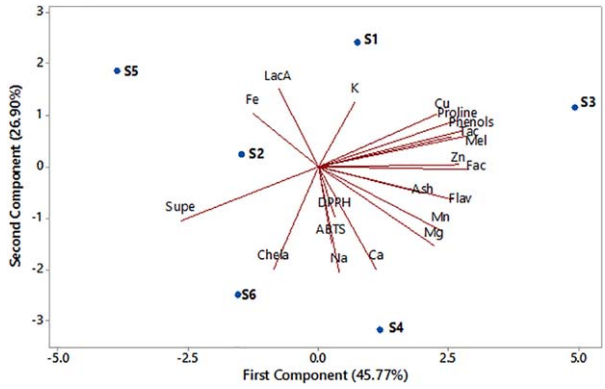

FIGURE 2 Scatterplot of honey samples and measured parameters on the plane defined by the two first components of principal component analysis.
Note. ash = ash content; FAC = free acidity; LACA = lactonic acidity; TAC = total acidity; PROLINE = proline content; MEL = melanoidins; PHENOLS = phenols content; FLA = flavonoids content; ABTS = ABTS radical scavenging; DPPH = DPPH free radicals; SUPE = superoxide; CHEL = chelating; mineral content = Ca; Na; Mg; K; Fe; Zn; Cu; Mn

The values found in the present work were within the range detected for Moroccan honeys, regardless of the floral origin (Aazza et al., 2013). Since the methodology followed in all assays was the same because they were performed by the same team in the same laboratory, we can conclude that for the same floral origin of honeys, the geographical origin has a very important role on the antioxidant activity. Such is in accordance to that observed in the present work: for the same floral origin of honeys (predominance of *Eragrostis* spp. pollen), diverse activities were found.

3.4 | Variance explained by principal component analysis

The data of all measured parameters were subjected to the principal component analysis (PCA). As a first step, all the variables were used to run the PCA (data not showed), and only the variables correlating the resulted factors with, at least, 0.7 as second step were chosen. The results are shown in the Figure 2, where the first two principal components accounted for 72.7% of the total variance, however, in the third component (total of 87.3%) the content of K and Fe with the DPPH free radicals correlated well.

The plot of the honey samples in the plane defined by the two components exhibits the separation of all samples reported a variability between samples. The first component split into two groups (Group 1: S2, S5, and S6; Group 2: S1, S3, and S4). The second component which accounted for 26.9% of the total variance, separated samples S4 and S6 from the others. In fact, the two components are responsible for different groups of characteristics of honey. Component 1 correlated well the ash content, acidity, proline, melanoidins, phenols, and flavonoids content and the minerals Mg, Zn, Cu, and Mn together and superoxide in inverse order. The sample S3 was characterized by a higher amount in these components (Figure 2).

Component 2 correlated well ABTS, chelating, Ca and Na and in inverse order the lactonic acidity. Samples S1 and S5 have an important amount of K and Fe and S4 and S6 have the higher values of ABTS and DPPH.

4 | CONCLUSIONS

The analyzed samples showed great variability in their physicochemical parameters, and antioxidant profiles, independently on the high representability of *Eragrostis* spp. pollen species. The changes in the analyzed parameters seem to be more sensitive to the secondary important pollen species, suggesting that the melliferous plant *Eragrostis* spp. is a weak nectar delivering plants. In addition, to eliminate the hypothesis of other possible influencing factors (meteorological) on nectar secretion, further work needs to be carried out on several apicultural season samples.

Approaching this honey type for possible industrial or medicinal uses seems to be delicate, and high attention needs to be payed to secondary important pollen, and if possible apply a preliminary tool to assess its antioxidant activity.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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