Physicochemical Characterization and Antioxidant Activity of Commercial Portuguese Honeys

Smail Aazza, Badiaa Lyoussi, Dulce Antunes, and Maria Graça Miguel

Abstract: The present study evaluated the physicochemical characteristics and antioxidant activity of 13 commercial honeys from diverse floral origin, produced in Portugal. The values of electrical conductivity of cardoon and pennyroyal honeys were superior to the maximum limits defined by European legislation. Citrus, strawberry tree, and 1 sample of lavender honeys had values of diastase activity below those determined by European legislation. Strawberry tree, pennyroyal, and cardoon honeys had the highest amounts of potassium that coincided with the highest electrical conductivity. Strawberry tree honey was the most effective as antioxidant along with cardoon and heather honeys. This ability was strongly correlated with the amounts of phenols and flavonoids and not with the levels of vitamin C or proline.

Keywords: antioxidant activity, honey, physicochemical attributes, Portugal

Introduction

Honey is a naturally sweet substance that is produced by honeybees (*Apis mellifera* L.) from the nectar of blossoming plants, exudates secreted by certain trees and plants, or from excretion of plant sucking insects that live on plants (honeydew honey). Honeybees collect this material, transform it by combining it with their own specific substances, and then deposit, dehydrate, store, and leave it in the honey comb to ripen and mature (Codex Alimentarius 2001; EU Council 2002).

Honey is a source of natural antioxidants with application in human health and in prevention of deteriorative oxidative reactions in foods. Such antioxidant capacity is strongly related to the chemical composition that, in turn, is dependent on the floral source, and seasonal and environmental factors (Lachman and others 2010). A correlation between antioxidant activity with total phenolic content has been established (Gheldof and Engeseth 2002; Al and others 2009, Ferreira and others 2009). Proline and ascorbic acid contents have also been reported as contributing to the antioxidant activity of honey (Meda and others 2005; Khalil and others 2012).

The antioxidant activity of Portuguese honey has been checked by Ferreira and others (2009). Such surveys have been focused on honey from Northeast Portugal. The characterization of commercial honeys available in the Portuguese market with respect to floral origin, physicochemical parameters, microbial safety, and commercial quality assessment has been performed by Gomes and others (2010). In the present work, the antioxidant activity of 13 commercial honeys of diverse botanical origin was checked, particularly the ability for scavenging free radicals (ABTS, peroxyl, and nitric oxide) and for chelating metal ions. At the same time, the physicochemical characterization of 13 honey brands of a stated botanical origin from Portugal was evaluated.

MS 20130337 Submitted 3/10/2013, Accepted 5/26/2013. *Author Aazza is with Faculdade de Ciências e Tecnologia, IBB-Centro de Biotecnologia Vegetal, Univ. do Algarve, Edif. 8, Campus de Gambelas, 8005-139 Faro, Portugal; and Laboratory of Physiology, Pharmacology and Environmental Health, Faculty of Sciences Dhar El Mehraz, Univ. Sidi Mohamed Ben Abdallah, BP 1796 Atlas, Fez 30 000, Morocco. Author Lyoussi is with Laboratory of Physiology, Pharmacology and Environmental Health, Faculty of Sciences Dhar El Mehraz, Univ. Sidi Mohamed Ben Abdallah, BP 1796 Atlas, Fez 30 000, Morocco. Authors Antunes and Miguel are with Faculdade de Ciências e Tecnologia, IBB-Centro de Biotecnologia Vegetal, Univ. do Algarve, Edif. 8, Campus de Gambelas, 8005-139 Faro, Portugal. Direct inquiries to author Miguel(E-mail: mgmiguel@ualg.pt).*

Materials and Methods

Honey samples and chemicals

A total of 13 commercial honeys (3 replicates for each sample) of Portuguese origin were obtained from supermarkets in Algarve, Portugal, in 2012. The classification of honey brands was according to their respective labels (Table 1).

Analytical procedures

Free acidity, pH, lactone acidity, and total acidity; ash; electrical conductivity; moisture; proline content; diastase activity, and hydroxymethylfurfural content. The measurements were performed according to the Intl. Honey Commission IHC (Bogdanov 2002).

Color. Color was determined by reading the absorbance in aqueous solutions at 635 nm (10 g honey in 20 mL water) (Naab and others 2008) using a spectrophotometer Shimadzu 160–UV (Shimadzu, Kyoto, Japan) and mm Pfund values were obtained using the following algorithm:

$$\text{mm Pfund} = -38.7 + 371.39 \times \text{absorbance}$$

Mineral elements. Potassium and sodium were determined by flame photometry using an air/butane flame. Calcium and magnesium were determined by atomic absorption spectrometry (Terrab and others 2004).

Sugar content. The quantification of carbohydrates was performed according to Al and others (2009) and the chromatographic conditions were those reported by Ciulu and others (2011).

Vitamin C. Homogenized honey (10 g) was dissolved in 10 mL ultra-pure water. Then, 1 mL of 2 M NaOH and 12.5 mL of 1 M phosphate buffer (pH = 5.5) were added and the solution was

doi: 10.1111/1750-3841.12201

Journal of Food Science A Publication of the Institute of Food Technologists

Table 1–Common name, Latin name, and year of production of honey samples.

Common name	Latin name	Year of production
Strawberry tree	*Arbutus unedo*	2011
Cardoon	*Carlina racemosa*	2011
Carob	*Ceratonia siliqua*	2011
Citrus	*Citrus sinensis*	2011
Heather	*Calluna vulgaris*	2011
Eucalyptus	*Eucalyptus globulus*	2011
Sunflower	*Helianthus annus*	2011
Lavander-1	*Lavandula stoechas*	2011
Lavander-2	*Lavandula stoechas*	2011
Pennyroyal	*Mentha pulegium*	2011
Multifloral-1*	–	2011
Multifloral-2	–	2011
Thyme	*Thymus vulgaris*	2011

*Multifloral is not a common name of a plant.

topped up to the mark with ultra-pure water in a 25-mL volumetric flask. The chromatographic conditions were those reported by Ciulu and others (2011).

Total polyphenols. Polyphenols content was determined according to Singleton and Rossi (1965).

Total flavonoids. A method described by Popova and others (2005) was used for total flavonoids determination.

Antioxidant activity

Capacity for scavenging 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The determination of ABTS radical scavenging was carried out as reported by Miguel (2010).

Oxygen radical activity capacity (ORAC). The ORAC method with fluorescein (FL) as the fluorescent probe was followed as described by Ou and others (2001) and ORAC values were calculated according to Cao and Prior (1999).

Nitric oxide-scavenging capacity. The nitric oxide (NO) scavenging activity was measured according to the method described by Ho and others (2010).

Chelating metal ions. The degree of chelating of ferrous ions by honey was evaluated according to Miguel and others (2010).

Statistical analysis

Statistical analysis was carried out by ANOVA through the SPSS 18.0 program (SPSS Inc., Chicago Ill., U.S.A.) and using the Tukey posthoc test at $P < 0.05$. Correlations between phenol and flavonoid contents and antioxidant activity were achieved by Pearson correlation coefficient (r) at a significance level of 99% ($P < 0.01$).

Results and Discussion

Physicochemical characterization

The obtained pH values ranged from 3.61 for citrus honey to 4.49 for strawberry tree honey (Table 2). Such values are within the range accepted for honey (Bogdanov and others 2004).

Gluconic acid is the main organic acid present in honey and responsible for its acidity, which is in a variable equilibrium with the respective glucono-lactone (Bogdanov and others 2004). Strawberry tree honey and citrus honey had the lowest and similar free acidities values, in contrast to the highest free acidity detected in pennyroyal honey (Table 2). Such free acidity values were within the limits of Portuguese and European legislations (EU Council 2002; DR 2003) and indicate the absence of undesirable fermentation. Lactonic acidity ranged from a minimum detected in thymus honey (8.17 meq/kg) to a maximum in eucalyptus honey (26.83 meq/kg). Total acidity varied between 32.32 (lavander-1 honey) and 58.00 meq/kg (pennyroyal honey). This last value was relatively high when compared to the maximum value (51.5 meq/kg) found in 1 sample of multifloral honey (*Eucalyptus*, *Erica*, and *Rubus*) from Portugal (Silva and others 2009).

Ash represents the direct measure of inorganic residues after honey carbonization. Honey richness in mineral content constitutes a quality parameter, which depends mainly on its floral origin (Marchini and others 2010). Ash values must not exceed 0.6% for nectar honeys (Codex Alimentarius 2001). Some honeys of this work had ash contents equal to or even more than that value: strawberry tree and pennyroyal honeys. The lowest values were found for lavender-1 (0.11%) and citrus (0.13%) honeys (Table 2), as reported for citrus honeys (Felsner and others 2004).

Electrical conductivity is a useful parameter for determining the botanical origin of honey and differentiating between nectar honey and honeydew (Bogdanov and others 2004). The values found in honey samples ranged from 199.20 μS/cm (lavender-1) to 941.67 μS/cm (pennyroyal). Two honey samples had values of electrical conductivity higher than the maximum limits defined by Portuguese legislation (cardoon and pennyroyal) (DR 2003).

Moisture percentage ranged from 17.2 in cardoon honey to 19.9% in multifloral-2 honey (Table 2) and was below the imposed limit of $\leq$20% for blossom honeys (Codex Alimentarius 2001). Such requirement will prevent the growth of molds on a honey surface, thus avoiding fermentation during storage (Mendes and others 1998).

The free proline content of the samples ranged from 341.82 (strawberry tree honey) to 1118.55 mg/kg (thyme honey) (Table 3). Values found were similar to those reported by others for the same unifloral honeys from several countries (Costa and others 1999; Hermosín and others 2003; Terrab and others 2003a, c; Serrano and others 2004; Saxena and others 2010) and always higher than 200 mg/kg as stated by Hermosín and others (2003).

Diastase activity indicates if honey was submitted to inadequate storage conditions or processing. Its values ranged from 2.2 to 30.0 Schade units/g (Table 3). Strawberry tree, citrus, and lavander-1 honeys had 2.2, 6.0, and 6.7 Schade units/g, respectively. The hydroxymethylfurfural (HMF) contents for the same samples were 8.1, 28.7, and 0.6 mg/kg, respectively (Table 2). According to Portuguese legislation, 3 samples had inappropriate diastase activity (<8 Schade units/g honey) (DR 2003). A minimal of 3 units is permitted, but only for citrus honey that is characterized by low levels of enzymes. Nevertheless, the HMF content should not exceed 15 mg/kg. This sample had 28.7 mg/kg. Some authors have suggested that such rules should be replaced, preferably to introduce a list of honeys that are low in enzymes and not connect diastase activity with HMF content (Thrasyvoulou and others 2012).

International regulations set a maximum HMF content of 40 mg/kg (Codex Alimentarius 2001; EU Council 2002). Only the carob honey exceeded this concentration. On the opposite side, only traces were possible to be found in cardoon honey, and impossible to quantify in our work.

Honey color is closely related to the content of minerals, phenols, pollen, and storage conditions and is characteristic of floral origin (Bertoncelj and others 2007; Naab and others 2008; Stawiarz and Wróblewska 2010). Citrus honey was white in

Table 2–Physicochemical results obtained from 13 Portuguese commercial honeys purchased in supermarkets of Algarve (Portugal).

Sample	pH	Free acidity (mEq/kg)	Lactonic acidity (mEq/kg)	Total acidity (mEq/kg)	Ash (%)	Electrical conductivity (μS/cm)	Moisture (%)
Strawberry tree	4.49 ± 0.05^{a}	20.17 ± 0.39^{j}	12.25 ± 0.65^{efg}	32.42 ± 0.80^{f}	0.70 ± 0.01^{a}	875.67 ± 2.80^{b}	19.8 ± 0.2^{ab}
Cardoon	4.43 ± 0.05^{a}	41.37 ± 0.39^{a}	11.67 ± 0.65^{fgh}	53.03 ± 0.80^{b}	0.55 ± 0.01^{c}	833.67 ± 2.80^{c}	17.2 ± 0.2^{g}
Carob	4.07 ± 0.05^{cd}	31.72 ± 0.39^{e}	12.42 ± 0.65^{efg}	44.13 ± 0.80^{de}	0.43 ± 0.01^{e}	627.33 ± 2.80^{f}	18.6 ± 0.2^{cde}
Citrus	3.61 ± 0.05^{gh}	20.20 ± 0.39^{j}	17.33 ± 0.65^{b}	37.53 ± 0.80^{f}	0.13 ± 0.01^{ij}	218.60 ± 2.80^{k}	18.2 ± 0.2^{def}
Heather	4.09 ± 0.05^{cd}	35.23 ± 0.39^{d}	10.00 ± 0.65^{hi}	45.23 ± 0.80^{d}	0.48 ± 0.01^{d}	738.67 ± 2.80^{d}	19.0 ± 0.2^{bcd}
Eucalyptus	3.84 ± 0.05^{ef}	21.77 ± 0.39^{i}	26.83 ± 0.65^{a}	48.60 ± 0.80^{c}	0.26 ± 0.01^{g}	471.00 ± 2.80^{g}	18.6 ± 0.2^{cde}
Sunflower	3.84 ± 0.05^{ef}	25.50 ± 0.39^{h}	13.83 ± 0.65^{de}	39.33 ± 0.80^{f}	0.15 ± 0.01^{hi}	234.93 ± 2.80^{j}	19.2 ± 0.2^{abc}
Lavender-1	3.57 ± 0.05^{h}	21.82 ± 0.39^{i}	10.50 ± 0.65^{gh}	32.32 ± 0.80^{g}	0.11 ± 0.01^{j}	199.20 ± 2.80^{l}	18.1 ± 0.2^{efg}
Lavender-2	4.27 ± 0.05^{b}	27.38 ± 0.39^{g}	15.17 ± 0.65^{cd}	42.55 ± 0.80^{e}	0.18 ± 0.01^{h}	374.67 ± 2.80^{h}	19.2 ± 0.2^{abc}
Pennyroyal	4.18 ± 0.05^{bc}	42.17 ± 0.39^{a}	15.83 ± 0.65^{bc}	58.00 ± 0.80^{a}	0.61 ± 0.01^{b}	941.67 ± 2.80^{a}	18.1 ± 0.2^{ef}
Multifloral-1	3.87 ± 0.05^{ef}	39.75 ± 0.39^{b}	13.00 ± 0.65^{ef}	52.75 ± 0.80^{b}	0.33 ± 0.01^{f}	468.67 ± 2.80^{g}	19.0 ± 0.2^{cde}
Multifloral-2	3.75 ± 0.05^{fg}	28.62 ± 0.39^{f}	11.17 ± 0.65^{fgh}	39.78 ± 0.80^{f}	0.17 ± 0.01^{h}	358.00 ± 2.80^{i}	19.9 ± 0.2^{a}
Thyme	3.96 ± 0.05^{de}	37.20 ± 0.39^{c}	8.17 ± 0.65^{a}	45.37 ± 0.80^{d}	0.45 ± 0.01^{de}	665.67 ± 2.80^{e}	17.5 ± 0.2^{fg}

Values in the same column followed by the same letter are not significant different ($P < 0.05$) by the Tukey's multiple range test.

Table 3–Honey color, proline, diastase, and HMF results obtained from 13 Portuguese commercial honeys purchased in supermarkets of Algarve (Portugal).

Sample	Pfund scale (mm)	Honey color	Proline (mg/kg)	Diastase (Schade units/g)	HMF (mg/kg)
Strawberry tree	88.9 ± 6.5	Amber	341.82 ± 14.36^{h}	2.25 ± 2.19^{h}	8.2 ± 0.6^{e}
Cardoon	131.9 ± 6.1	Dark amber	813.82 ± 14.36^{c}	33.75 ± 2.19^{a}	–
Carob	153.2 ± 5.3	Dark amber	682.18 ± 14.36^{d}	13.75 ± 2.19^{defg}	41.8 ± 0.6^{a}
Citrus	30.5 ± 3.5	White	453.09 ± 14.36^{g}	6.15 ± 2.19^{gh}	28.2 ± 0.6^{b}
Heather	208.2 ± 2.3	Dark amber	1044.36 ± 14.36^{b}	20.70 ± 2.19^{bcd}	22.8 ± 0.6^{c}
Eucalyptus	54.9 ± 3.0	Light amber	462.55 ± 14.36^{g}	16.10 ± 2.19^{def}	1.8 ± 0.6^{g}
Sunflower	97.6 ± 3.1	Amber	647.73 ± 14.36^{d}	21.55 ± 2.19^{bcd}	8.1 ± 0.6^{e}
Lavender-1	39.4 ± 1.8	Light extra amber	470.54 ± 14.36^{g}	6.90 ± 2.19^{gh}	0.6 ± 0.6^{g}
Lavender-2	49.4 ± 3.5	Light extra amber	1029.82 ± 14.36^{b}	11.55 ± 2.19^{efg}	12.8 ± 0.6^{d}
Pennyroyal	98.8 ± 7.7	Amber	665.45 ± 14.36^{d}	28.25 ± 2.19^{ab}	4.1 ± 0.6^{f}
Multifloral-1	65.2 ± 2.4	Light amber	543.27 ± 14.36^{f}	18.95 ± 2.19^{cde}	10.2 ± 0.6^{e}
Multifloral-2	170.1 ± 3.3	Dark amber	604.37 ± 14.36^{e}	10.55 ± 2.19^{fg}	23.1 ± 0.6^{c}
Thyme	68.5 ± 3.0	Light amber	1118.54 ± 14.36^{a}	26.25 ± 2.19^{bc}	1.6 ± 0.6^{g}

Values in the same column followed by the same letter are not significant different ($P < 0.05$) by the Tukey's multiple range test.

Table 4–Mineral content in 13 Portuguese commercial honeys purchased in supermarkets of Algarve (Portugal).

Sample	Sodium (mg/kg)	Potassium (mg/kg)	Calcium (mg/kg)	Magnesium (mg/kg)	Na + K + Ca + Mg (mg/kg)
Strawberry tree	161.02 ± 1.54^{c}	1736.29 ± 9.82^{a}	24.92 ± 0.59^{g}	50.00 ± 0.71^{c}	1972.23
Cardoon	208.10 ± 1.54^{b}	1341.16 ± 9.82^{c}	46.63 ± 0.59^{d}	42.12 ± 0.71^{e}	1638.02
Carob	138.10 ± 1.54^{e}	723.28 ± 9.82^{e}	46.89 ± 0.59^{d}	79.09 ± 0.71^{a}	987.36
Citrus	56.34 ± 1.54^{i}	170.07 ± 9.82^{j}	9.81 ± 0.59^{j}	28.18 ± 0.71^{h}	264.40
Heather	155.45 ± 1.54^{d}	1196.31 ± 9.82^{d}	31.51 ± 0.59^{f}	45.15 ± 0.71^{d}	1428.41
Eucalyptus	156.06 ± 1.54^{d}	483.81 ± 9.82^{g}	20.27 ± 0.59^{h}	51.82 ± 0.71^{c}	711.97
Sunflower	87.93 ± 1.54^{g}	276.86 ± 9.82^{i}	24.92 ± 0.59^{g}	68.18 ± 0.71^{b}	457.9
Lavender-1	41.47 ± 1.54^{j}	78.09 ± 9.82^{k}	6.84 ± 0.59^{k}	17.88 ± 0.71^{i}	144.27
Lavender-2	62.53 ± 1.54^{h}	173.17 ± 9.82^{j}	14.46 ± 0.59^{i}	32.43 ± 0.71^{g}	282.58
Pennyroyal	161.64 ± 1.54^{c}	1582.56 ± 9.82^{b}	70.92 ± 0.59^{b}	39.09 ± 0.71^{f}	1854.21
Multifloral-1	241.55 ± 1.54^{a}	735.11 ± 9.82^{e}	61.87 ± 0.59^{c}	16.06 ± 0.71^{i}	1054.59
Multifloral-2	132.53 ± 1.54^{f}	560.68 ± 9.82^{f}	41.20 ± 0.59^{e}	44.55 ± 0.71^{d}	778.96
Thyme	61.30 ± 1.54^{h}	341.91 ± 9.82^{h}	74.15 ± 0.59^{a}	68.79 ± 0.71^{b}	546.15

Values in the same column followed by the same letter are not significant different ($P < 0.05$) by the Tukey's multiple range test.

color, immediately followed by lavender honey (light extra amber) (Table 3). These lighter colors are in accordance with those reported by González-Miret and others (2005). Heather, multifloral, and carob honeys were dark amber (>140 mm Pfund). This dark color of heather and carob honeys is a characteristic already reported by Terrab and others (2003a). A positive correlation was found between phenol, flavonoid content, and color of honey samples ($r = 0.685$, $P < 0.01$; $r = 0.843$, $P < 0.01$) (Table 7). Such correlation has also been reported for other honey samples (Bertoncelj and others 2007; Stawiarz and Wróblewska 2010; Isla and others 2011).

Mineral content is a potential indicator of geographical origin of honey (Anklan 1998). Potassium was quantitatively the most important mineral among the 4 evaluated (Table 4). Strawberry tree honey had the highest *K* concentration, while lavender had the lowest. Three more honeys also had relative high concentrations of

Table 5–Sugar content (g/kg) in 13 Portuguese commercial honeys purchased in supermarkets of Algarve (Portugal).

Sample	Fructose	Glucose	Sucrose	Turanose	Maltose	Trehalose	Melezitose	Glucose + Fructose
Strawberry tree	359.63 ± 4.47^{f}	267.43 ± 3.35^{de}	3.75 ± 0.39^{e}	16.49 ± 1.51^{fg}	21.53 ± 0.66^{f}	10.43 ± 0.66^{de}	5.38 ± 0.43^{de}	627.06
Cardoon	357.03 ± 4.47^{f}	250.20 ± 3.35^{f}	3.70 ± 0.39^{e}	25.98 ± 1.51^{bc}	33.23 ± 0.66^{ab}	17.50 ± 0.66^{a}	37.11 ± 0.43^{a}	607.23
Carob	379.64 ± 4.47^{d}	263.72 ± 3.35^{e}	3.23 ± 0.39^{e}	25.96 ± 1.51^{bc}	30.01 ± 0.66^{c}	10.54 ± 0.66^{de}	5.85 ± 0.43^{de}	643.36
Citrus	401.80 ± 4.47^{bc}	271.10 ± 3.35^{de}	3.33 ± 0.39^{e}	27.76 ± 1.51^{b}	29.45 ± 0.66^{cd}	12.12 ± 0.66^{cd}	10.35 ± 0.43^{c}	672.9
Heather	359.63 ± 4.47^{f}	267.43 ± 3.35^{de}	2.76 ± 0.39^{e}	26.83 ± 1.51^{bc}	32.88 ± 0.66^{ab}	10.02 ± 0.66^{de}	1.07 ± 0.43^{g}	627.06
Eucalyptus	365.61 ± 4.47^{def}	275.93 ± 3.35^{cd}	3.54 ± 0.39^{e}	20.95 ± 1.51^{def}	34.68 ± 0.66a	13.19 ± 0.66^{bc}	3.99 ± 0.43^{f}	641.54
Sunflower	412.44 ± 4.47^{ab}	380.90 ± 3.35^{a}	23.48 ± 0.39^{b}	14.09 ± 1.51^{g}	15.12 ± 0.66h	4.42 ± 0.66^{g}	–	793.34
Lavender-1	398.12 ± 4.47^{c}	264.06 ± 3.35^{e}	3.70 ± 0.39^{e}	26.26 ± 1.51^{bc}	29.92 ± 0.66c	12.72 ± 0.66^{bc}	11.91 ± 0.43^{b}	662.18
Lavender-2	416.61 ± 4.47^{a}	285.21 ± 3.35^{c}	5.04 ± 0.39^{c}	18.68 ± 1.51^{efg}	23.69 ± 0.66e	10.48 ± 0.66^{de}	9.83 ± 0.43^{c}	701.82
Pennyroyal	364.11 ± 4.47^{ef}	246.68 ± 3.35^{f}	28.60 ± 0.39^{a}	33.09 ± 1.51^{a}	12.60 ± 0.66i	7.94 ± 0.66^{f}	–	610.79
Multifloral-1	392.71 ± 4.47^{c}	296.63 ± 3.35^{b}	3.82 ± 0.39^{e}	22.04 ± 1.51^{cde}	27.83 ± 0.66d	14.37 ± 0.66^{b}	6.44 ± 0.43^{d}	689.34
Multifloral-2	378.20 ± 4.47^{de}	296.90 ± 3.35^{b}	3.74 ± 0.39^{e}	17.66 ± 1.51^{efg}	18.83 ± 0.66g	9.73 ± 0.66^{ef}	4.75 ± 0.43^{ef}	675.1
Thyme	370.46d ± 4.47^{ef}	247.94 ± 3.35^{f}	4.07 ± 0.39^{cd}	24.08 ± 1.51^{bcd}	31.38 ± 0.66bc	13.85 ± 0.66^{bc}	5.20 ± 0.43^{def}	618.4

Values in the same column followed by the same letter are not significant different ($P < 0.05$) by the Tukey's multiple range test.

Table 6–Vitamin C, phenols, and flavonoid contents and antioxidant activities of 13 Portuguese commercial honeys purchased in supermarkets of Algarve (Portugal).

Sample	Vitamin C (mg/kg)	Phenols (mg GAE/100 g)	Flavonoids (mg QE/100 g)	TEAC (IC_{50} = mg/mL)	ORAC (μmol TE/g	Chelating (IC_{50} = mg/mL)	NO (IC_{50} = mg/mL)
Strawberry tree	71.57 ± 3.16^{g}	117.65 ± 2.21^{a}	9.66 ± 0.80^{c}	0.44 ± 0.03^{i}	39.55 ± 0.61^{b}	2.60 ± 0.34^{e}	9.83 ± 2.34^{g}
Cardoon	93.43 ± 3.16^{cde}	102.82 ± 2.21^{b}	13.32 ± 0.80^{b}	0.85 ± 0.03^{h}	42.75 ± 0.61^{a}	2.32 ± 0.34^{e}	27.14 ± 2.34^{e}
Carob	138.87 ± 3.16^{a}	74.20 ± 2.21^{cd}	13.34 ± 0.80^{b}	1.55 ± 0.03^{f}	9.99 ± 0.61^{e}	6.40 ± 0.34^{b}	39.73 ± 2.34^{d}
Citrus	77.70 ± 3.16^{fg}	32.10 ± 2.21^{g}	1.73 ± 0.80^{e}	4.04 ± 0.03^{a}	3.28 ± 0.61^{g}	3.79 ± 0.34^{cd}	–
Heather	109.73 ± 3.16^{b}	117.59 ± 2.21^{a}	21.16 ± 0.80^{a}	0.86 ± 0.03^{h}	22.58 ± 0.61^{c}	4.37 ± 0.34^{cd}	17.51 ± 2.34^{f}
Eucalyptus	79.27 ± 3.16^{fg}	54.25 ± 2.21^{f}	5.28 ± 0.80^{d}	2.86 ± 0.03^{c}	7.40 ± 0.61^{f}	3.16 ± 0.34^{de}	73.07 ± 2.34^{a}
Sunflower	98.57 ± 3.16^{c}	36.69 ± 2.21^{g}	1.93 ± 0.80^{e}	3.17 ± 0.03^{b}	0.41 ± 0.61^{h}	4.29 ± 0.34^{cd}	53.60 ± 2.34^{bc}
Lavender-1	87.63 ± 3.16^{def}	31.85 ± 2.21^{g}	3.09 ± 0.80^{de}	3.98 ± 0.03^{a}	7.43 ± 0.61^{f}	9.09 ± 0.34^{a}	–
Lavender-2	83.87 ± 3.16^{ef}	34.13 ± 2.21^{g}	3.15 ± 0.80^{de}	4.03 ± 0.03^{a}	7.57 ± 0.61^{f}	4.25 ± 0.34^{cd}	45.56 ± 2.34^{d}
Pennyroyal	140.37 ± 3.16^{a}	79.58 ± 2.21^{c}	9.27 ± 0.80^{c}	1.35 ± 0.03^{g}	5.66 ± 0.61^{f}	4.86 ± 0.34^{c}	55.54 ± 2.34^{b}
Multifloral-1	95.73 ± 3.16^{cd}	53.76 ± 2.21^{f}	5.30 ± 0.80^{d}	1.73 ± 0.03^{e}	11.73 ± 0.6^{e}	0.61 ± 0.34^{f}	45.16 ± 2.34^{d}
Multifloral-2	135.13 ± 3.16^{a}	67.99 ± 2.21^{de}	8.06 ± 0.80^{c}	2.00 ± 0.03^{d}	14.21 ± 0.61^{d}	8.57 ± 0.34^{a}	24.03 ± 2.34^{ef}
Thyme	84.53 ± 3.16^{ef}	62.91 ± 2.21^{e}	5.62 ± 0.80^{d}	1.59 ± 0.03^{f}	10.58 ± 0.61^{e}	3.19 ± 0.34^{de}	46.62 ± 2.34^{cd}

Values in the same column followed by the same letter are not significant different ($P < 0.05$) by the Tukey's multiple range test

Table 7–Pearson correlation coefficients.

	TEAC	ORAC	NO	Chelating activity	Phenols	Flavonoids	Na + K + Ca + Mg
Phenols	−0.917**	0.822**	−0.735**	–	1	0.861**	0.886**
Flavonoids	−0.750**	0.580**	−0.570**	–	0.861**	1	0.680**
Na + K + Ca + Mg	−0.887**	0.699**	−0.497*	–	0.886**	0.680**	1
Ascorbic acid	–	–	–	–	–	–	–
Proline	–	–	–	–	–	–	–
Color	−0.655**	0.420*	−0.623**	–	0.685**	0.843**	0.494**

**Correlation is significant at the $P < 0.01$; *Correlation is significant at the $P < 0.05$; – not significant.

potassium: pennyroyal, cardoon, and heather. With the exception of thyme honey, sodium was the second mineral more important. The concentration of calcium was superior to that of magnesium in strawberry tree, carob, citrus, heather, eucalyptus, sunflower, and lavender samples. The opposite was found in pennyroyal and multifloral honeys. Similar concentrations of both minerals were detected in cardoon, multifloral-1, and thyme honeys. All values found in the samples were within the ranges reported for honeys from other countries (Terrab and others 2003b; González-Miret and others 2005) and even from Portugal, particularly of the Luso region (Silva and others 2009).

A correlation between honeys color and their mineral content has been stated by González-Miret and others (2005) and Nalda and others (2005). According to these authors, lower amounts of minerals originate lighter honeys as found in our samples of lavender and citrus. A positive correlation was found between the sum of sodium, potassium, calcium, and magnesium contents and the color of honeys ($r = 0.494$, $P < 0.01$) (Table 7).

Guler and others (2007) demonstrated that electrical conductivity has a strong positive correlation with potassium. Honey samples with the highest potassium amounts also had the highest electrical conductivity: strawberry tree, pennyroyal, and cardoon honeys (Table 2).

Fructose and glucose were the main sugars present in honey samples as expected. The concentration values of fructose ranged from 357.03 g/kg in cardoon honey to 416.61 g/kg in lavender-2 honey (Table 5). The lowest concentration of glucose was found in pennyroyal honey, whereas the highest concentration was detected in sunflower honey. The total fructose and glucose content in honey samples ranged from 607.23 g/kg in cardoon honey to 793.34 g/kg in sunflower honey. The highest contents of glucose and fructose are in accordance with those reported by Mateo and Bosch-Reig (1997).

According to the obtained results, all samples had the minimal concentrations of glucose and fructose permitted by law for blossom honeys (>60%). Sucrose cannot exceed 5%, except for some exceptions (Codex Alimentarius 2001). In our case, all samples meet the requirements for honeys. Pennyroyal and sunflower honeys had the lowest amounts of maltose. Pennyroyal had the highest amount of turanose. The lowest level of this disaccharide was found in sunflower honey. This also had the lowest concentration of trehalose in contrast to cardoon honey. It is noteworthy to refer the relative high concentration of melezitose in cardoon honey that was not possible to quantify in sunflower and pennyroyal honeys. According to Bogdanov and others (2004), melezitose is absent in blossom honeys, whereas honeydew honeys have relatively high concentrations of that trisaccharide. Nevertheless, Mateo and Bosch-Reig (1997) and Ruoff (2006) found melezitose in some unifloral honeys. In our case, sunflower honey and pennyroyal honey were the sole samples in which no melezitose was found as reported for sunflower honey (Mateo and Bosch-Reig 1997).

Vitamin C, phenolic, and flavonoid content, and antioxidant activity

Pennyroyal, carob, and the multifloral-2 honeys had high concentrations of vitamin C (Table 6), in contrast to that found in the strawberry tree honey. The concentrations found were within the range described for other floral honeys from north Portugal (Ferreira and others 2009). In Spanish honeys, León-Ruiz and others (2011) found a wide range of concentrations of vitamin C. The ascorbic acid contents described by these authors, as well as those found in the present work, were far from those reported by Ciulu and others (2011). Nevertheless, other samples of the same floral origin had significantly lower concentrations (2 mg/kg).

Total phenolic content (mg of gallic acid equivalent/100 g of honey) varied from 31.85 in lavender-1 honey to 117.65 in strawberry tree and heather honeys. The flavonoid content (mg of quercetin equivalent/100 g of honey) ranged from 1.93 in sunflower to 21.16 in heather honey (Table 6). Others also reported that polyphenols in honey varied according to its floral origin (Küçük and others 2007; Escuredo and others 2011). A study performed by Rosa and others (2011) found that strawberry tree honey was the richest in total phenols when compared to citrus, eucalyptus, and heather honeys, as was found in the present work. Nevertheless, total phenols in our heather honey were substantially higher than that reported by those authors. Citrus and eucalyptus honeys from Mexico had higher phenol contents than those reported here (Rodríguez and others 2012). These results, along with those of Rosa and others (2011), reveal that the geographical origin is also important for the phenol content of honeys of the same floral origin.

The studied honey had phenol and flavonoid contents similar to those of different origin (Beretta and others 2005; Saxena and others 2010; Escuredo and others 2011). Nevertheless, the amounts of flavonoids in the present work were significantly lower when compared to those from Trás-os-Montes (Portugal) (Estevinho and others 2012) but within the range found by Sant'Ana and others (2012) for various unifloral honeys from Brazil.

A correlation between total flavonoid content and total phenols ($r = 0.861$, $P < 0.01$) was found (Table 7) as reported by Al and others (2009), but contrarily to Meda and others (2005) and Kamboj and others (2013) who found no correlation.

The determination of minerals in honeys is a good procedure for the botanical and geographical authentication, because minerals are linked to the soil and vegetation from the area where honey is produced (Marghitas and others 2010). Such may partly explain the correlation between the sum of sodium, potassium, calcium, and magnesium and the content of phenols ($r = 0.886$, $p < 0.01$) and flavonoids ($r = 0.680$, $P < 0.01$) (Table 7) found in the present work. Nevertheless, such must be confirmed in further studies.

In the present work, the antioxidant ability was evaluated by the capacity for scavenging free radicals (ABTS, peroxyl, and nitric oxide); and by the capacity for chelating metal ions. All samples showed antioxidant activities. The capacity for scavenging free radicals had already been reported by Estevinho and others (2008) for northeast Portugal honeys. Such capacity was less than those reported in the present work, which may be attributed to the different methods used.

Strawberry tree honey was significantly the most effective as antioxidant, immediately followed by cardoon and heather honeys (Table 6). Citrus and both lavender honeys had the lowest capacity for scavenging ABTS free radicals. Cardoon and strawberry tree honeys were also the most effective for scavenging peroxyl radicals, as measured by the ORAC method, immediately followed by the heather honey. The lowest capacity was found in sunflower and citrus honeys.

Concerning the capacity for scavenging nitric oxide, strawberry tree honey was significantly the most active in contrast to that of citrus and lavender-1. In conclusion, strawberry tree honey had the best antioxidant activity, independent of the method used for its determination. The lowest activity detected for citrus honey had already been reported by Perna and others (2012). Rosa and others (2011) reported that strawberry tree honey was also the most active honey in the capacity for scavenging free radicals in contrast to citrus or eucalyptus honeys.

Nitric oxide is formed during infections and inflammations at high concentrations. In aerobic conditions, the NO molecule reacts with the oxygen to produce NO_2, N_2O_4, N_3O_4, nitrate, and nitrite. NO is also able to react with superoxide originating peroxynitrite. All of these products formed are genotoxic (Jagetia and others 2004). Therefore, strawberry tree honey being able to scavenge NO shows an important role in the protection of human health.

Multifloral-1 honey had the best chelating capacity followed by cardoon and strawberry tree honeys, in contrast to that of lavender-1. Zhou and others (2012) detected weak chelating activity in their samples, which could be explained by the absence of some type of flavonoids mainly those possessing *o*-diphenolic groups in the 3,4-dihydroxy position in ring B and the ketol structure, 4-oxo, 3-OH or 4-oxo, 5-OH in the C ring of the flavonols. According to this statement, a greater proportion of this kind of flavonoids in Multifloral-1 honey may partially explain our results.

The capacity for scavenging free radicals by honey samples correlated well with the contents of phenols and flavonoids. The Pearson correlation coefficients (r) showed a strong relationship between the phenol content and peroxyl-scavenging ability of samples ($r = 0.822$, $P < 0.01$) and a negative correlation between phenol contents and IC_{50} values for ABTS and NO for which values detected were $r = -0.917$, $P < 0.01$; and $r = -0.735$, $P < 0.01$, respectively. The activities also correlated with the content of flavonoids: $r = 0.580$, $P < 0.01$, between scavenging peroxyl radicals and flavonoid content, and $r = -750$ and $r = -0.570$ in both cases with $P < 0.01$, between flavonoid content and IC_{50} values for the capacity of scavenging ABTS and NO radicals, respectively.

Some authors have demonstrated that minerals exert synergism effect in antioxidant ability when combined with phenols because the charges formed in metals after donating electrons are readily stabilized by the phenolic compounds (Sant'Ana and others 2012). Escuredo and others (2013) also found that the antioxidant capacity of honeys was strongly related to the flavonoid and mineral contents. In our case, a relationship was also found between the sum of sodium, potassium, calcium, and magnesium and the capacity for scavenging free radicals: ABTS radicals ($r = -0.887$, $P < 0.01$), peroxyl radicals ($r = 0.699$, $P < 0.01$), NO radicals ($r = 0.497$, $P < 0.05$) (Table 7).

In our work, the antioxidant activity correlated well with amounts of phenols, flavonoids, and minerals but not with proline or ascorbic acid contents as Meda and others (2005) and Khalil and others (2012) had reported for honey samples.

Since a relationship was found between phenol, flavonoid contents, and color, as well as between phenols, flavonoids, and antioxidant activity, a correlation between color and antioxidant activity will be expectable. In fact, our results showed correlations between color and the capacity for scavenging ABTS and NO radicals ($r = -0.655$ and $r = -0.623$, respectively, in both cases with $P < 0.01$) (Table 7), whereas between color and the capacity for scavenging peroxyl radicals, the correlation was $r = 0.420$, $P < 0.05$. In this way, darker honeys will present better antioxidant activities than the lighter ones. Relationships between antioxidant activity and color of honeys have also been reported by other authors (Frankel and others 1998; Beretta and others 2005; Bertoncelj and others 2007; Isla and others 2011).

Conclusion

Strawberry tree, cardoon, and heather honeys were the most effective for scavenging ABTS, peroxyl, and NO free radicals. Concerning the ability for chelating metal ions, 1 sample of multifloral honey had the best capacity followed by cardoon and strawberry tree honeys.

Electrical conductivity and diastase activity were out of the limits permitted by European legislation in some honey samples. Citrus, strawberry tree, and 1 sample of lavender honeys had values of diastase activity below that recommended by European legislation.

The present work reinforces the opinion of some researchers who ask that a list of honeys with low enzymatic activity be introduced for replacing the actual legislation that defines a relationship between diastase activity and HMF amount.

References

Al ML, Daniel D, Moise A, Bobis O, Laslo L, Bogdanov S. 2009. Physico-chemical and bioactive properties of different floral origin honeys from Romania. Food Chem 112:863–7.

Anklan E. 1998. A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chem 63:549–62.

Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal Chim Acta 533:185–91.

Bertoncelj E, Dobersek U, Jamnik M, Golob T. 2007. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem 105:822–8.

Bogdanov S. 2002. Harmonized Methods of the International Honey Commission. (http://www.apiculturacluj.com/ApiculturaCluj/italiano/Documents/IHCmethods_e.pdf) (most recent access date).

Bogdanov S, Ruoff K, Oddo LP. 2004 Physico-chemical methods for the characterisation of unifloral honeys: a review. Apidologie 35:S4-17.

Cao GH, Prior RL. 1999. Measurement of oxygen radical absorbance capacity in biological samples. Meth Enzymol 299:50–62.

Ciulu M, Solinas S, Floris I, Panzanelli A, Pilo MI, Piu PC, Spano N, Sanna G. 2011. RP-HPLC detection of water-soluble vitamins in honey. Talanta 82:924–9.

Codex Alimentarius C. 2001. *Revised Codex Standard for Honey*, Codex STAN 12-1981, Rev. 1 1987, Rev. 2.

Costa LSM, Albuquerque MLS, Trugo LC, Quinteiro LMC, Barth OM, Ribeiro M, de Maia CAB. 1999. Determination of non-volatile compounds of different botanical origin Brazilian honeys. Food Chem 65:347–52.

DR. 2003 Decreto-lei N° 214/2003 (Portuguese Legislation) 18 September 2003.

Escuredo O, Míguez M, Fernández-González M, Seijo MC. 2013. Nutritional value and antioxidant activity of honeys produced in na European Atlantic area. Food Chem 138: 851–6.

Escuredo O, Seijo MC, Fernández-González M. 2011. Descriptive analysis of Rubus honey from the north-west of Spain. Intl J Food Sci Technol 46:2329–36.

Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E. 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem Toxicol 46:3774–9.

Estevinho LM, Feás X, Seijas JA, Vázquez-Tato MP. 2012. Organic honey from Trás-os-Montes region (Portugal): chemical, palynological, microbiological and bioactive compounds characterization. Food Chem Toxicol 50:258–64.

EU Council. 2002 Council directive 2001/11 O/EC of 20 December 2001 relating to honey. Off J Eur Commun L10:47–52.

Felsner ML, Cano CB, Bruns RE, Watanabe HM, Almeida-Muradian LB, Matos JR. 2004. Characterization of monofloral honeys by ash contents through a hierarchical design. J Food Compos Anal 17:737–47.

Ferreira ICFR, Aires E, Barreira JCM, Estevinho LM. 2009. Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. Food Chem 114:1438–43.

Frankel S, Robinson GE, Berenbaum MR. 1998. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. J Apic Res 37:27–31.

Gheldof N, Engeseth NJ. 2002. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. J Agric Food Chem 50:3050–5.

Gomes S, Dias LG, Moreira LL, Rodrigues P, Estevinho L. 2010. Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food Chem Toxicol 48:544–8.

González-Miret ML, Terrab A, Hernanz D, Fernández-Recamales MA, Heredia FJ. 2005. Multivariate correlation between color and mineral composition of honeys and by their botanical origin. J Agric Food Chem 53:2574–80.

Guler A, Bakan A, Nisbet C, Yavuz O. 2007. Determination of important biochemical properties of honey to discriminate pure and adulterated honey with sucrose (*Saccharum officinarum* L.) syrup. Food Chem 105:1119–25.

Hermosín I, Chicón RM, Cabezudo MD. 2003. Free aminoacid composition and botanical origin of honey. Food Chem 83:263–8.

Ho SC, Tang YL, Lin SM, Liew YF. 2010. Evaluation of peroxynitrite scavenging capacities of several commonly used fresh spices. Food Chem 119:1102–7.

Isla MI, Craig A, Ordoñez R, Zampini C, Sayago J, Bedascarrasbure E, Alvarez A, Salomón V, Maldonado L. 2011. Physico chemical and bioactive properties of honeys from Northwestern Argentina. LWT-Food Sci Technol 44:1922–30.

Jagetia GC, Rao SK, Baliga MS, Babu KS. 2004. The evolution of nitric oxide scavenging activity of certain herbal formulations *in vitro*: a preliminary study. Phytother Res 18: 561–5.

Kamboj R, Bera MB, Nanda V. 2013. Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. Intl J Food Sci Technol 48: 578–87.

Khalil Md I, Moniruzzaman M, Boukraâ L, Benhanifia M, Md Islam A, Islam Md, Sulaiman SA, Gan SH. 2012. Physicochemical and antioxidant properties of Algerian honey. Molecules 17:11199–215.

Küçük M, Kolayl S, Karaoglu S, Ulusoy E, Baltac C, Candan F. 2007. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem 100:526–34.

Lachman J, Orsák M, Hejmánková A, Kovářová E. 2010. Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT 43:52–8.

León-Ruiz V, Vera S, González-Porto AV, Andrés MPS. 2011. Vitamin C and sugar levels as simple markers for discriminating Spanish honey sources. J Food Sci 76:C356–61.

Marchini PV, Moreti ACCC, Otsuk IP Sodré GS. 2007. Physicochemical composition of *Apis mellifera* honey samples from São Paulo, Brazil. Quim Nova 30:1653–7.

Marghitas LA, Dezmirean DS, Pocol CB, Ilea M, Boleis O, Gergen I. 2010. The development of biochemical profile of *Acacia* honey by identifying biochemical determinants of its quality. Not Bot Hort Agrobot Cluj 38:84–90.

Mateo R, Bosch-Reig F. 1997. Sugar profiles of Spanish unifloral honeys. Food Chem 60: 33–41.

Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 91:571–7.

Mendes E, Proença EB, Ferreira IMPLVO, Ferreira MA. 1998. Quality of evaluation of Portuguese honey. Carbohydr Polym 37:219–23.

Miguel MG. 2010. Antioxidant activity of medicinal and aromatic plants. A review. Flavour Fragr J 25:291–312.

Miguel MG, Nunes S, Dandlen SA, Cavaco AM, Antunes MD. 2010. Phenols and antioxidant activity of hydro-alcoholic extracts of propolis from Algarve, South of Portugal. Food Chem Toxicol 48:3418–23.

Naab OA, Tamame MA, Caccavari MA. 2008. Palynological and physicochemical characteristics of three unifloral honey types from central Argentina. Span J Agric Res 6:566–76.

Nalda MJN, Yagüe JLB, Calva JCD, Gómez MTM. 2005. Classifying honeys from the Soria Province of Spain via multivariate analysis. Anal Bioanal Chem 382:311–9.

Ou B, Hampsch-Woodill M, Prior RL. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J Agric Food Chem 49:4619–26.

Perna A, Simonetti A, Intaglietta I, Sofo A, Gambacorta F. 2012. Metal content of southern Italy honey of different botanical origins and its correlation with polyphenol content and antioxidant activity. Intl J Food Sci Technol 47:1909–17.

Popova M, Silici S, Kaftanoglu O, Bankova V. 2005. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine 12:221–8.

Rodríguez BA, Mendoza S, Iturriga MH, Castaño-Tostado E. 2012. Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. J Food Sci 71:C121–7.

Rosa A, Tuberoso CIG, Atzeri A, Melis MP, Bifulco E, Dessì MA. 2011. Antioxidant profile of strawberry tree honey and its marker homogentisic acid in several models of oxidative stress. Food Chem 129:1045–53.

Ruoff K. 2006. Authentication of the botanical origin of honey. [PhD thesis], ETH, Zurich.
Sant'Ana LDO, Sousa JPLMS, Salgueiro FB, Lorenzon CMF, Castro RN. 2012. Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. J Food Sci 71:C135-40.
Saxena S, Gautam S, Sharma A. 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem 118:391–7.
Serrano S, Villarejo M, Espejo R, Jodral M. 2004. Chemical and physical parameters of Andalusian honey: classification of *Citrus* and *Eucalyptus* honeys by discriminant analysis. Food Chem 87:619–25.
Silva LR, Videira R, Monteiro AP, Valentão P, Andrade PB. 2009. Honey from Luso region (Portugal): physico-chemical characteristics and mineral contents. Microchem J 93:73–7.
Singleton VL, Rossi JA Jr. 1965. Colorunetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144–58.
Stawiarz E, Wróblewka A. 2010. Melissopalynological analysis of multifloral honeys from the Sandomierska upland area of Poland. J Apic Sci 54:65–75.
Terrab A, Díez MJ, Heredia FJ. 2003a. Palynological, physico-chemical and colour characterization of Moroccan honeys: III. Other unifloral honey types. Intl J Food Sci Technol 38:395–402.
Terrab A, Díez MJ, Heredia FJ. 2003b. Palynological, physico-chemical and colour characterization of Moroccan honeys: II. Orange (*Citrus* sp.) honey. Intl J Food Sci Technol 38: 387–94.
Terrab A, Díez MJ, Heredia FJ. 2003c. Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (*Eucalyptus camaldulensis* Dehnh) honey. Intl J Food Sci Technol 38:379–86.
Terrab A, Recamales AF, Hernanz D, Heredia FJ. 2004. Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. Food Chem 88: 537–42.
Thrasyvoulou A, Tananaki C, Dimou M, Karazafiris E, Goras G, El Taj HF, Liolios B, Kanelis D. 2012. http://pt.scribd.com/doc/106951928/Legislation-Thrasyvoulou-2012-SUGGESTION-FOR-IMPROVEMENTS-OG-EUROPEAN-LEGISLATION-GOVERNING-BEE-PRODUCTS. Accessed 2012 March 11.
Zhou J, Li P, Cheng N, cao H, Wang B, Wei Y, Cao W. 2012. Protective effects of buckwheat honey on DNA damage induced by hydroxyl radicals. Food Chem Toxicol 50: 2766–73.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Antioxidant activity of commercial honey