Arabian Journal of Chemistry (2018) **11**, 265–274

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia

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Received 7 April 2013; accepted 20 August 2014
Available online 29 August 2014

KEYWORDS

Tunisian honeys;
Physicochemical parameters;
Bioactive compounds;
Antioxidant activity

Abstract The present study was undertaken to determine the physicochemical, biochemical, and antioxidant activities of Tunisian honey samples. All the extracted honey samples appeared to conform to the European Legislation (EC Directive 2001/110) for all parameters. Mint honey, for instance, possesses significant pH value ($p < 0.05$), invertase activity, water, and protein contents. In addition, this study demonstrates that the color of the Tunisian honeys is highly variable and ranges from pale yellow to dark brown. The total phenolic, flavonoid and carotenoid contents significantly vary ($p < 0.05$). The highest values were found in mint honey, which has a very dark color. Correlations between the analyzed parameters are statistically significant ($p < 0.05$). The DPPH radical scavenging activity of rosemary honey was determined as lower ($p < 0.05$) than the other analyzed honey samples. Yet, the highest activity was detected in mint honey. The results suggest that Tunisian honeys could be beneficially used as a functional or nutraceutical substance as they prevent or moderate oxidative stress-related diseases.

1. Introduction

Honey is defined as "the sweet substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honey combs" (Codex Alimentarius Commission, 2001). Naturally, honey has been traditionally recognized as a valuable source of energy. It has also been recognized for its antimicrobial and antioxidant characteristics (Alvarez-Suarez et al., 2010;

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.08.011
1878-5352

Basualdo et al., 2007). It is a concentrated aqueous solution of invert sugar that contains a mixture of other carbohydrates, amino and organic acids, minerals, aromatic substances, pigments, waxes and pollen grains to make it complex (Alvarez-Suarez et al., 2010; Ajlouni and Sujirapinyokul, 2010; Manzanares et al., 2011; Rashed and Soltan, 2004). Many studies have demonstrated that honey serves as a source of natural antioxidants, which are effective in reducing the risk of heart disease, cancer, immune system deficiency, cataracts, different inflammatory processes, etc. (National Honey Board, 2002). In recent years, the antibiotic and wound healing properties of honey have been scientifically proven (Molan and Betts, 2004).

In the past three decades, the large number of published studies concerning the physicochemical characteristics of honeys of different botanical and geographical origins illustrates the importance of determining honey's quality. Very few studies, however, have analyzed honey's physicochemical properties, and none of them has determined the physicochemical characteristics and antioxidant activities of any Tunisian honey variety. It is known that the physicochemical parameters of natural honeys, such as pH, moisture, sugar composition and hydroxymethylfurfural (HMF) contents, color, acidity and specific conductivity, are strictly defined and represent the quality indicators that characterize each individual honey variety. It is important to note that glucose and fructose are the major honey sugars while sucrose remains very scarce (Ajlouni and Sujirapinyokul, 2010; Fallico et al., 2004; Tosi et al., 2008). HMF is practically not present in fresh food, but it is naturally generated in sugar-containing food during heat-treatments like drying or cooking. HMF can be used as an indicator for excess heat-treatment. For instance, fresh honey only has low amounts of HMF less than 15 mg/kg, and the European Union (EU, 2001) requires an HMF limit in honey of 40 mg/kg and 80 mg/kg for honey coming from countries or regions with tropical temperatures, 15 mg/kg for honey with low enzymatic level (8–3 Schade Units). This standard guarantees that the honey has not undergone heating during processing (Codex Alimentarius, 2000). The geographical origin of honey has previously been studied by many researchers around Europe, especially in Slovenia, Romania, Spain, Denmark and Portugal (Bertoncelj et al. 2011; De la Fuente et al. 2011; Stolzenbach et al., 2011; Feás et al., 2010), in Africa mainly in Morocco, Burkina Fasan and Algeria (Terrab et al., 2002; Meda et al., 2005; Ouchemoukh et al., 2007), in South America mainly in Argentina, Cuba and Brazil (Alvarez-Suarez et al., 2010; Chirifie et al., 2006; Moreira et al., 2010), and in Australia and New Zealand (Ajlouni and Sujirapinyokul, 2010; Vanhanen et al., 2011). The authors have determined the physicochemical parameters including water content, pH, conductivity and sugar composition. They found that the geographical area influences and distinguishes the physicochemical properties of honey to a large extent.

In Tunisia, nevertheless, honey has always had a valued place in traditional medicine. It has been principally employed for wound healing and diseases of the gut. Unfortunately, there are no ample investigations regarding its quality and/or its biochemical characteristics. Besides, there are few Tunisian varieties that have never been analyzed before, i.e. mint, orange, eucalyptus, thyme, rosemary and horehound.

The present study aims at identifying natural honey varieties harvested in Northwest Tunisia with respect to their floral origin, physicochemical properties such as moisture, ash, pH, free acidity, electrical conductivity, HMF content, reducing sugars and invertase activity. Note that the total carotenoids, total flavonoids and antioxidant activities were also identified.

2. Materials and methods

2.1. Chemicals and reagents

Glucose, fructose, sucrose, maltose, Folin–Ciocalteu reagent, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl, (+)-catechin, L-proline and methanol were purchased from Sigma Chemical Co. (St. Louis, USA). Ultrapure water was made using a Milli-Q water purification system (Millipore, Bedforde, MA, USA). All the other used chemicals were high purity analytical grade reagents.

2.2. Samples

There are six honey samples coming from different botanical origins and produced in Tunisia. They were collected throughout a whole year in different regions in Tunisia (see Fig. 1). Table 1 shows different origins, and type of the examined honeys. Obviously, all honey samples were directly obtained from beekeepers. The beekeepers directly extracted the samples into 250 mL sterilized glass sample bottles with glass caps. The samples were then stored in a dry and dark place at a temperature of 20 °C. Subsequently, the honey samples were classified into six categories as they belong to the following diverse floral origin: eucalyptus, orange, thyme, mint, rosemary, and horehound.

2.3. Proximate composition analyses

Water content was measured by a Carl Zeiss 16531 refractometer at 20 °C and the corresponding water content was calculated using the Association of Official Analytical Chemists method (AOAC, 1990). While the total nitrogen (TN) was determined using the Kjeldahl method from which the crude protein could be calculated as % $N \times 6.25$. The ash content was determined by burning the samples in a muffle furnace at 500 °C for 6 h. The proline content was determined according to the Bogdanov method (Bogdanov et al., 1997). The pH, however, was measured using a Mettler Toledo pH meter (California, USA). The pH probe was immersed in a 250 mL beaker that contained a solution of 4 grams of honey dissolved in 30 mL of ultrapure water. Free acidity was determined according to the AOAC method (AOAC, 2000), by the titrimetric method. To make things clear, the electrode of the pH meter was immersed in the solution, stirred with a magnetic stirrer and titrated to pH 8.5 by adding a 0.05 N of NaOH solution. The Electrical conductivity (EC) of a honey solution, at 20% (w/v) (dry matter basis) in ultrapure water, was measured at 20 °C using a Consort conductometer (Consort C830, Belgium). The results were expressed as milli Siemens per centimeter (mS/cm). The water activity (a_w) was determined at 25 ± 0.02 °C using an AquaLab water activity meter (AquaLab CX2T, Decagon Devices, USA). Viscosity was determined by an Ostwald viscometer at 25 °C and at a shear rate of 5 rpm.

Note that all the tests were performed in triplicate and were expressed as mean ± standard deviation.

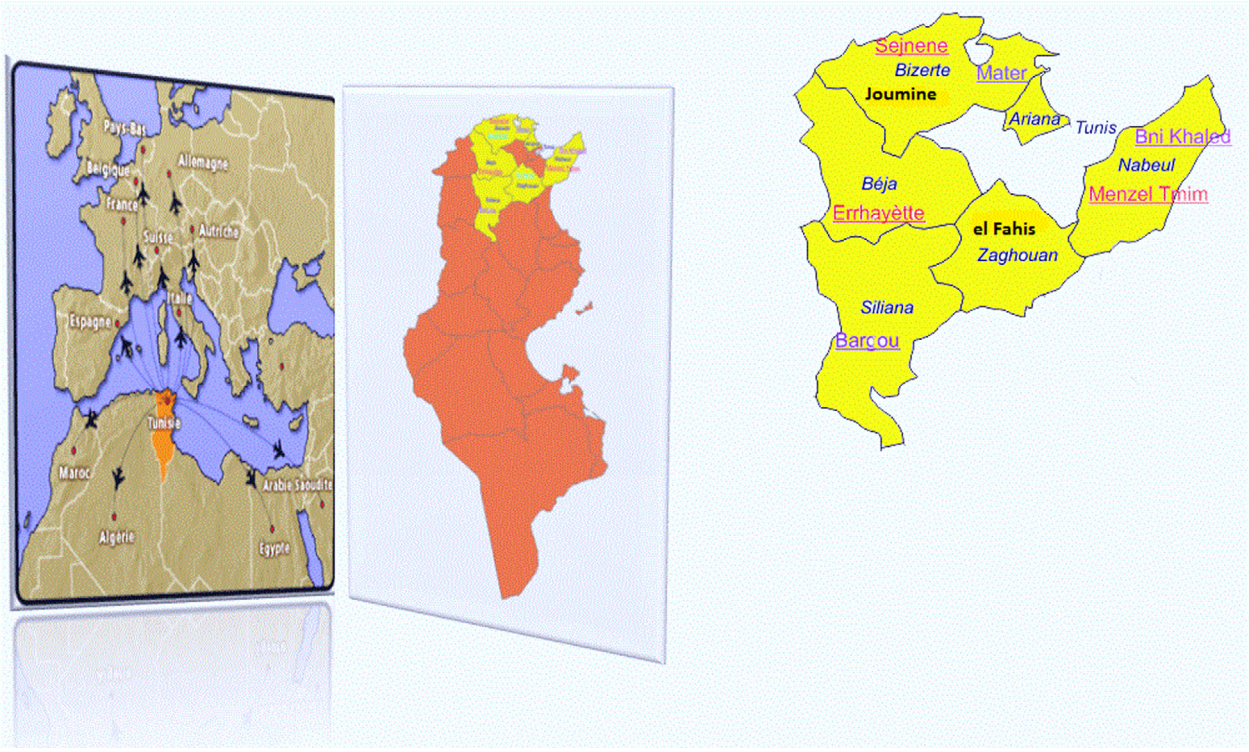

Figure 1 Map of North Tunisia indicating the regions where honey samples were collected.

Table 1 Honey samples from different regions of Tunisia.

Honeys	Location	Geographic origin
Mint	Bizerte	North (Sejnene, Joumine)
Rosemary	Nabeul	North Est (Bni Khaled)
Eucalyptus	Siliana	North ouest (Bargou)
Horehound	Beja	Centre ouest (Errhayette)
Thyme	Zaghouan	Centre East (El Fahis)
Orange	Ariana	North (Ariana)

2.4. Invertase activity

Invertase activity was spectrophotometrically measured with 4-nitrophenyl-a-D-glucopyranoside and the results are expressed in international units (IU).

2.5. Mineral analyses

Mineral elements were analyzed using a Varian Spectra Atomic Absorption Spectrophotometer (Model 220, Varian, USA) after ash drying. The sodium (Na) and potassium (K) contents were determined by emission photometry (AOAC, 1990). The contents of magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn) and copper (Cu) were determined by flame atomic absorption spectrophotometry. Lead (Pb), cadmium (Cd) and chromium (Cr) contents were determined by electro-thermal atomic absorption spectrometry (23). Phosphorus (P) content was measured by a UV–visible spectrophotometer (Model U2001, Hitachi Co., Tokyo, Japan) according to the method described by the AOAC (AOAC, 1990).

2.6. Color measurement

CIELAB $L^* a^* b^*$ color parameters were measured in a Minolta colorimeter (Minolta, Model CM-3600 d, UK) controlled by a computer that calculated color from the reflectance spectrum. Samples were placed in Petri dishes and filled to the brim. Each Petri dish was placed directly on the colorimeter sensor (3.5 cm in diameter) and in order to be measured there were five measurements taken per treatment every week. The instrument was calibrated using a white tile ($L^* = 94.52$, $a^* = -0.36$ and $b^* = 1.04$) as standard. The L^* parameter (lightness index scale) ranges from 0 (black) to 100 (white). However, the a^* parameter indicates the degree of red ($+a^*$) or green ($-a^*$) colors, whereas the b^* parameter measures the degree of the yellow ($+b^*$) or blue ($-b^*$) colors. The results were expressed in accordance with the CIELAB system with reference to illuminant D65 and with a visual angle of 10°.

2.7. Sugar composition determination

The sugar composition was determined by an HPLC (Shimadzu, Tokyo, Japan) equipped with a refractive index detector (HPLC-RI) at 30 °C. A honey sample (1 g) was dissolved in 19 mL Milli-Q water, filtered through a 0.22 mL nylon filter into an HPLC vial, capped and injected (20 µl) into the HPLC. The HPLC column was Supelcogel C-610H, 30 cm, 7.8 mm I.D. fitted with a guard column Supelguard C-610H 5 cm,4.6 mm I.D. Col: H + 11,855. The mobile phase was 0.005 M sulfuric acid at a flow rate of 0.75 mL/min. The external calibration curves produced by standard solutions were used to quantify the amount of sugars in the samples (Bonta et al., 2007). Honey sugars are identified and quantified by

the method of external etalonanage by comparing their retention times and their peak areas with those of standard sugars. The results were expressed as gram sugar per 100 g of honey.

2.8. Hydroxymethylfurfural (HMF) analysis

HMF was determined according to the European commission honey method (Bogdanov et al., 1997). Briefly, 20 g of honey samples was dissolved in 100 ml Milli-Q water, filtered through a 0.22 mL nylon filter into an HPLC vial, capped and injected (20 µl) into the HPLC. HMF concentration in the honey is calculated by comparing the peak area to that of the standards taking into account the dilutions. There are a linear relationship between the concentration and the peak area and the results were expressed as mg/kg honey.

2.9. Biochemical analyses

2.9.1. Total phenolic content

The total phenolic content was determined according to the method described by Singleton et al. (1999), with a slight modification. Thus, 1 g of honey sample was diluted with ultrapure (10 mL) of water and filtered through a Minisart filter of 45 µm. Then 0.5 mL of this solution was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent and 2 mL of 0.7 M sodium carbonate (Na_2CO_3) for 7 min. After incubation in the dark at ambient temperature (≈25 °C) for 2 h, the absorbance of the reaction mixture was measured at 760 nm against the sugar analog using a Beckman Du 640 spectrophotometer (Instruments Inc., Fullerton, CA, USA). Gallic acid was used as a standard to produce the calibration curve (80–250 mg/L). The total phenolic content was expressed in mg of gallic acid equivalents (mg GAE/100 g of honey).

2.9.2. Total flavonoid content

The Total flavonoid content of Tunisian honey samples was determined according to Blasa et al. (2007). Subsequently, 1 mL of a honey solution (1 mg/mL) was mixed with 0.3 mL NaNO2 (5%), and a solution of 0.3 mL $AlCl_3$ (10%) was added in five minutes. The honey samples were mixed and in six minutes they were neutralized with a 2 mL of NaOH solution (1 M). The absorbance was measured for all samples at 510 nm and the calibration curve was used in order to do quantification. Similarly, different concentrations of (+)-catechin (5–114 mg/L) were used for calibration. The results were expressed in mg for quercetin equivalents (CE)/100 g of honey, as the average of triplicate measurements.

2.9.3. Carotenoid content

The total carotenoid content (TCC) extraction was carried out as previously reported by Ferreira et al. (2009), with minor modifications. 1 g of sample was vigorously shaken with 10 mL of an *n*-hexane–acetone mixture (6:4) for 10 min at room temperature (20 °C) and was then filtered through a No. 4 Whatman filter paper. The absorbance of the filtrate was measured at 450 nm in comparison to a blank one using a spectrophotometer (Beckman Du 640, USA). Beta-carotene was used for the calibration curve (0.015–0.6 l g/mL). Thus, the total carotenoid content was expressed as mg of β-carotene equivalents (mg β-carotene/kg of honey).

2.10. DPPH radical scavenging activity

The DPPH radical scavenging activity was determined by the method described by Ferreira et al. (2009), with slight modification. That is to say, 0.30 mL of a honey sample (0.5 g/mL) was added to 2.7 mL of 0.20 mM DPPH-methanol solution, and the absorbance of the sample solution (A_{sample}) was measured at 517 nm against a blank after 60 min of incubation period at room temperature (≈22 °C). The sample concentration required to scavenge 50 % of free radicals (IC_{50}) was calculated on the basis of the regression equation.

$$\text{Scavenging effect}\,(\%) = \left(1 - \left[\frac{A_1 - A_2}{A_0}\right]\right) \times 100$$

where A_0 is the absorbance of the control (water instead of the sample test solution), A_1 is the absorbance of the sample. A_2 blank stands for the absorbance of the samples under identical conditions as A_1 and with water instead of $DPPH^{\cdot}$ solution.

Honey sample concentration providing 50% inhibition (IC_{50}) was calculated according to the linear regression algorithm of the plotted inhibition graph percentage in comparison to the honey sample concentration. For the calculation of these values, a Sigma plot (version 10) was used.

2.11. Statistical analysis

All the tests were performed in triplicate using the SPSS statistical package (SPSS Inc., Chicago, IL), version 18.0. The result was expressed as mean ± standard deviation. The Duncan's multiple-range test was used to evaluate significance of difference ($p < 0.05$) between results.

3. Results and discussion

3.1. Physicochemical properties

The physicochemical parameters of the six Tunisian honeys namely, rosemary, horehound, orange, thyme, eucalyptus, and mint were analyzed as shown in Table 2. The water content found in the above honey samples ranged from 17.27% to 19.80%. Evidently, these samples are within the range of the required standards (⩽20%) according to the International Regulations of Quality (Codex Alimentarius Commission, 2001). However, it is prominent to state that the highest water content value was found in mint and orange honeys ($p < 0.05$). Nevertheless, there are no significant differences ($p > 0.05$) between horehound and thyme honeys in terms of water content. These results were similar to those previously reported for this monofloral honey and whose corresponding values ranged from 15.20% to 20.60% (Manzanares et al., 2011; Feás et al., 2010; Vanhanen et al., 2011). It has been reported that higher water content might cause undesirable honey fermentation during storage and formation of acetic acid (Chirifie et al., 2006). Conspicuously, honey's water content depends on various factors, such as the harvesting season, the degree of maturity reached in the hive, and the geographic and environmental factors (Acquarone et al., 2007; Kahraman et al., 2010).

Similarly, protein contents of most of the Tunisian honeys were also convergent (0.13 and 0.16 g/100 g honey) but higher

Table 2 Physico-chemical parameters of analyzed honey samples.

Honeys	Water (%)	Protein (%)	Proline (mg/kg)	Ash (%)	Electrical conductivity (EC) mS cm^{-1}	pH	Total acidity (meq/kg)	Water activity	Viscosity (Pa.s)	HMF mg/kg	Invertase activity (unit/kg)
Mint	19.80 ± 0.10^{a}	0.16 ± 0.03^{a}	102.22 ± 4.20^{a}	0.13 ± 0.01^{c}	0.43 ± 0.03^{c}	4.11 ± 0.02^{a}	27.03 ± 1.06^{a}	0.63 ± 0.01^{a}	6.83 ± 0.05^{e}	12.07 ± 1.00^{d}	184.68 ± 4.16^{a}
Rosemary	17.27 ± 0.01^{b}	0.13 ± 0.02^{a}	39.62 ± 2.58^{b}	0.39 ± 0.10^{b}	0.65 ± 0.04^{b}	4.02 ± 0.03^{b}	7.11 ± 0.20^{b}	0.56 ± 0.01^{b}	9.62 ± 0.06^{b}	12.91 ± 0.63^{d}	52.29 ± 2,44^{b}
Eucalyptus	19.12 ± 0.07^{e}	0.14 ± 0.01^{a}	102.60 ± 2.08^{a}	0.14 ± 0.01 ^{c}	0.52 ± 0.03^{c}	3.68 ± 0.17^{c}	26.60 ± 0.20^{a}	0.60 ± 0.02^{a,b}	10.14 ± 0.10^{a}	27.43 ± 1.50^{a}	46.25 ± 1.08^{f}
Horehound	18.20 ± 0.20^{c}	0.15 ± 0.01^{a}	85.94 ± 4.48^{e}	0.11 ± 0.02^{e}	0.41 ± 0.02^{cd}	3.67 ± 0.01^{c}	27.20 ± 0.20^{a}	0.64 ± 0.02^{a}	8.01 ± 0.04^{c}	19.63 ± 1.11^{c}	92.66 ± 1.53^{c}
Thyme	18.16 ± 0.02^{c}	0.14 ± 0.02^{a}	68.70 ± 2.46^{e}	0.08 ± 0.02^{d}	0.39 ± 0.02^{d}	3.87 ± 0.01^{e}	26.20 ± 0.20^{a}	0.60 ± 0.01^{a,b}	10.23 ± 0.10^{a}	25.49 ± 1.31^{a}	73.74 ± 0.65^{e}
Orange	19.73 ± 0.23^{a}	0.14 ± 0.03^{a}	59.12 ± 2.51^{d}	0.69 ± 0.03^{a}	0.89 ± 0.06^{a}	3.82 ± 0,03^{d}	21.41 ± 0.10^{c}	0.63 ± 0.01^{a}	7.26 ± 0.04^{d}	22.56 ± 1.39^{b}	82.01 ± 1.72^{d}

Results are reported as means ± standard deviation. Means in the same column with different letters are significantly different at $p < 0.05$.

than the rosemary honey. This variation may be attributed to the type of flora. Hence, there are no significant differences ($p < 0.05$) in terms of protein content of all the examined honeys. As previously demonstrated, honey contains about 0.20% protein, as α-amylase, invertase, catalase, glucose oxidase, and phosphatase. This is related to the plant origin as well as pollens and nectars (Anklam, 1998). It is important to remark now that the results are in total conformity with the results obtained by Azeredo et al. (2003). The proline content in the honey samples varied from 39.62 to 102.60 mg/kg and the highest was observed in mint and eucalyptus honeys all of which were not significant at 95%. However, rosemary honey had a significantly lower ($p < 0.05$) of proline content than the other observed honeys samples.

Ash content is also another parameter used for the determination of the botanical origin such as flora mix (White, 1978). In the present study, the ash content of Tunisian honeys ranged from 0.08% to 0.69%. Note that the results are within the average percentage for floral honeys (0.60%). Hence, there are no significant differences between mint, horehound and eucalyptus in terms of ash.

The electrical conductivity (EC) of honey is closely related to the concentrations of minerals or total ash, salts, organic acids and proteins, and is a parameter that shows great variation according to the honey's floral origin (Terrab et al., 2003; León-Ruiz et al., 2011). The results obtained after examining the six Tunisian honey samples show that the values for EC ranged from 0.39 to 0.89 mS cm^{-1}. There is a positive correlation between electrical conductivity and ash ($r = 0.971$). Hence, the above results demonstrate a large variation that depends on the floral origin. The orange honey sample has the highest values for EC (0.89 mS cm^{-1}), compared with mint, horehound, rosemary, thyme and eucalyptus honeys. These results conform with previous studies (Kaškonien ≐ et al., 2010).

The water activity values obtained in this study ranged between 0.56 and 0.64. There are no significant differences between honey samples in terms of water activity ($p < 0.05$). In addition, the viscosity of honeys was also examined, and the highest viscosity was observed in thyme, followed by eucalyptus, rosemary, horehound, orange, and mint. These results showed that thyme and eucalyptus honeys are not significantly different ($p > 0.05$) in terms of viscosity. Tunisian honeys have high viscosities in comparison to other honeys (El-Bialee and Sorour, 2011).

Another important parameter during the extraction and storage of honey is the pH value. It influences honey texture, stability and shelf life (Terrab et al., 2002). As shown in Table 2, all the examined honeys had an acidic character. Their pH values ranged from 3.67 to 4.11. These values were similar to those previously reported in Algerian, Spanish and Portugal honeys that have been found to vary between pH 3.50 and pH 4.58 (Rashed and Soltan, 2004; Feás et al., 2010; Ouchemoukh et al., 2007). In general, a low pH of honey inhibits the growth and proliferation of microorganisms.

In the six Tunisian honey samples, the total acidity of honey ranged from 7.11 to 27.20 meq/kg honeys (Table 2). No significant difference ($p > 0.05$) was observed in terms of acidity value in mint and horehound honeys. But it must be noted that the lowest acidity value was observed in rosemary honey (7.11 meq/kg honey). These data illustrate the significant influence of floral type on the total acidity of honey.

According to the obtained results of free acidity, none of the analyzed samples exceeded the limit of 40 meq kg^{-1} as required by the international regulations (Bogandov et al., 1999). Thus, all samples were in compliance with the standards. Variation in free acidity among different honeys could be due to the different floral origins or to the variation in harvest seasons (Perez-Arquillué et al., 1994).

It has been reported that high free acidity values may indicate the fermentation of honey sugar by yeasts. It is well known that during fermentation, glucose and fructose are converted into carbon dioxide and alcohol. Alcohol is further hydrolyzed in the presence of oxygen and converted into acetic acid. This, obviously, greatly contributes to the level of free acidity in honey (Ajlouni and Sujirapinyokul, 2010).

3.2. HMF content

HMF is, undoubtedly, an excellent indicator of honey freshness and purity (Codex Alimentarius, 2000). High concentrations of HMF in honey indicate overheating or poor storage conditions. According to the International Trade Guidelines (European Economic Commity, 2002), the honey's HMF content should not exceed 40 mg/kg. In the present study, the HMF of the six examined Tunisian honeys ranged from 12.07 to 27.43 mg/kg. The highest HMF was observed in eucalyptus followed by thyme, orange, horehound and rosemary honeys. There are no significant differences ($p > 0.05$) between mint and rosemary. All honey samples had an HMF value lower than the above limit, and none showed values higher than 40 mg/kg. Hence, the honey samples herein investigated are to be deemed as fresh honeys. It has been demonstrated that the HMF parameter is related to the honey's quality and its heat processing but not to its origin (Anklam, 1998). Several factors influence the formation of HMF, such as storage conditions (e.g. temperature) and floral sources (Fallico et al., 2004; Terrab et al., 2002; Meda et al., 2005). In addition to these facts, the HMF level in honey also depends on the sugar type present in honey itself like fructose: glucose ratio (Doner, 1979). Similarly, it is well known that honey heating results in the formation of HMF, which is produced during acid-catalyzed dehydration of hexoses, such as fructose and glucose (Fallico et al., 2004; Tosi et al., 2008).

3.3. Invertase activity

Invertase is a natural honey enzyme which is commonly used in Europe as a determinant of freshness. Its level depends on the geographic and floral origins of the product, as well as on its freshness. Table 2 illustrates that invertase activity values ranged from 46.25 to 184.68 unit/kg honey. Besides, all honey samples contained a significant invertase activity. The highest value was found in mint honey while the lowest was detected in eucalyptus. Note that all investigated samples herein conform to the required standards (40 unit/kg honey) (Codex Alimentarius Commission, 2001). Obviously, this suggests optimal storage or processing conditions.

3.4. Mineral composition

The mineral composition of the six Tunisian honeys is shown in Table 3. Generally, the most abundant elements found in

Table 3 Mineral analysis of Tunisian honey samples (mg/kg fresh weight).

Minerals													
Honeys	Calcium	Potassium	Magnesium	Sodium	Phosphore	Sulfur	Copper	Iron	Zinc	Lead	Nickel	Chromium	Cadmium
Mint	221.07 ± 5.16^{a}	976.75 ± 11.98^{a}	78.12 ± 6.55^{a}	343.58 ± 12.50^{a}	59.31 ± 2.25^{a}	36.49 ± 1.07^{a}	nd	3.54 ± 0.02^{a}	1.10 ± 0.03^{a}	0.05 ± 0.01^{a}	0.40 ± 0.01a	0.04 ± 0.00^{a}	nd
Rosemary	187.09 ± 3.46^{b}	944.24 ± 12.65^{a}	74.90 ± 2.15^{a}	406.52 ± 10.98^{b}	48.81 ± 3.76^{b}	30.88 ± 1.60^{b}	0.34 ± 0.01^{a}	1.41 ± 0.03^{b}	0.42 ± 0.02^{b}	0.02 ± 0.01^{b}	nd	nd	nd
Eucalyptus	180.11 ± 4.39^{b}	326.13 ± 14.51^{b}	58.22 ± 2.32^{bc}	453.55 ± 16.44^{c}	29.31 ± 0.94^{c}	32.17 ± 1.65^{a,b}	0.20 ± 0.01^{b}	1.20 ± 0.11^{b}	2.06 ± 0.04^{c}	0.01 ± 0.01^{b}	0.10 ± 0.01^{b}	nd	nd
Horehound	150.13 ± 5.26^{c}	782.06 ± 10.69^{c}	68.44 ± 3.28^{a,b}	521.22 ± 15.34^{d}	72.58 ± 2.26^{d}	34.14 ± 1.17^{a,b}	nd	2.84 ± 0.07	1.45 ± 0.03^{d}	nd	0.04 ± 0.00^{c}	nd	nd
Thyme	113.85 ± 3.10^{d}	172.48 ± 14.44^{d}	37.32 ± 2.31^{d}	251.34 ± 10.93^{e}	61.32 ± 1.70^{a}	21.37 ± 1.97^{c}	0.12 ± 0.01^{c}	1.35 ± 0.05^{b}	nd	nd	0.04 ± 0.00^{c}	0.02 ± 0.00^{a}	nd
Orange	129.04 ± 5.51^{e}	483.78 ± 8.72^{e}	49.15 ± 3.45^{c}	351.71 ± 10.93^{a}	26.30 ± 1.19^{c}	19.34 ± 1.61^{c}	0.17 ± 0.02^{b}	0.83 ± 0.05^{c}	0.55 ± 0.03^{e}	0.02 ± 0.01^{b}	0.19 ± 0.01^{d}	0.32 ± 0.02^{c}	nd

Results are reported as means ± standard deviation. Means in the same column with different letters are significantly different at $p < 0.05$.
nd. not detected.

the investigated honey samples were potassium, sodium and calcium, and which ranged between 976.75 and 172.48; 521.22 and 251.34 and 221.07 and 113.85 mg/kg honey, respectively. Other minerals were meagerly present such as sulfur, copper, iron and zinc. These results are in total agreement with the previous studies (Vanhanen et al., 2011; Rodriguez-Otero et al., 1994). On the other hand, lead, nickel and chromium levels ranged from 0.01 to 0.05, 0.04 to 0.40 and 0.04 to 0.32 mg/kg in the Tunisian honey samples, respectively. The highest lead content was observed in the mint honey sample. Cadmium was not detected in all honey samples. It has been reported that cadmium and lead are non-essential elements in plant nutrition and are one of the most toxic substances that accumulate in biological systems (Mena et al., 1996).

3.5. Color

Color is the physical property that is immediately perceived by the consumer. The determination of the color of honeys is a useful criterion of classification for unifloral honeys. The color parameters of the six honeys are illustrated in Table 4. The lightness values (L^*) of the six honey samples ranged from 36.64 to 51.37. Mint and thyme honeys testify lower lightness values (36.64 and 42.49, respectively). The lightness of ingredients plays a major role in the honey assessment due to consumer preferences. It is clear that the analyzed honey samples had orange, yellow and green components. The green components (negative a^* values) were present in rosemary honey, whereas mint honey showed the highest redness and then comes eucalyptus honey second in rank. The brownish values attested a great disparity among the different honey samples. The orange honey had the lowest value and the highest value was observed in the mint honey. The yellow hue (b^*) had a great variation, but two main groups could be distinguished. The honeys that had b^* values higher than ten (between 10 and 20) are thyme, orange, eucalyptus, horehound and mint, but rosemary honey had a value lower than 10.

3.6. Major sugar composition

The sugar composition of the six Tunisian honeys was analyzed as shown in Table 4. This study affirms that in all the examined samples the percentage of fructose and glucose ranged from 35.78% to 37.84 % and from 31.07% to 36.58 %, respectively. Yet, eucalyptus and rosemary honeys specifically contained a significant amount of fructose and glucose. The predominant sugar of the six investigated honeys was fructose followed by glucose, while sucrose and maltose were present in low amounts in all other samples. Obviously, a high sucrose concentration usually means a premature harvest of honey as sucrose has not been fully developed to glucose and fructose by the action of invertase (Özcan et al., 2006). Note that the sucrose amount of all the honey samples was below the maximum conventional limit of 5% recommended by the European Community (European Economic Commity, 2002). In addition, the fructose/glucose ratio was calculated for all the six honey samples. This ratio tells about the crystallization state of honey, i.e. when fructose is higher than glucose the honey is fluid (Ouchemoukh et al., 2010; Venir et al., 2010). It has also been reported that the fructose/glucose ratio may also have an impact on the honey flavor since fructose is much

Table 4 Color, sugars, and spectrophotometric analysis of biochemical characteristics (total phenolic, total flavonoid, total carotenoid contents) and antioxidant properties of Tunisian honeys.

Honeys	Color			Major sugar composition (%)					Biochemical characteristics			
	L^*	a^*	b^*	Fructose	Glucose	Sucrose	Maltose	F/G	TPC mg Gallic acid equivalent (GAE)/100 g	TF (mg CE/kg)	TC (mgβ-carotene/kg)	Antioxidant activities IC_{50}(mg/mL)
Mint	36.64 ± 0.11^a	4.41 ± 0.03^a	17.67 ± 0.07^a	36.49 ± 0.73^a	31.07 ± 0.04^a	4.60 ± 0.03^a	4.34 ± 0.04^d	1.17	119.42 ± 0.25^a	22.45 ± 0.10^a	3.93 ± 0.09^a	11.08 ± 0.32^a
Rosemary	51.37 ± 0.12^b	−0.67 ± 0.01^b	6.06 ± 0.06^b	37.73 ± 0.05^b	36.58 ± 0.03^b	n.d	n.d	1.03	89.31 ± 0.10^b	16.24 ± 0.08^b	1.16 ± 0.02^b	17.51 ± 0.54^b
Eucalyptus	42.87 ± 0.06^c	2.27 ± 0.01^c	15.78 ± 0.03^c	37.84 ± 0.04^c	33.48 ± 0.02^c	2.23 ± 0.03^b	4.31 ± 0.05^a	1.13	32.17 ± 0.09^c	9.58 ± 0.03^c	2.69 ± 0.03^c	93.26 ± 0.37^c
Horehound	46.18 ± 1.12^d	0.57 ± 0.02^d	16.92 ± 0.04^d	36.77 ± 0.06^d	35.15 ± 0.05^d	0.20 ± 0.02^c	2.87 ± 0.02^b	1.04	42.40 ± 0.11^d	11.03 ± 0.07^d	3.05 ± 0.03^d	71.49 ± 0.53^d
Thyme	42.49 ± 0.06^e	0.59 ± 0.01^d	13.68 ± 0.04^e	36.96 ± 0.04^e	32.09 ± 0.07^e	3.94 ± 0.04^d	2.95 ± 0.02^c	1.15	63.08 ± 0.10^e	14.77 ± 0.04^e	2.04 ± 0.04^e	44.34 ± 0.34^e
Orange	46.95 ± 0.03^f	0.34 ± 0.01^e	15.79 ± 0.05^c	35.78 ± 0.04^f	32.58 ± 0.08^f	0.37 ± 0.02^e	1.36 ± 0.04^d	1.09	63.00 ± 0.07^e	11.12 ± 0.03^d	4.72 ± 0.03^f	52.72 ± 0.45^f

Results are reported as means ± standard deviation. Means in the same column with different letters are significantly different at $p < 0.05$.
TPC: total phenolic content; **TF**: total flavonoids; **TC**: total carotenoids; **IC_{50}**: Inhibitory concentration at 50 %. F: fructose; G: glucose; **n.d:** not detected.

sweeter than glucose (Alvarez-Suarez et al., 2010). Subsequently, all the examined Tunisian honey samples were fluid (ratio great than 1). This result is asserted by the viscosity value of the above samples.

3.7. Biochemical characteristics

3.7.1. Total polyphenol content

The results of the total polyphenols of the six Tunisian honeys are portrayed in Table 4. It might be deduced that the total phenolic substances were higher in mint honey (119.42 mg Gallic acid/100 g honey), followed by rosemary (89.31 mg Gallic acid/100 g honey), thyme (63.08 mg Gallic acid/100 g honey), orange (63 mg Gallic acid/100 g honey), horehound (42.40 mg Gallic acid/100 g honey) and eucalyptus (32.17 mg Gallic acid/100 g honey). No significant variation ($p > 0.05$) was observed in orange and thyme honeys in terms of total phenol content. A high correlation was observed between L^* parameter and the total phenol content ($r = 0.964$, $p < 0.05$). This implies that the amount and type of polyphenolic substances in honey are variable and essentially depend on the floral origin (Küçük et al., 2007).

3.7.2. Total flavonoid content

The total flavonoid contents of the six Tunisian honeys are illustrated in Table 4. A close look at the results reveals that mint honey contains a significant amount of total flavonoids (22.45 mg CE/kg honey), followed by rosemary (16.24 mg CE/kg honey), thyme (14.77 mg CE/kg honey), orange (11.12 mg CE/kg honey), horehound (11.02 mg CE/kg honey), and eucalyptus (9.58 mg CE/kg honey). A high correlation was found between total flavonoids and total phenol content ($r = 0.915$). Yet, there are no significant differences in horehound and orange honeys in terms of total flavonoids. The total flavonoids are also related to the floral sources as discussed by Amiot et al. (1989).

3.7.3. Total carotenoid content

The total carotenoids of the six investigated honey samples are shown in Table 4. It is evident that orange honeys have the highest carotenoid content (4.72 and mg β-carotene/kg of honey), followed in rank by mint, Horehound, eucalyptus, thyme and rosemary honeys respectively. A linear correlation was found between the total carotenoids and $+b^*$ ($r = 0.827$, respectively). Similarly, the total carotenoids depend on the geographical area, the environmental factors and the changing seasons.

3.8. Antioxidant activity

The DPPH free radical is a stable free radical, which has been widely used as a tool to determine the free radical-scavenging activity of antioxidants. Antioxidants, when interacting with DPPH, transfer either electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character (Devasagayam et al., 1995). The color of the reaction mixture changes from purple to yellow, and its absorbance decreases at 517 nm. Note that the DPPH radical scavenging activities of the Tunisian honey samples (expressed by IC_{50}) are shown in Table 4. Note that lower IC_{50} value indicates a higher DPPH free radical scavenging activity. Therefore, the present study demonstrates that the DPPH radical scavenging activity of mint honey is significantly ($p < 0.05$) higher (11.08 mg/mL) than all other honey samples (rosemary, horehound, orange, thyme, and eucalyptus). Note that a significant difference ($p < 0.05$) between all honey samples was found. Yet, higher correlations were observed between the DPPH radical scavenging activity and the total polyphenol ($r = -0.945$, $p < 0.01$), antioxidant activities and total flavonoids ($r = -0.866$, $p < 0.01$), and between total flavonoids and total polyphenols ($r = 0.957$, $p < 0.01$). These results are in perfect accordance with the works of Alvarez-Suarez et al. (2010), Sant'Ana et al. (2012), and Ferreira et al. (2009) who found that there is a positive correlation between DPPH radical scavenging activity and total polyphenols, and total flavonoids.

4. Conclusion

In the present study, six Tunisian honey samples were unprecedentedly investigated for their physicochemical properties, such as pH, conductivity, sugar composition, and HMF.

The results allow us to assess the quality of six Tunisian honeys and establish some guidelines that assert their quality. The results also point out that the six Tunisian honeys were characterized by the prevalence of total polyphenols, total flavonoids and total carotenoids. This fact is of great economic and/or industrial interests on account of the applications of these components in the food, cosmetics and pharmaceutical industries. Likewise, the DPPH radical scavenging activity indicated that mint honey possessed excellent antioxidant properties. Eventually, honey is such a natural product with a number of salient therapeutic properties. Yet, it is recommended that further meticulous studies should bring to light the other hidden properties of honey.

Acknowledgements

Thanks are due to all the beekeepers who have provided the honey samples of this study.

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