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POLLEN PROFILES, PHYSICOCHEMICAL CHARACTERISTICS, AND ANTIOXIDANT ACTIVITIES OF TWO HONEY SAMPLES FROM JIJEL CITY (ALGERIA)

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Abstract

Honey is a sweet and flavorful animal product that comes from nectar and/or honeydew. It is used in different nutritional and therapeutic fields. This study aimed at the determination of the botanical origin, the physicochemical characteristics, and the antioxidant activities of two honey samples from two locations in Jijel City (Algeria). The analyzed honey complies with the standards of the Codex Alimentarius Commission. Pollen analysis showed that honey sample H1 was polyfloral and honey sample H2 was monofloral. Quality parameter analysis revealed that moisture content varied from 13.21 to 16.44%. The average pH was 4.34, and the electrical conductivity was 0.56 mS/cm. Protein, proline, and HMF contents were 32.08 mg EBSA/ 100 g, 435.47 mg/kg, and 3.49 mg/kg, respectively. Both analyzed kinds of honey were levorotatory. Phenolic compounds and flavonoids were found to be higher in honey H1 with 60.93 mg of EAG/100g and 20.92 mg of EC/100g, respectively. Results of antioxidant activities showed that honey H1 was much more effective in reducing iron and copper than honey H2 and gave the best total antioxidant capacity. In addition, the study of the antiradical activity against DPPH and ABTS revealed that honey H1 was able to scavenge DPPH and ABTS radicals with 48.88 and 18.21 %, respectively.

Keywords: honey, physicochemical properties, pollen analysis, phytochemical analysis, antioxidant activity

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Introduction

The diversity of the Algerian flora and the relative mildness of the climate, make it an excellent pole for the development of beekeeping. This sector puts at the disposal of the consumer several products of the hive, such as honey, pollen, wax, propolis, etc. (Ouchemoukh *et al.*, 2007). Among the remedies of the hive, honey is the most common product used since ancient times as an ingredient in some manufactured foods. It is a food with high nutritional value that is distinguished by its characteristic aroma and pleasantly sweet taste.

It is the result of the work of *Apis mellifera* domestic bees, which collect nectars of flowers and honeydew around the hive and which the bees collect, transform by assembling them with their secretion of specific substances, deposit, dehydrate, store, and let mature in the hive combs, or combine with their own materials, preserve, and let mature. This food can be thick, fluid, or crystallized (Blanc, 2010; Odoux *et al*., 2014; Sari *et al.*, 2019).

Honey is a natural product whose composition varies according to the floral source, the nature of the soil, and the meteorological conditions, the bee itself, free to choose its floral source to forage, the presence or not of other insects (aphids, mealybugs), and the treatment methods used by the beekeeper (Ballot-Flurin, 2010). It is composed mainly of carbohydrates and water, as well as other less abundant constituents such as: pollen, phenolic compounds, minerals, proteins, and amino acids, vitamins, carotenoids, enzymes, and organic acids (Belhaj *et al.*, 2015). Aspects of its use indicate that honey functions as a food preservative as it contains bioactive components that act as preservatives, namely phenolic compounds, α-tocopherols, flavonoids, and some enzymes (Maaria *et al.*, 2018; Ren *et al.*, 2019; Sari *et al.*, 2019). It also plays an important role in reducing the risk of immune system decline, the prevention of heart disease, cancer, and inflammation (Swapna *et al.*, 2017; Liu *et al.*, 2019).

Due to its peculiar characteristics, this product has aroused great interest in current functional food research, which is a trend in food chemistry. Hence, to contribute more to the knowledge of Algerian honey and considering the lack of detailed study in the literature, the present research aimed (i) to characterize the botanical origin (blossom and/or honeydew) of honey, (ii) to determine the physicochemical and authenticity parameters, and (iii) to quantify the amounts of phenolic compounds that are likely responsible for most of the bioactivity in honey and are considered as potential markers for their botanical origin, and finally (iiii) to evaluate the antioxidant properties with the use of different methods to determine the potential functional value of these kinds of honey.

Materials and methods

Honey samples

The present study was carried out on two honey samples (H1 and H2) harvested in July 2019, in two different regions in Jijel City (Algeria). Samples were preserved

in a sterile glass bottle covered with aluminum, hermetically closed, and kept at room temperature in order to protect sensitive compounds from heat and light.

Sample H1 was kindly given by beekeeping from the Ziama-Mansouriah region and honey H2 from the Texenna region. The extraction of honey was carried out by manual pressure on the wax frames of traditional hives.

Pollen analysis

Pollen analysis was carried out using the methods previously reported by Louveaux *et al.*, 1978). A quantity of 10 g of honey was dissolved in 20 ml of water acidified with sulphuric acid. The obtained solution was centrifuged for 10 minutes at 3000 rpm. The supernatant was eliminated and 10 ml of acidified water was added to the pellet. The mixture was then centrifuged under the same conditions. The recovered pellets were spread on a glass slide and dried in the oven. After drying, the slides were examined with a photonic microscope under x 40 magnification (Zeiss Axiolab, Göttingen, Germany). This apparatus was coupled to a computer for image processing. The identification of the pollen grains was done by referring to general palynological databases, such as CETAM (Bee Studies Center of Moselle, France) and the existing information about the flora of Algeria.

Physico-chemical analysis

The following physico-chemical parameters: moisture, Brix, pH, electrical conductivity, and HMF were analyzed according to the method reported by Bogdanov (1997).

Moisture and Brix

The humidity of honey was determined according to the measurement of the refractive index, which allows one to establish the water content. The measurement of this index was carried out as follows: a drop of honey at a temperature of 20 °C was spread on the surface of the prism of the refractometer, previously calibrated with distilled water. The reading was made through the eyepiece of the apparatus after an adjustment so as to have a horizontal line of division between a clear and a dark zone identical. This line intersects a vertical scale that is directly graduated in refractive index and Brix percentage in honey. The calculations were made with reference to the CHATAWAY table.

Electrical conductivity (EC)

Electrical conductivity was measured in a 20% (w/v) solution of honey in deionized water with low electrical conductivity meter. The EC was calculated as follows:

$$EC\ (\text{milli-Siemens/cm}) = \text{measured value} - A \qquad (1)$$

where: EC is the value measured by the conductivity meter; A is calculated by this equation: $A = (EC \times 0.032) \times (T - 20°)$; 0.032 is the correction factor; T is the ambient temperature of measurement.

pH and acidity measurement

An aqueous solution of honey at 10 % was carried out: 2.5g of honey was dissolved in 25 ml of distilled water in order to determine its pH. The result was read on the pH meter.

The determination of the acidity of honey was carried out according to the method published in the Official Journal of the French Republic (1977). 10g of honey were dissolved in a volume V (75ml) of distilled water, then this last one was titrated with NaOH at 0.05N until the increase of pH to 8.5. a volume of 10ml of NaOH (0.05N) was added to the solution and titrated with HCl at 0.05N to decrease the pH to 8.3. The result of free acidity was calculated according to the following formulas:

$$\text{Free acidity } (m_{eq}/\text{kg honey}) = (V_{NaOH\ (sample)})\cdot C_{NaOH}\cdot 1000\ /m \qquad (2)$$

where: m : Test sample (g); $V_{NaOH\ (sample)}$: Volume of NaOH necessary to reach the equivalent point ; C_{NaOH} : NaOH normality (0.05N).

$$\text{Combined acidity } (m_{eq}\ /\text{kg of honey}) = (10 - V_{Hcl}\cdot C_{Hcl})\cdot 1000\ /m \qquad (3)$$

where: m : Test sample (g) ; V_{Hcl} : Volume of HCl necessary to reach the equivalent point ; C_{Hcl} : Normality of HCl (0.05N).

The total acidity was obtained by adding the free acidity and the bound acidity.

Hydroxymethyl furfural (HMF)

The HMF is an indicator determining the freshness and quality of honey. A quantity of 5g of honey samples was dissolved in 25 ml of distilled water. 0.5 ml of Carrez I solution (15% potassium hexanocyanoferrate solution) and 0.5 ml of Carrez II solution (30% zinc acetate solution) were added.

The mixture was adjusted to 50 ml with distilled water. After filtration, the first 10 ml of the filtrate were discarded. Two aliquots of 5ml each were then introduced into two tube tests, one with 5ml of distilled water (analysis aliquot) and the other with 5ml of sodium bisulphate at 2% (reference aliquot). The absorbance was read at 284nm and 336nm. The difference between the absorbance of a clear aqueous honey solution (clarified with Carrez I and II solutions) and the same solution after the addition of bisulphite was determined to prevent other components from interfering. The HMF content was calculated as follows:

$$(\text{HMF})\ (\text{mg}/\text{kg}) = ((A_{284}-A_{336})\cdot 149{,}7\cdot 5)\ /\ W \qquad (4)$$

where A_{284} is the absorbance at 284nm; A_{336} is the absorbance at 336nm; W is the weight of honey sample in grams; 149.7 is a constant.

Proline

A 0.5 ml volume of a 5% (w/v) honey solution was introduced into a test tube. 1 ml of formic acid and 1 ml of 3% ninhydrin solution were added to the reaction mixture. The tube was closed, shaken for 15 minutes, and then placed in a water bath at 100°C for 15 minutes, then transferred to another water bath at 70°C for 10 minutes. 5 ml of the aqueous 2-propanol solution (50%) was added to the tube and the absorbance was read at 510 nm, after 45 minutes (Bogdanov *et al.*, 2002). Proline concentrations were determined according to the following formula:

$$\text{Proline } (\text{mg}/\text{Kg}) = (E_S\ /\ E_a)\cdot(E_1/\ E_2)\cdot 80 \qquad (5)$$

where: ES : Absorbance of the honey sample ; Ea : Absorbance of the standard solution of proline ; E1 : mg of proline for the standard solution ; E2 : Quantity of honey (Kg) ; 80 : Dilution factor.

Color

Honey color intensity was determined according to the method described by Al *et al.* (2009). In 4 ml of distilled water, 1 gram of honey sample was dissolved. After homogenization, the absorbance was read at 450 nm.

Protein content

Proteins content was determined by the method reported by Azeredo *et al.* (2003). This colorimetric method was based on the use of 5 ml of Bradfords solution which were added to 100 µl of the prepared 50% honey solution. After 2 min, Coomassie blue G250 binds to the (NH^{3+}) groups of the proteins. This reaction gives a blue color to the reaction medium. The absorbance was read at 595 nm. The results were expressed in mg equivalents of bovine serum albumin per 100 g of honey by reference to the calibration curve with BSA (Bovin Serum Albumin) (mg BSAE/100g) ($y = 0.693\ x + 0,04$; $R^2 = 0.999$).

Determination of sugar

The reducing sugars and total sugars were determined by the Bertrand method. The determination was based on the property of sugars to reduce the copper alkaline liquor. The amount of copper oxide formed was determined by manganometry. A table gives the correspondence between the mass of copper and the mass of sugar. The reaction must be carried out at a high temperature for 3 min from boiling (Audigie *et al.*, 1984).

Total sugars (TS)

A mass of 0.5 g of honey was dissolved in 20 ml of distilled water, which contained 2 ml of HCl (2.2N). The reaction mixture was placed in a water bath at 65°C for 45 minutes, then neutralized with NaOH (2N) in the presence of phenolphthalein until the pink coloration appeared, after which it was adjusted with distilled water to 100 ml.

To a volume of 10 ml of the prepared dilution, 10 ml of Fehling A and 10 ml of Fehling B (a blue solution is obtained) were added. The mixture has been heated until boiling for 3 min, or until a brick red precipitate was obtained. Then the precipitate was recovered from the filter with 10ml of the ferric solution, or a clear green solution was obtained. In the end, the solution was titrated with $KMnO_4$ (0.02N) (to obtain a pink color). The results were calculated as follows:

$$m\,IS_{(cu)} = 5 \cdot V_{KMnO4} \cdot N_{KMnO4} \cdot M_{cu} \qquad (6)$$

From the table of invertible sugars (IS) (using the $m_{(Cu)}$ calculation to determine the total sugars in P%)

$$P\ (TS) = mIS \cdot 10^{-3} \cdot (100/10) \cdot (100/\ me)\ \% \qquad (7)$$

Reducing sugars (RS)

To a volume of 10 ml of a 0.5% (w/v) honey solution, 10 ml of Fehling A and 10 ml of Fehling B were added. From this mixture, the same steps were followed for

recovery of the precipitate and titration with $KMnO_4$ (0.02N) used for the total sugars. The results were calculated as follows:

$$m\ RS_{(cu)} = 5V_{KMnO4} \cdot N_{KMnO4} \cdot M_{cu} \quad (8)$$

From the glucose conversion table (using the $m_{(Cu)}$ calculation to determine total sugars in P%)

$$P\ (RS) = mRS \cdot 10^{-3} \cdot (100/10) \cdot (100/ me)\ \% \quad (9)$$

Sucrose was determined from the following formula:

$$Sucrose = 0.95(TS- RS) \quad (10)$$

Measurement of ash content

Ash level was determined by the standard procedure described by Bogdanov *et al.* (1999). Two grams of honey (m_0) were weighed into a porcelain crucible previously dried and weighed empty (m_2). The whole was incinerated in an electric oven at 600°C for 3 hours. The crucible containing the ashes was then weighed (m_1) and the rate of ashes (W_A), expressed in g/ 100g of honey, was calculated according to the following formula:

$$W_A = (m_1 - m_2) \cdot 100 / m_0 \quad (11)$$

Determination of antioxidants

Total phenolic compounds

The content of phenolic compounds was evaluated according to the method described by Naithani *et al.* (2006). 100 µl of honey solution (0.5 g/ml) was added along with 100 µl of Folin-Ciocalteu reagent (50%) and 2 ml of sodium carbonate solution (Na_2CO_3, 2%). After 30 min in the dark, the absorbance was read at 750nm. Gallic acid was used as standard (0.05g mg/ml), and the results were expressed in mg gallic acid equivalents per 100 grams of honey (mg GAE/ 100g) ($y = 2.79x + 0.02$; $R^2 = 0.996$).

Total flavonoids

The flavonoid content of the samples was estimated according to the method described by Al *et al.* (2009). The reaction medium contained 1000 µl of honey solution (0.5g/ml) and 300 µl of sodium nitrite (5%). After 5 min, an equivalent volume of aluminum chloride (10%) was added. After 6 min, 2 ml of sodium hydroxide (1M) was added. The absorbance was read at 510 nm and the results were expressed in mg quercetin equivalents (0.5- 0.25 mg/ml) per 100g of honey (mg EQ/ 100g) ($y = 1.580\ x + 0.02$; $R^2 = 0.994$).

Antioxidant activities

Reducing power

The analysis of the reducing power of honey was performed according to the method reported by Li and Lin (2010). A volume of 0.5 ml of sample extract (0.2g / ml) was added to 0.5 ml of phosphate buffer (pH 6.6; 0.2 M) and 0.5 ml of potassium hexacyanoferrate (1%). The mixture was incubated for 20 min in a water

bath at 50°C. Afterward, 0.5 ml of trichloroacetate solution (TCA10%) was added to this mixture. In a test tube, 0.5 ml of the reaction mixture was taken and added to 0.8 ml of distilled water and 0.1 ml of ferric chloride (0.1%).

The absorbance was read at 700 nm after 10 min. The results were expressed in mg of gallic acid (0.2- 0.03 mg/ml) equivalents per 100 g of honey (mg GAE/ 100g)

($y = 26.273x + 0.002$; $R^2 = 0.999$).

Ferric reducing antioxidant power assay (FRAP test)

The FRAP test was used to determine the antioxidant capacity of honey as reported by Alvarez-Suarez *et al.* (2010). A volume of 750 µl of FRAP solution [300 mM sodium acetate solution, 10 mM TPTZ in 40 mM HCl, and 20 mM iron chloride solution ($FeCl_3$, $6H_2O$)], was mixed with 500 µl of honey solution. The absorbance was read at 593 nm after incubation for 5 min at 37°C. The results were expressed as mg gallic acid (0.2- 0.1 mg/ml) equivalents (mg GAE) per 100g of honey ($y = 5.18x + 0.162$; $R^2 = 0.993$).

CUPRAC test (Cupric reducing antioxidant capacity)

CUPRAC assay was estimated according to Apak *et al.* (2004) method. A volume of 300 µl of $CuCl_2$ copper chloride (10^{-2}M), was mixed with 300 µl of ammonium acetate (1M), 300 µl of Neocuproin ($7.5 .10^{-3}$M), 150 µl of honey solution (2.5%) and 150 µl of distilled water. After 30 min in the dark, the absorbance was read at 450 nm. Results were expressed as mg Trolox equivalent (0.2- 0.05 mg/ml) per 100 g of honey (mg TRE/ 100g) ($y = 15.27x + 0.030$; $R^2 = 0.996$).

Total antioxidant capacity (TAC)

A mixture of 100 µl of honey solution and 1ml of phosphomolybdate reagent [ammonium heptamolybdate, sulfuric acid, and sodium phosphate] was incubated for 90 min at 90°C. The absorbance was read at 695 nm (Mcanalley *et al.*, 2003). The results were expressed in mg of gallic acid equivalents (0.2-0.1 mg/ml) per 100 g of honey (mg GAE/100 g) ($y = 3.636x$; $R^2 = 0.997$).

DPPH (2,2-diphenyl-1-picrylhydrazyl) test

The evaluation of the antiradical capacity by the DPPH assay was performed by the method described by Meda *et al.* (2005). A volume of 1 ml of the ethanolic solution of DPPH (6×10^{-2} mM) was mixed with 0.5 ml of the honey solution (0.025 g/ml). After 15 min, the absorbance was read at 517 nm. The percentage of inhibition was calculated according to the following formula:

$$\text{Antiradical DPPH activity (\%)} = ((Abs_{(c)} - Abs_{(s)})/ Abs_{(c)})\cdot 100 \quad (12)$$

where: $Abs_{(c)}$ is Absorbance of the control; $Abs_{(s)}$ is Absorbance of the sample.

ABTS [2,2-azinobis (3-ehtylbenzothiazoline-6-sulfonic acid) diamonium salt] test

The evaluation of the antioxidant capacity with ABTS was performed according to the method reported by Re *et al.* (1999). A volume of 0.1 ml of honey solution (0.025 g/ml) was added to 1 ml of ABTS solution (7 mM). After 7 min of incubation at room temperature, the absorbance was read at 734 nm. The percentage of ABTS reduction was given according to the following formula:

$$\text{Antiradical ABTS activity (\%)} = ((Abs_{(c)} - Abs_{(s)} / Abs_{(c)}))\cdot 100 \quad (13)$$

where: $Abs_{(c)}$: is absorbance of the control ; $Abs_{(s)}$: is absorbance of the sample.

Statistical analysis

All experiments were performed in triplicates, and the results were presented as mean ± standard deviation (S.D.). Statistical analyses were performed using elementary statistics. A *Student test* was applied in order to highlight the significant differences between the analyzed samples. Differences between means were considered significant at $*p < 0.05$, 0.01 or 0.001.

Results and discussion

Pollen analysis

All kinds of honey intrinsically possess the signature of their origins. It is a kind of "fingerprint" full of information. Pollen analysis is the average way to decipher this "fingerprint". Indeed, it relies on the identification and quantification of pollen observed under an optical microscope by comparing them to a pollen bank (Chefrour *et al.*, 2007; Makhloufi *et al.*, 2015).

Pollen grain frequency classes are given as predominant pollen (> 45%), secondary pollen (16 - 45%), minor pollen (3 - 15%), and rare or isolated pollen (1 - 3%) (Ouchemoukh *et al.*, 2007; Makhloufi *et al.*, 2010). Honey is considered monofloral when the number of dominant pollen from one flower species is greater than or equal to 45%.

A melissopalynology test allowed the identification of honey H1 as a polyfloral honey. This honey contained accompanying pollens including *Fabaceae* and *Myrtaceae*, but it contained a remarkable number of minor pollens (*Lamiaceae, Ericaceae, Convolvulaceae, Cornaceae, Brassicaceae*) and rare pollens (*Rosaceae, Asteraceae, Oleaceae, Papaveraceae, Liliaceae, Malvaceae, Rononculaceae, Apiaceae*) (Table 1).

This polyfloral honey was evidence of floral diversity, but it could be explained by the absence of large-scale monocultures in the regions where the honey was harvested.

The pollen analyses also revealed that honey H2 was monofloral honey whose dominant pollen was *Myrtaceae*. This sample presented some secondary pollens (*Fabaceae*), minor (*Ericaceae, Convolvulaceae*), and rare pollens (*Rononculaceae, Cistaceae*).

The pollen content of honey differed according to the botanical richness of the region, climatic conditions, and the distance from the hive to the flower field (Makhloufi *et al.*, 2010; Ouchemoukh, 2012). Photography of analyzed pollens were shown in Figures 1 and 2.

Physico-chemical analysis

Physicochemical parameters are necessary for the identification of the quality of honey. The obtained physicochemical results were presented in table 2.

Table 1. Pollen spectrum and pollen percentages of analyzed honeys.

Honey	Botanical origin	Predominant pollen (≥45%)	Secondary pollen (15-45%)	Minor pollen (3-15%)	Important minor pollen (≤3%)	Others ≤1
		Pollen type %	Pollen type %	Pollen type %	Pollen type %	
H1	**Poly-floral**	/	*Fabaceae* 37.58 *Myrtaceae* 16.29	*Lamiaceae 3.7* *Ericaceae 6.13* *Convolvulaceae 11.45* *Cornaceae 4.67* *Brassicaceae 3.22*	*Rosaceae* 1.93 *Asteraceae* 1.29 *Oléaceae 1.29* *Papavéraceae 2.74* *Liliaceae 1.29* *Malvaceae 1.77* *Rononculaceae 2.42* *Apiaceae 1.61*	*Buxaceae* *Géraniaceae* *Caprifoliaceae* *Mimosaceae* *Fagaceae*
H2	**Mono-floral**	*Myrtaceae* 46.27	*Fabaceae 38.47*	*Ericaceae* 6.14 *Convolvulaceae* 3.65	*Rononculaceae 1.66* *Cistaceae* 2.99	*Caprifoliaceae* *Mimosaceae* *Brassicaceae*

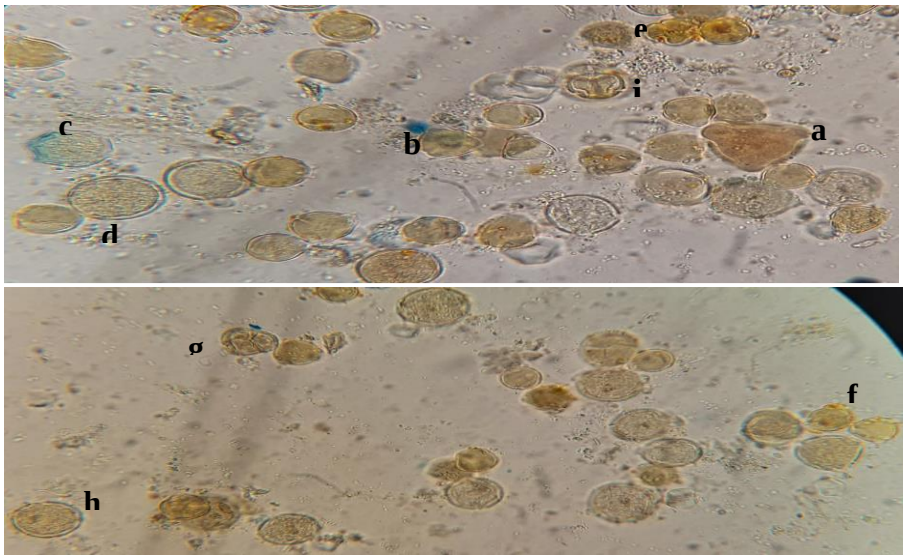

Figure 1. Pollen grains of honey H1 under optical microscope (G x 40). **a:** *Ulex europaeus* (*Fabaceae*); **b:** *Robinia pseudacacia* (*Fabaceae*); **c:** *Mentha pulegium* (*Lamiaceae*); **d:** *Anemone nemorosa* (*Renonculaceae*); **e:** *Quercus ilex* (*Fagaceae*); **f:** *Myrathus communis* (*Myrtaceae*); **g:** *Arbutus unedo* (*Ericaceae*); **h:** *Filipendula ulmaria* (*Rosaceae*); **i:** *Sambuscus nigra* (*Caprifoliaceae*).

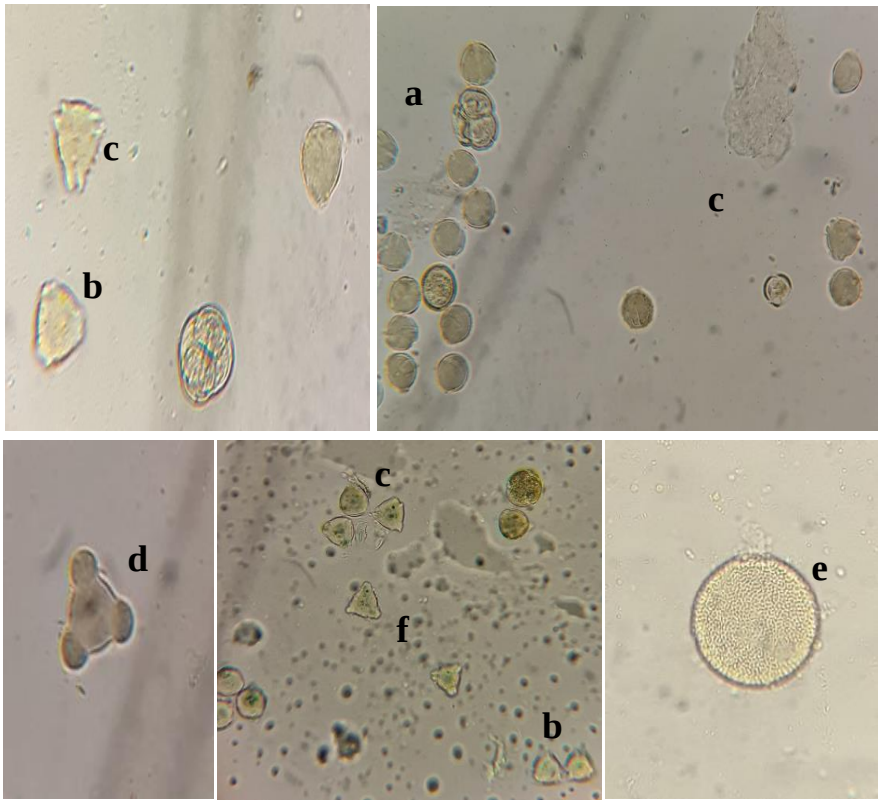

Figure 2. Pollen grains of honey H2 under optical microscope (G x 40).

a: *Arbutus unedo* (*Ericaceae*); **b:** *Callistemon lanceolatus* (*Myrtaceae*); **c :** *Eucalyptus camaldulensis* (*Myrtaceae*) ; **d :** *Cytisus laburnum* (*Fabaceae*); **e :** *Convolvulaceae* ; **f :** *Ulex europaeus* (*Fabaceae*).

Humidity is an important element in the evaluation of the degree of maturity of the honey and its life span (Doukani *et al.*, 2014). The humidity of the analyzed samples was lower than the maximum limit fixed by the European Commission (2002) and the Codex Alimentarius (2001) which is 20%. The values recorded for this parameter were 13.21% and 16.44% for H1 and H2, respectively, which corresponded respectively to refractive indices of 1.5038 and 1.4955.

The obtained moisture of the analyzed samples was similar to those obtained by Tornuk *et al.* (2013) (8.99 to 17.40%), Doukani *et al.* (2014) (13.4 to 17.2%), and Habib *et al.* (2014) (13.63 to 20.60%), and lower than that of Mouhoubi-Tafinine *et al.* (2018) (17.28 to 21.34%).

The variation in moisture could be explained by honey composition, floral origin, bee colony strength, harvesting method and season, degree of maturity reached in the hive, as well as hygrometric conditions of the hive.

The Brix degree, or percentage of dry matter, indicates the quantity of sugars contained in honey (Conti *et al.,* 2014). The obtained Brix degree was 84.50 ± 0.66 % for honey H1 and 81.90 ± 0.1% for honey H2. These values were in an inverse relationship with the water content.

Table 2. Physico-chemical analysis of the studied honeys.

Parameters		**H1**	**H2**
Humidity (%)		13.21 ± 0.10^{b}	16.44 ± 0.20^{a}
Refractive index		1.5038 ± 0.00^{a}	1.4955 ± 0.00^{b}
Brix (%)		84.50 ± 0.66^{a}	81.90 ± 0.10^{b}
CE (mS / cm)		0.89 ± 0.07^{a}	0.23 ± 0.01^{b}
pH		4.26 ± 0.12^{b}	4.43 ± 0.22^{a}
Acidity (meq/Kg)	Free acidity	39.66 ±0.05^{a}	25.64 ± 0.80^{b}
	Combined acidity	7.82 ± 0.30^{a}	0.83 ± 0.30^{b}
	Total acidity	47.67 ± 0.03^{a}	26.47 ± 0.01^{b}
HMF (mg / kg)		3.19 ± 0.34^{b}	3.79 ± 0.73^{a}
Proline (mg / kg)		553.29± 0.77^{a}	317.66± 0.77^{b}
Color		0.81 ± 0.00^{a}	0.27 ± 0.00^{b}
Protein (mg EBSA / 100 g)		47.25 ± 0.00^{a}	16.91 ± 0.80^{b}
Sugars (%)	Total sugar	58.19 ± 0.01^{b}	61.64 ± 0.00^{a}
	Reducing sugar	55.32 ± 0.01^{b}	57.41 ± 0.00^{a}
	Sucrose	2.73 ± 0.01^{b}	4.01 ± 0.005^{a}
Ash (%)		0.28 ± 0.01^{a}	0.20 ± 0.01^{b}

Values are mean ± standard deviation (n= 3). Different letters indicate significant differences within rows ($p<0.05$).

These values were in agreement with that set by the Codex Alimentarius (2001) and higher than those obtained by Islam *et al.* (2012) on Bangladesh honey (42.8 to 60.6%) as well as those of Malaysian honey (55.33 to 64.93%) reported by Moniruzzaman *et al.* (2013), but close to those given by Belay *et al.* (2013) on Ethiopian honey (60 to 85%) and those of Habib *et al.* (2014) on the study of honey from arid regions (79 to 84.10%).

The electrical conductivity of the analyzed kinds of honey varied from 0.89± 0.07 mS / cm (H1) to 0.23 ± 0.01 mS/cm (H2). According to Gonnet (1986), nectar honey has a conductivity ranging from 0.1 to 0.5 mS/cm. On the other hand, honeydew honey from 1 to 1.5 mS/cm, the median values correspond to mixtures of two origins (nectar + honeydew). Whereas the H2 honey had a value lower than 0.5 mS / cm, so this honey was of floral origin. However, the H1 honey had a value superior to 0.5 mS/cm and inferior to 1 mS/cm, therefore this honey was of nectar and not to be considered as honey of mixtures, after the confirmation by the other analyses: the pH and the rate of ash. The results obtained are similar to those given by Ouchemoukh (2012) (0.21 to 0.89 mS/cm) and Bettar *et al.* (2015) (0.22 to 0.87 mS/cm).

Differences in electrical conductivity of the studied kinds of honey could be explained by the variability in botanical origin and the chemical composition (the content of minerals, proteins, and other ionizable substances).

The pH plays a very important role during the extraction of honey, it influences its texture and its stability during conservation. Honey H1 and H2 were acidic. The obtained pH could be due to the foraged flora, the salivary secretion of the bee, and the enzymatic and fermentative processes during the transformation of the raw material.

Acidity is a very important quality criterion during the extraction and storage of honey, because of its influence on texture and stability. The obtained results were 39.66 meq/kg for H1 and 25.64 meq/kg for H2 and were in conformity with the European standard (2002), which fixed for honey a maximum value of 50 meq/kg.

The obtained acidities were included in the range reported by Belay *et al. (*2013) on Harrena forest honey (25.49 - 48.81) and were different from that obtained by Chakir *et al.* (2011) on Moroccan honey (15.70 meq/kg- 21.71 meq/kg) and Ouchemoukh (2012) on Algerian honey (3.90 to 31.37 meq/kg).

Combined or lactone acidity is considered the reverse acidity when honey becomes alkaline (Bettar *et al.,* 2015). The lactone acidity values of the analyzed kinds of honey were 7.82 meq/Kg (H1) and 0.83 meq/ Kg (H2). These results were included in the range found by Bettar *et al.* (2015) on honey from Morocco (0.5-16.65 meq/kg), and were different from that reported by Fallico *et al.* (2004) (9.5 meq/kg) on Italian honey and Ouchemoukh (2012) (9.23 and 30.37) on Algerian ones.

The total acidity is the sum of free and bound acidities and was found for H1 at 47.67 meq/kg and for H2 at 26.47 meq/kg. These acidities were in the range of those obtained by Ouchemoukh (2012) (24.40 to 59.10 meq/kg) on polyfloral kinds of honey and were different from those of *Erica arborea* honey (38.23 to 41 meq/kg).

HMF is a parameter used for the evaluation of the freshness and overheating of honey (Doukani *et al.,* 2014). The spectrometric analysis of the honey samples revealed HMF levels, 3.19 ± 0.34 mg/kg for H1 and 3.79 ± 0.73 mg/ g for H2. The values of HMF of the analyzed samples were in accordance with the standards established by the Codex Alimentarius (2001) (< 40 mg/kg). HMF results were in agreement with those reported by Habib *et al.* (2014) (0.17 to 79.26 mg/kg) and Bettar *et al.* (2015) (2.54 to 85.48 mg/kg). HMF content is influenced by factors including sugar type, concentration, pH, floral source, temperature, heating time, and storage conditions, which are factors influencing HMF levels (Habib *et al.,* 2014).

Proline is an indicator of honey maturity and adulteration. In general, its level should not be lower than 180 mg/kg (Codex Alimentarius, 2001).

The proline contents of the analyzed samples were 553.29 mg/kg for H1 and 317.66 mg/kg for H2, indicating that these kinds of honey were mature and

authentic. These obtained values were within the range reported by Ouchemoukh (2012) (302.33 ± 2.52 to 1420.07 ± 16.5 mg/kg).

The color of the analyzed kinds of honey varied between light yellow and brown. The optical densities obtained were 0.81 for H1 and 0.27 for H2 and were in the range reported by Moniruzzaman *et al.* (2013) and Das *et al.* (2015).

The color of honey is closely related to its botanical origin, chemical composition, mineral content, pollen, and mainly to the presence of pigments (carotenoids, flavonoids, tannin derivatives, and polyphenols) (Ribeiro *et al.*, 2014). It plays a very important role in determining the antioxidant capacity of honey, the darker honey is the higher the content of total phenols, minerals, and acids is (Ouchemoukh *et al.*, 2007; Doukani *et al.*, 2014; Amessis-Ouchemoukh *et al.*, 2021).

The protein concentrations of the studied kinds of honey were 47.25 and 16.91 mg EBSA per 100 g for H1 and H2, respectively. These values were within the range set by Alvarez-Suarez *et al.* (2010) on Cuban honey (12-92.3 mg EBSA / 100 g), Yucel and Sultanoglu (2013) (13 to 115 mg EBSA / 100 g) and lower than those of Chefrour *et al.* (2007) (220 - 960 mg / 100 g) on Algerian honey.

Protein concentrations of honey varied according to their botanical and geographical origins, the conditions and time of their storage, the presence of enzymes added by bees during the ripening process, and the pollen grains present (Moniruzzaman *et al.*, 2013; Habib *et al.*, 2014; Da Silva *et al.*, 2016).

Sugars represent the main components of all types of honey. Reducing agents (invert sugar), mainly fructose and glucose, were found to be major constituents of honey (Küçük *et al.*, 2007). The values of reducing sugars were 55.32% for H1 and 57.41% for H2 and they were found to meet the standards required by the Codex Alimentarius (2001), which is above 45%.

The amounts of reducing sugars were lower than those found by Ouchemoukh *et al.* (2007) and Doukani *et al.* (2014), who reported a rate of reducing sugars ranging successively from 67.83 to 80.25% and 61.4 to 79.9%.

The composition of sugars allows, in some cases, to identify the botanical origin of some monofloral honey, and the proportion of the various sugars present in honey is very variable. It depends, indeed, directly on the type of flowers foraged by the bees (Louveaux, 1968).

The saccharose is a mixture of fructose and glucose and its content in honey is a parameter of authenticity. According to the Codex Alimentarius, the content of saccharose must be lower than 5g/100g, with the exception of certain types of honey (*hedysarum*, *Eucalyptus camaldulensis*, etc.) which requires a maximum limit of 10 g/ 100 g. The contents of saccharose were 2.73% (H1) and 4.01% (H2). The values of total sugar obtained were 58.19% (H1) and 61.64% (H2).

The ash content is an indicator of the mineral content and is considered a quality criterion that determines the botanical and geographical origin of honey (Belay *et al.*, 2013). The results were found to be 0.28% for H1 and 0.20% for H2. According to the European Commission (2002), the ash content of nectar honey

does not exceed 0.6% and it is between 0.6 and 1% for honeydew honey or mixed with flower honey. The ash content of the studied honey samples was less than 0.6%, which means that the analyzed samples are nectar honey.

These values were similar to those obtained by Ouchemoukh (2012), who studied some samples of Algerian honey (0.16% to 0.32%). Ash content is mainly related to climate and soil characteristics (Oroian *et al.,* 2013; El Sohaimy *et al*. 2015).

Indeed, the increase of the intensity of the color seems to be related to an increase of the ash content, the more honey is of dark color the higher the mineral content is (Ouchemoukh *et al.,* 2007; Doukani *et al.*, 2014).

Antioxidants content

The results of the antioxidant contents are presented in Figure 3.

The levels of total phenolic compounds of the analyzed kinds of honey were 32.11 and 60.93 mg of GAE /100g for H2 and H1, respectively (Figure 3, (A)). These results were higher than those found by Perna *et al.* (2013) (10.65-15.05 mg GAE/100g) on southern Italian honey and Osés *et al.* (2016) (21.188-22.762 mg GAE/100g) when assaying polyphenols in honey alone but also lower than the results of Doukani *et al.* (2014) (166.11-427.14 mg EAG/100g), Mouhoubi-Tafinine *et al.* (2016) (171.72-5351.22 mg EAG/100g) for Algerian honey and El-Haskoury *et al.* (2018) (75.52-245.22 mg EAG / 100 g).

Khalil *et al.* (2012) reported that phenolics are responsible for honey's coloring; dark honey contains a significant amount of phenolic compounds, which was confirmed with dark honey H1.

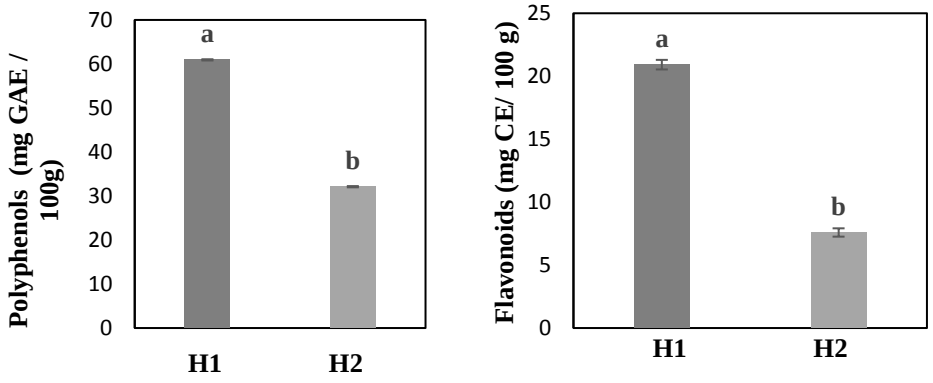

Figure 3. Antioxidant contents of the analyzed honeys.

Different letter(s) indicate the values are significantly different (*p< 0.05). Vertical bars represent standard deviations. **GAE:** Gallic Acid Equivalents; **CE:** Catechin Equivalents.

Levels of flavonoids in the different kinds of honey were 7.59 and 20.92 mg CE / 100 g for H2 and H1, respectively (Figure 3, (B)). These results were different from those obtained by Bueno-Costa *et al.* (2016), who found a value between 2.98 and 10.46 mg CE /100 g, and similar to those reported by Ouchemoukh (2012) (0.30- 35.61 mg/100 g) on Algerian honey.

Doukani *et al.* (2014) showed that most phenolic compounds were in the form of flavonoids whose concentration depended on various factors, including plant species used by bees, plant health, season, and environmental factors.

Antioxidant activity

The results of the antioxidant activities were presented in Table 3.

Table 3. Antioxidant activities of the analyzed honeys.

Parameters	H1	H2
Reducing power (mg GAE / 100 g)	24.97 ± 0.55^{a}	12.54 ± 0.23^{b}
FRAP (mg GAE/100g)	75.93 ± 0.44^{a}	31.15 ± 0.90^{b}
CUPRAC (mg TRE/100g)	49.16 ± 0.15^{a}	12.92 ± 0.30^{b}
CAT (mg GAE/g)	104.19 ± 0.21^{a}	87.24 ± 0.21^{b}
DPPH (%)	48.88 ± 0.59^{a}	38.91 ± 0.17^{b}
ABTS (%)	18.21 ± 0.08^{a}	8.90 ± 0.21^{b}

Values are mean ± standard deviation (n = 3). Different letters indicate significant differences ($_{P}$<0.05). **GAE:** Gallic Acid Equivalents; **TRE:** Trolox Equivalents.

Analysis of the reducing powers of the samples gave values of 12.54 and 24.97 mg GAE/100g for H2 and H1, respectively (Table 3). These results were lower than those reported by Čanadanović-Brunet *et al.* (2014) (39.06 to 120.00 mg EAG /100g). The reducing powers of the samples could be explained by the presence of antioxidants with reducing activities such as polyphenols, vitamin C, and carotenoids. According to Küçük *et al.* (2007), the reducing power of honey is due to the sum of the partial reducing activities of its reducing compounds. In another study, phenolic compounds were found to be the main components responsible for the antioxidant activity of honey (Bueno-Costa *et al.*, 2016).

The FRAP test is a method used to determine and estimate the antioxidant capacity of honey. The reduction of ferric iron to ferrous iron (Fe^{3+}/ Fe^{2+}) is possible by the presence or not of honey components capable of inhibiting this free radical (Moniruzzaman *et al.*, 2013).

The results obtained for the FRAP assay were 31.15 and 75.93 mg GAE/100 g for H2 and H1, respectively (Table). These results were similar to those found by Doukani *et al.* (2014) (8.3 to 240 mg GAE/100 g).

Honey H2 showed the lowest antioxidant potential, which could be due to its non-richness in antioxidant compounds, while the sample (H1) showed the highest antioxidant activity and contained high amounts of phenols. The difference in the antioxidant powers of the studied kinds of honey could be explained by the nature of the antioxidant compounds contained in these different kinds of honey. In fact, the antioxidant activity of honey is attributed to the quantitative and qualitative nature of its phenol content (Beretta *et al.*, 2005).

According to Moniruzzaman *et al.* (2013), the botanical origin of honey was the cause of variations in antioxidant activity, while processing during handling and

storage affects the antioxidant activity of honey to some extent only. On the other hand, Doukani *et al.* (2014) reported that honey species from different floral sources possessed high antioxidant activities.

The copper reducing antioxidant power of the samples was estimated by the method described by Apak *et al.* (2004). This method is based on the measurement of absorbance at 450 nm by the formation of a stable complex between neocuproin and copper (I). The latter is formed by the reduction of copper (II) in the presence of neocuproin.

The results obtained showed that sample H1 (49.16 mg TR/100g) had higher activity than H2 (12.92 mg TR/100g) (Table 3). This difference in reducing activity between the two kinds of honey may be explained by the richness of H1 in phenolic compounds compared to H2. These results were different from those found by Ulusoy *et al.* (2010), on nine honey samples from the Black Sea region of Turkey, with values between 124.8 and 532 (µmol TR/g). The increase in the number of phenolic compounds could be considered directly proportional to the total antioxidant capacity.

The capacity of molybdate reduction by the studied kinds of honey were 87,24 and 104,19 mg GAE/g for H2 and H1, respectively (Table 3). These results were similar to those given by Imtara *et al.* (2018) in the study of Palestinian honey (87.29 mg EAA/g for honey S9 and 102.66 mg EAA/g for honey S7), and higher than those reported by Tornuk *et al.* (2013), namely, 58.92 to 80.80 mg EAA/ g.

The DPPH radical is one of the most widely used substrates for the evaluation of the antioxidant activity of biological molecules, due to its stability in radical form and the simplicity of the analysis.

The DPPH radical scavenging activities of the two studied kinds of honey were 38.91 % for H2 and 48.88% for H1 (Table 3). These values were similar to those obtained by Al *et al.* (2009) on honey from Romania (35.80 - 64.83%), Alvarez-Suarez *et al.* (2012) (38.15 - 58.4%) and Ochemoukh, (2012) (2.97 - 87.68%), but were not included in the range found by Doukani *et al.* (2014) (3.42 - 22.06%) and Wilczyńska (2014) (47.2 - 83.4 %).

Honey H2 showed the lowest antiradical activity, which could be explained by its lower content of antioxidant components. The antioxidant activity depends, in general, on the chemical composition of the plant, such as flavonoids, phenols, enzymes, organic acids, amino acids, and carotenoids (Flores *et al.*, 2015).

The antiradical ABTS activity was another method tested to evaluate the ability of honey to inhibit the cationic radical $ABTS^{+}$ and reduce it to its neutral form ABTS. The obtained results were 8.90 % for H2 and 18.21 for H1 (Table 3).

The obtained scavenging activities were within the ranges found by Wilczyńska (2014) (6 to 79%), and Bueno-Costa *et al.* (2016) (8.24 to 111.48%), and were different from those obtained by Perna *et al.* (2013) (59.17±1.69%), and Habib *et al.* (2014) (40 to 80%).

The antioxidant properties of honey were, on one hand, related to enzymatic substances (catalase, glucose oxidase, and peroxidase) and on the other hand, were

related to non-enzymatic compounds (ascorbic acid, carotenoids, amino acids, proteins, flavonoids, and phenolic acids). The amount and type of these antioxidants depend largely on the variety of honey (Khalil *et al.*, 2012). Thus, the antioxidant activity depends on the type and amount of antioxidants present in honey.

Conclusions

The present study focused on the analysis of two samples of honey through the study of their botanical origin, their physicochemical properties, and their antioxidant capacities. The pollen analyses showed that H1 was polyfloral and H2 monofloral. Most of the physico-chemical parameters analyzed were in agreement with the Codex Alimentarius standards. Consequently, the analyzed samples were of floral origin, and this was confirmed by pH and ash tests. The HMF levels of the studied kinds of honey were below the maximum limit of 40%, which proved that the honey was fresh and of good quality. The color of the honey varied from yellow to brown, which played an important role in the antioxidant capacity of the honey. These quality criteria were influenced by the environmental conditions, the type of foraged flora, the strength of the bee colony, and the beekeeping skills.

Honey H2 contained low levels of polyphenols and flavonoids and was light in color, in contrast to the very dark honey H1, which had high levels of polyphenols and flavonoids.

The study of the antioxidant capacity by six different methods (reducing power, phosphomolybdate reduction, CUPRAC and FRAP tests, and antiradical activities against DPPH and ABTS) reveals that the analyzed kinds of honey have appreciable antioxidant activities that differ from one honey to another. These variations are due to the botanical origin of the analyzed honey samples and depend on the type and quantity of antioxidants present in the honey.

The study of the physicochemical characteristics and phenolic compounds of honey from the Jijel region deserves to be pursued to identify and quantify the biologically active components, used for the treatment of several diseases and future work could also be envisaged to determine other biological activities such as anti-inflammatory, hypoglycemic, antibacterial, and antifungal activities.

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