Pol. J. Food Nutr. Sci., 2020, Vol. 70, No. 2, pp. 169–178
DOI: 10.31883/pjfns/118528
http://journal.pan.olsztyn.pl

Original article
Section: Food Quality and Functionality

Altitude Effect on the Properties of Honeys from the Region of Jijel (Algeria)

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Key words: Jijel, coastal and mountain honeys, physicochemical properties, antioxidants, antioxidant activity, antibacterial activity

Coastal and mountainous honey samples were collected from different regions in Jijel (Algeria) to evaluate their moisture content, electrical conductivity, ash content, pH, acidity, protein content, color parameters, antioxidants content, radical scavenging activity, reducing power, and antibacterial activity, to reveal the differences between coastal and mountain honeys and to determine the correlation between altitude and different parameters. The results indicate that Jijelian honeys were dark with acceptable physicochemical properties and a good bioactive potential. *Escherichia coli* was sensitive to Jijelian honeys while *Staphylococcus aureus* and *Pseudomonas aeruginosa* were more resistant. Coastal honeys had statistically significantly higher pH, electrical conductivity, ash content, color intensity, hydroxymethylfurfural (HMF) content, and reducing power than the mountainous samples ($p<0.05$), while the total acidity was higher in the mountain honeys ($p<0.05$). The altitude was significantly negatively correlated with HMF content, electrical conductivity, ash content, and pH. The correlation coefficients were -0.510, -0.405, -0.360, and -0.355, respectively.

INTRODUCTION

Honey is a natural product that honeybees produce from some plant parts or excretions of some insects that feed on plant sap [Karabagias *et al.*, 2014]. More than two hundred components have been found in honey; it is an important source of energy due to its high sugar content, mainly fructose (38%) and glucose (31%) [Alvarez-Suarez *et al.*, 2010; Bueno-Costa *et al.,* 2016]. Moreover, it has small amounts of amino acids, proteins, phenolic compounds, carotenoids, organic acids, ascorbic acid, enzymes, α-tocopherol, and oligosaccharides [Alvarez-Suarez *et al.*, 2010]. The composition and characteristics of honey are primarily determined by the food source (plants); however, environmental factors, processing, and storage affect its composition as well [Saxena *et al.*, 2010].

Phenolic compounds content and antioxidant activity have been widely used as indicators to evaluate the characteristics and bioactive properties of honey [Tahir *et al.*, 2017]. Honey contains a variety of phenolics, and is rich in antioxidants, which increases its usability potential for therapeutic purposes [Küçük *et al.*, 2007]. In addition, several other authors have mentioned the antimicrobial potential of honey [Alvarez-Suarez *et al,* 2010; Bueno-Costa *et al.*, 2016; Küçük *et al.,* 2007; Liu *et al.*, 2013]. The concentration of hydrogen peroxide, which is determined according to the level of glucose oxidase (from bees) and catalase (pollen source), in honey mainly predicts its antimicrobial potential, however, lysozyme, phenolic acids, and flavonoids are the major non-peroxide contributing factors [Tenore *et al.*, 2012]. On the other hand, the correlation of the color with bioactive compounds and antioxidant and antibacterial activities has been revealed in other studies [Bueno-Costa *et al.*, 2016]. In recent years, many authors have studied the physicochemical and bioactive properties of honeys from different regions in the world including Algeria [Bueno-Costa *et al.*, 2016; Mouhoubi-Tafinine *et al.*, 2016; Ouchemoukh *et al.*, 2007; Tahir *et al.*, 2017; Tenore *et al.*, 2012], using different analytical methods.

Many scientists have studied the characteristics and the properties of mono-floral honeys produced from different plants by honeybees [Alvarez-Suarez *et al.*, 2010; Karabagias *et al.*, 2014; Küçük *et al.*, 2007; Tenore *et al.*, 2012]. However, the aim of this study was to evaluate the characteristics (physicochemical properties, protein content, color parameters, contents of total phenolics and total flavonoids, DPPH radical scavenging activity, reducing power, and antibacterial activity) of honeys from the greenest region of Algeria (Jijel) from different altitudes, to determine the differences between coastal and mountain honeys and to reveal the correlation between the altitude and different parameters.

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MATERIALS AND METHODS

Samples

Twenty-two honey samples were collected from different regions in Jijel (Algeria). Half of these samples were collected from regions close to the Mediterranean Sea and the other half from mountain regions. All these samples were produced from hives placed in areas with diverse vegetation in order to get poly-floral honeys that are more representable of this region. In addition, honeys from hives placed in vast monoculture fields were avoided. All samples were stored at 4–5°C in airtight glass containers until analyses.

Physicochemical analyses

The physicochemical analyses were determined according to the International Honey Commission [2009]. Moisture and ash contents were expressed in g/100g. Acidity, electrical conductivity, and hydroxymethylfurfural (HMF) content were expressed in milliequivalents of sodium hydroxide required to neutralize 1 kg of honey (meq/kg), mS/cm, and mg/kg, respectively.

Protein content

The protein content was analyzed according to the Bradford method reported by Azeredo *et al.* [2003]. The absorbance was measured at 595 nm (UV-1800 UV-Vis Spectrophotometer from Shimadzu, Kyoto, Japan), against a standard solution of bovine serum albumin (0.1–1.4 mg/mL).

Color analysis

Color analysis was reviewed according to Ferreira *et al.* [2009]. Honeys in distilled water solutions of 50% (*w/v*) were centrifuged at 3000×*g* for 10 min (centrifuge Model 3–16P, Sigma Laborzentrifugen GmbH, Osterode, Germany). The color was measured spectrophotometrically at 635 nm. The Pfund scale was used to classify the honeys as follows: mm Pfund = -38.70 + 371.39 ×Abs.

Total phenolics content

The following method described by Bueno-Costa *et al.* [2016] was used to determine the total phenolics content (TPC): a honey solution of (0.1 g/mL) was centrifuged at 3000×*g* for 10 min. Then, 0.5 mL of supernatant and 2.5 mL of 0.2 N Folin–Ciocalteu reagent were mixed for 5 min. Afterwards, 2 mL of a sodium carbonate solution (75 g/L) was added and the mixture was incubated for 2 h in dark. The absorbance was measured using a spectrophotometer at 765 nm. The TPC was expressed as mg gallic acid equivalent per 100 g of sample (mg GAE/100 g).

Total flavonoids content

The total flavonoids content (TFC) was determined according to the method described by Chaikham *et al.* [2016]. A solution of honey in ddH_2O (1 mL; 0.5 g/mL) was mixed with 300 μL $NaNO_2$ (5.0%). A volume of 300 μL of $AlCl_3$ (10%) was added to the mixture, and after 6 min, 2 mL of 1M NaOH was added. A spectrophotometer was used at 510 nm to measure the absorbance. A standard curve was defined by the known concentrations of quercetin (0–40 mg/L), and the results were expressed as mg quercetin equivalent per 100 g of sample (mg QE/100 g).

DPPH radical scavenging activity

Assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) was performed according to Meda *et al.* [2005] procedure. The 0.75 mL of each honey solution in methanol (2.5–160 mg/mL) was mixed with 1.5 mL of DPPH in methanol (0.02 mg/mL). The mixture was left in the dark for 15 min and then its absorbance was measured at 517 nm. The DPPH radical solution without the sample served as the blank sample. The results were calculated based on the following formula: %Inhibition = [(blank absorbance – sample absorbance)/blank absorbance] ×100. The half maximal inhibitory concentration (IC_{50}) value of each honey sample was estimated from the plot of % inhibition *vs.* honey concentration.

Reducing power

The following method of reducing power (RP) determination was used [Küçük *et al.*, 2017]: 1 mL of a honey solution (5.0%) was added to 2.5 mL of a phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 min. Afterwards, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000×*g* for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm. Ascorbic acid (1.0 mg/mL) was used as a reference standard.

Antibacterial activity

Agar disc diffusion assay of 100% honey concentration was used against three strains of bacteria, which were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 (Pasteur Institute of Algeria, Algeria), according to Alderman & Smith [2001]. The results were expressed in zone of growth inhibition (mm).

Statistical analysis

All tests were performed in triplicate and the results were expressed as mean ± standard deviation. The parameters of the descriptive statistics were calculated using the Microsoft Excel 2007 program. A one-way analysis of variance (ANOVA) was carried out with the STATISTICA 7.1 software to highlight the presence or absence of a significant difference between the samples of honey, which was considered statistically significant at the level of 0.05. LSD test was used as post-hoc ANOVA test ($p<0.05$). The relationships between the parameters were determined by the correlation matrix ($p<0.01$), while the comparison between means obtained for the coastal and mountain honeys was determined by Student's *t*-test using XLSTAT 2014.

RESULTS AND DISCUSSION

Physicochemical parameters of honeys

Moisture content

The Jijelian honeys had a moisture content varying between 16.7 and 19.8 g/100 g (Table 1). The results reported differed significantly ($p<0.05$) among the samples, and all

TABLE 1. Physicochemical parameters of honeys from coastal (C1-C11) and mountain (M1-M11) of the Jijel region of Algeria.

Samples locations	Altitude (m)	Moisture (g/100 g)	pH	Free acidity (meq/kg)	Lactonic acidity (meq/kg)	Total acidity (meq/kg)	EC (mS/cm)	Ash (g/100 g)
Coastal honeys								
Beni Belaid (C1)	6	17.2±0.05^{jk}	4.59±0.07^{a}	18.8±0.65^{ef}	9.2±0.51^{fgh}	28.0±1.11^{fgh}	1.13±0.01^{a}	0.81±0.02^{b}
Oued Zhour (C2)	9	18.5±0.05^{c}	4.14±0.04^{ef}	24.1±2.00^{bc}	8.5±1.41^{ghi}	32.6±3.41^{cde}	0.67±0.01^{g}	0.19±0.02^{mn}
El Janah (C3)	10	18.3±0.05^{d}	4.26±0.05^{bcd}	10.8±0.55^{j}	9.6±0.30^{fg}	20.4±0.98^{kl}	0.89±0.01^{d}	0.66±0.01^{d}
Achouat (C4)	12	18.1±0.05^{e}	4.01±0.02^{ef}	22.1±0.75^{cd}	9.2±0.45^{fgh}	31.3±1.2^{def}	0.65±0.00^{h}	0.38±0.02^{g}
El Balouta (C5)	30	19.8±0.10^{a}	4.34±0.03^{b}	12.5±0.36^{hij}	11.6±0.96^{de}	24.1±1.27^{ijk}	0.51±0.01^{l}	0.13±0.01^{o}
Boukartoum (C6)	40	18.9±0.05^{b}	4.58±0.01^{a}	14.3±2.42^{gh}	8.2±0.55^{hi}	22.5±2.94^{jkl}	1.06±0.01^{b}	0.83±0.01^{a}
Jijel (C7)	60	17.5±0.05^{g}	4.07±0.02^{fgh}	14.4±0.91^{gh}	11.2±1.25^{de}	25.6±2.16^{hij}	0.58±0.01^{j}	0.27±0.00^{j}
El Kennar (C8)	60	18.8±0.10^{b}	3.81±0.04^{i}	20.8±0.30^{de}	8.4±0.36^{ghi}	29.2±0.65^{efgh}	0.62±0.01^{i}	0.21±0.01^{lm}
El Aouana (C9)	65	17.1±0.05^{k}	4.25±0.02^{bcd}	26.2±1.40^{ab}	8.4±0.34^{ghi}	34.6±1.65^{bcd}	0.91±0.01^{c}	0.62±0.01^{e}
Timizer (C10)	70	17.3±0.05^{ij}	4.27±0.01^{bcd}	22.2±1.00^{cd}	10.4±0.65^{ef}	32.6±1.65^{cde}	0.84±0.01e	0.25±0.01^{k}
Ziama Mansouriah (C11)	120	17.1±0.05^{k}	4.19±0.01^{cde}	14.7±0.45^{gh}	12.5±0.36^{d}	27.2±0.80^{ghi}	0.43±0.01^{n}	0.17±0.01^{n}
Mean C	**44**	**18.1±1.36^{A}**	**4.23±0.23^{A}**	**18.3±5.16^{A}**	**9.7±1.47^{A}**	**28.0±4.56^{B}**	**0.75±0.23^{A}**	**0.46±0.28^{A}**
Mountain honeys								
El Milia (M1)	300	17.4±0.05^{hi}	3.64±0.03^{j}	18.8±4.45^{ef}	9.2±1.63^{fgh}	35.5±6.05^{fgh}	0.56±0.01^{k}	0.24±0.01^{k}
Ouled Yahia (M2)	310	18.5±0.05^{c}	3.80±0.01^{i}	28.3±2.45^{a}	7.6±0.55^{i}	35.9±2.95^{abc}	0.52±0.01^{l}	0.33±0.03^{i}
Ghebala (M3)	330	16.7±0.05^{l}	3.68±0.03^{j}	22.3±1.83^{cd}	16.5±0.95^{b}	38.8±2.71^{a}	0.48±0.01^{m}	0.35±0.00^{hi}
Bordj Thar (M4)	340	17.5±0.05^{gh}	3.89±0.06^{i}	20.7±0.80^{de}	10.4±0.72^{ef}	31.1±1.50^{def}	0.63±0.01^{i}	0.37±0.01^{gh}
Oudjana (M5)	400	18.8±0.10^{b}	4.28±0.02^{bc}	12.5±2.57^{hij}	7.2±0.43^{i}	19.7±3.00^{l}	0.62±0.00^{i}	0.41±0.01^{f}
Djimla (M6)	510	18.5±0.05^{c}	4.01±0.02^{h}	20.5±0.69^{de}	9.2±0.75^{fgh}	29.7±1.37^{efg}	0.82±0.01^{e}	0.62±0.01^{e}
Ouled Askeur (M7)	520	17.5±0.05^{gh}	4.01±0.00^{h}	13.6±1.56^{hi}	18.8±0.60^{a}	32.4±2.11^{cde}	0.75±0.01^{f}	0.33±0.00^{i}
Taksana (M8)	570	17.5±0.05^{g}	4.01±0.02^{h}	22.4±0.45^{cd}	9.6±0.75^{fg}	32±1.20^{de}	0.51±0.01^{l}	0.27±0.01^{j}
Selma (M9)	640	17.9±0.05^{f}	4.18±0.02^{de}	16.5±1.77^{fg}	11.2±1.27^{de}	27.7±2.96^{fghi}	0.29±0.01^{o}	0.14±0.02^{o}
Teyana (M10)	695	16.7± 005^{l}	4.04±0.01^{gh}	28.6±0.98^{a}	8.4±0.26^{ghi}	37.0±1.21^{ab}	0.65±0.01^{gh}	0.22±0.01^{kl}
Erraguen (M11)	700	18.8±0.05^{b}	4.12±0.03^{efg}	10.9±1.32^{ij}	14.5±0.85^{c}	25.4±2.12^{hij}	0.62±0.02^{i}	0.23±0.01^{k}
Mean M	**483**	**17.8±0.76^{A}**	**3.97±0.20^{B}**	**19.9±5.93^{A}**	**11.5±3.80^{A}**	**31.4±5.60^{A}**	**0.59±0.14^{B}**	**0.32±0.13^{B}**

EC: electrical conductivity. – Values are presented as mean ± standard deviation (n=3). Values C1-C11 and M1-M11 with lowercase superscript differ significantly (LSD test, $p<0.05$). Means for coastal and mountain honeys (C and M, respectively) marked with different capital letters in superscript are significantly different (t-test, $p<0.05$)

samples were within the limits (>20%) prescribed as per the Codex Alimentarius Commission Standard for honey [2001]. The moisture content of honey is related to different factors like the period of harvesting, ripening process, and climatic conditions [Finola *et al.*, 2007], and it is an important criterion because a higher water content could cause fermentation [Ribeiro *et al.*, 2014].

pH

Honey has an acidic nature with a pH level ranging between 3.2 and 4.5 [da Silva *et al.*, 2016]. The texture, the stability, and the shelf life of honey are affected by the pH level, and low pH usually prevents the development of microorganisms [Kumar *et al.*, 2018]. As can be seen from Table 1, the pH values ranged from 3.64 to 4.59 and were significantly different among the samples ($p<0.05$). The pH value was significantly higher in the coastal honeys than in the mountain ones with mean values of 4.23 against 3.97 ($p<0.05$). In addition, the pH value was significantly negatively correlated with the altitude (r=-0.355; $p<0.01$) (Table 3). On the other hand, Ribeiro *et al.* [2014] and Karabagias *et al.* [2014] reported different pH limits with 2.98–4.15 for Brazilian honeys and 3.40–5.31 for Greek unifloral honeys, respectively. Generally, plant source, soil, inorganic molecules, and honey ripening process can affect the pH level of honey [Ribeiro *et al.*, 2014].

Free, lactonic, and total acidities

In honey, organic acids represent less than 0.50% of the total composition. Nevertheless, they have a major impact on honey acidity, which influences honey flavor and boosts chemical reactions and bioactive activities [Cavia *et al.*, 2007]. In addition, gluconic acid is the most important acid presented in honey, and it comes originally from the activity of glucose oxidase provided by bees through the ripening process [Karabagias *et al.*, 2014]. Table 1 shows the results of measurements of free, lactonic, and total acidities of honey from the Jijel region of Algeria. The free acidity ranged from 10.8 to 28.6 meq/kg. All samples were within the allowed limits fixed by the European Honey Commission (under 50 meq/kg) [Karabagias *et al.*, 2014], showing the honey freshness and the absence of undesirable fermentations [Finola *et al.*, 2007]. On the other hand, Azonwade *et al.* [2018] reported a different range of free acidity within 35.7 and 40.5 meq /kg for Beninese honeys. The equilibrium between organic acids and their corresponding lactones and other mineral ions (*e.g.* phosphate) can be the main factor describing the level of free acidity [Finola *et al.*, 2007]. In addition, the lactonic acidity ranged from 7.2 to 18.8 meq/kg (Table 1). Fröschle *et al.* [2018] reported different lactonic acidity range (14.5±8.2 meq/kg) of Jatropha honey. Finally, the total acidity ranged from 19.7 to 38.8 meq /kg (Table 1). According to literature data, the total acidity ranged from 11.94 to 58.03 meq/kg as reported by Chakir *et al.* [2016] for Maroccan honeys and from 18 to 145.50 meq/kg as determined by Alqarni *et al.* [2016] for national and international Saudi honeys, which were higher than our results. The samples differed significantly ($p<0.05$) in free, lactonic, and total acidities. In addition, the mean value of total acidity was significantly ($p<0.05$) higher in the mountain honeys than in the coastal honeys (31.4 and 28.0 meq/kg). Moreover, free acidity correlated strongly with total acidity (r=0.856) (Table 3), although Kumar *et al.* [2018] observed even a stronger correlation between these parameters for Indian honeys (r=0.920). Finally, only lactonic acidity was negatively correlated with the altitude (r=-0.286; $p<0.05$) (Table 3).

Electrical conductivity (EC)

Electrical conductivity (EC) fell between 0.29 and 1.13 mS/cm (Table 1). The results observed for the honeys differed significantly ($p<0.05$), and EC negatively correlated with the altitude (r=-0.405; $p<0.01$) (Table 3). EC values of coastal and mountain samples were significantly different ($p<0.05$) with the mean values of 0.75 mS/cm against 0.59 mS/cm. However, Can *et al.* [2015] and Karabagias *et al.* [2014] reported higher EC with 0.3 to 1.5 mS/cm for Turkish honey and 0.31 to 2.49 mS/cm for Greek unifloral honeys, respectively. On the other hand, Flores *et al.* [2015] found the EC of honeydew honeys was higher than 0.8 mS/cm. Indeed, mineral salt, organic acid, and protein levels are the most important factors that influence the EC of honey. Moreover, it is an indicator used to distinguish floral honeys from honeydew honeys [Can *et al.*, 2015; Subbiah *et al.*, 2015]. Generally, honeydew honeys have EC greater than 0.8 mS/cm [Codex Alimentarius Commission Standard for Honey, 2001]. Hence, the tested honeys of Jijel included five coastal samples and one mountain sample with an EC value higher than 0.8 mS/cm, indicating that these samples are more likely to be honeydew honeys.

Ash content

Table 1 shows that ash content of honeys from the Jijel region of Algeria ranged from 0.13 to 0.83 g/100 g. Ouchemoukh *et al.* [2007] found a lower ash content for Algerian honey from a different region (0.06 to 0.54 g/100 g). Statistically, the results showed significant differences in ash content ($p<0.05$) and coastal honeys presented higher ash content than the mountain honeys. In addition, a negative correlation (r=-0.360; $p<0.01$) (Table 3) was noted between ash content and altitude. da Silva *et al.* [2016] stated that the ash content in honey ranged from 0.02 to 1.03 g/100 g. The ash indicates the inorganic components and it may be used to indicate environmental pollution [Karabagias *et al*., 2014] and to distinguish the floral origin of honey, which is ≤0.6 g/100 g for blossom honeys and ≤1.2 g/100 g for honeydew honeys [Kumar *et al.,* 2018]. Therefore, the five samples with ash content above 0.6 g/100 g can be determined as honeydew honeys. The correlation coefficient between electrical conductivity and ash content was 0.885 ($p<0.01$). Likewise, Ouchemoukh *et al.* [2007] and Saxena *et al.* [2010] obtained a higher correlation for some Algerian and Indian honeys (0.92 and 0.98), respectively.

Hydroxymethylfurfural content

Hydroxymethylfurfural (HMF) is a furanic compound indicating honey freshness. It is formed as a result of sugars dehydration in acidic conditions (caramelization) throughout heat treatment of food as an intermediate in the Maillard reaction [Pasias *et al.*, 2017] and its content is affected by the sugar content nature, organic acids, pH, water content, and plant source [da Silva *et al.*, 2016]. In this study, HMF content of all honeys was under the maximum limits (40 mg/kg) approved by the Codex Alimentarius Commission Standard for Honey [2001], and ranged from 2.4 to 10.8 mg/kg (Table 2), indicating the freshness of Jijelian honeys. In addition, HMF content was highly significantly negatively correlated with the altitude (r=-0.510; $p<0.01$) (Table 3). The statistical analysis shows that the honeys differed significantly in terms of HMF content ($p<0.05$), which was significantly higher in the coastal samples than in the mountain samples with the mean values at 7.3 mg/kg and 4.4 mg/kg, respectively.

Protein content

Protein represents between 0.2 and 1.6 g/100 g of honey produced by *Apis mellifera*. Both animal and vegetal sources contribute to the presence of proteins and amino acids in honey [da Silva *et al.*, 2016]. The protein content of honeys from the Jijel region of Algeria is presented in Table 2. It ranged from 35 to 900 mg/100g, which was similar to the results obtained by Ouchemoukh *et al.* [2007] for Algerian honey from a different region. On the other hand, Azeredo *et al.* [2003] and Saxena *et al.* [2010] reported lower protein contents for some Brazilian and Indian honeys, respectively. Protein content showed no significant correlation with the altitude.

TABLE 2. Hydroxymethylfurfural (HMF) content, protein content, color intensity and antibacterial activity of honey (from coastal (C1-C11) and mountain (M1-M11) of the Jijel region of Algeria.

Samples	HMF (mg/kg)	Protein (mg/100 g)	Pfund scale (mm)	Color	Zone of growth inhibition (mm)*		
					Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Coastal honeys							
C1	6.3 ± 1.57^{cdef}	309 ± 18^{gh}	101 ± 10^{g}	amber	9±1.0	0	0
C2	10.8 ± 3.81^{a}	900 ± 25^{a}	122 ± 9^{ef}	dark amber	16±0.6	8±0.6	11±1.0
C3	7.2 ± 0.95^{bcde}	89 ± 9^{m}	141 ± 12^{cd}	dark amber	10±1.7	0	0
C4	8.4 ± 1.66^{bc}	217 ± 22^{k}	79 ± 9^{hij}	light amber	8±0.0	0	0
C5	4.9 ± 1.00^{efgh}	81 ± 9^{m}	52 ± 4^{k}	light amber	10±0.6	0	0
C6	7.6 ± 2.35^{bcd}	582 ± 36^{b}	216 ± 15^{a}	dark amber	10±1.0	0	0
C7	8.2 ± 1.81^{bcd}	469 ± 24^{c}	149 ± 18^{cd}	dark amber	0	0	0
C8	6.1 ± 0.59^{cdef}	425 ± 26^{d}	96 ± 8^{gh}	amber	13±1.7	10±1.0	10±0.0
C9	7.9 ± 1.96^{bcd}	428 ± 38^{d}	179 ± 26^{b}	dark amber	8±1.0	0	0
C10	9.1 ± 1.78^{ab}	246 ± 18^{jk}	190 ± 14^{b}	dark amber	10±0.6	0	0
C11	3.3 ± 0.83^{hi}	98 ± 10^{m}	139 ± 11^{de}	dark amber	7±0.0	0	0
Mean C	**7.3 ± 2.05^{A}**	**349 ± 248^{A}**	**134 ± 50^{A}**	**dark amber**	–	–	–
Mountain honeys							
M1	4.3 ± 0.73^{fghi}	35 ± 6^{n}	23 ± 6^{l}	white	10±0.6	9±1.7	0
M2	3.0 ± 0.22^{hi}	312 ± 20^{gh}	137 ± 11^{de}	dark amber	8±0.0	0	0
M3	9.2 ± 1.15^{ab}	339 ± 8^{fg}	16 ± 3^{l}	extra white	10±1.0	0	0
M4	3.1 ± 0.5^{hi}	157 ± 19^{i}	91 ± 8^{ghi}	amber	9±1.0	0	0
M5	3.7 ± 0.65^{ghi}	294 ± 14^{hi}	158 ± 20^{c}	dark amber	9±0.6	0	0
M6	6.0 ± 1.45^{defg}	$362\pm13_{ef}$	90 ± 2^{ghi}	amber	10±0.0	0	0
M7	2.4 ± 0.45^{i}	374 ± 16^{e}	63 ± 6^{jk}	light amber	0	0	0
M8	4.2 ± 0.63^{fghi}	267 ± 17^{ij}	148 ± 6^{cd}	dark amber	12±1.0	0	0
M9	3.7 ± 0.88^{ghi}	136 ± 18^{i}	76 ± 8^{ij}	light amber	12±1.7	8±0.0	0
M10	7.0 ± 0.61^{bcde}	567 ± 29^{b}	105 ± 7^{fg}	amber	9±0.0	0	0
M11	2.4 ± 0.17^{i}	141 ± 5^{i}	108 ± 8^{fg}	amber	11±1.7	0	0
Mean M	**4.4 ± 2.12^{B}**	**271 ± 147^{A}**	**92 ± 46^{B}**	**amber**	–	–	–

* Undiluted honeys were analyzed. Values are presented as mean ± standard deviation (n=3). Values C1-C11 and M1-M11 with lowercase superscript differ significantly (LSD test, $p<0.05$). Means for coastal and mountain honeys (C and M, respectively) marked with different capital letters in superscript are significantly different (*t*-test, $p<0.05$).

Color analysis

Honey color is a strong indicator of pigments existence, like carotenoids and flavonoids, which provide a good antioxidant activity [Kek *et al.,* 2014]. Color and flavor of honey are connected to each other; while light colored honeys are mild, the darker ones present a strong flavor [Belay *et al.,* 2015]. The color of Jijelian honeys ranged from extra white to dark amber (Table1), and was arranged as follows (Table 2): dark amber (45.45%), amber (27.27%), light amber (18.18%), white color and extra white (4.54%). On the other hand, Bueno-Costa *et al.* [2016] reported light amber (41.7%), amber (25%), and dark amber (33.3%) for Brazilian honeys, while Finola *et al.* [2007] found white (27%), extra white (30%), white (27%), extra light amber (13%), and amber (3%) colors for Argentinian honeys. In addition, 63.63% of the coastal honeys were dark amber, while only 27.27% of the mountain honeys were in dark amber color. Honey color negatively correlated with the altitude ($r=-0.268$; $p<0.05$) (Table 3). Generally, darker honeys tend to have more ash, nutrients, and antioxidants according to their higher correlation with bioactive compounds and different bioactive activities compared to the light colored honeys. In addition, honey color is very important for commercialization, as it attracts the consumers and set their preferences [da Silva *et al.*, 2016].

TABLE 3. Pearson correlation coefficients among parameters.

	Altitude	Moisture	pH	EC	Ash	Free acidity	Lactonic acidity	Total acidity	Pfund scale	HMF	Protein	TPC	TFC	RSA IC_{50}	RP
Altitude	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Moisture	-0.168	1	–	–	–	–	–	–	–	–	–	–	–	–	–
pH	-0.355**	0.255*	1	–		–	–	–	–	–	–	–	–	–	–
EC	-0.405**	0.003	0.565**	1	–	–	–	–	–	–	–	–	–	–	–
Ash	-0.360**	-0.076	0.524**	0.885**	1	–	–	–	–	–	–	–	–	–	–
Free acidity	0.045	-0.391**	-0.336**	-0.011	0.005	1	–	–	–	–	–	–	–	–	–
Lactonic acidity	0.286*	-0.228	-0.187	-0.230	-0.239	-0.307*	1	–	–	–	–	–	–	–	–
Total acidity	0.202	-0.524**	-0.445**	-0.136	-0.124	0.856**	0.230	1	–	–	–	–	–	–	–
Pfund scale	-0.268*	0.102	0.552**	0.437**	0.501**	0.038	-0.415**	-0.187	1	–	–	–	–	–	–
HMF	-0.510**	-0.118	0.176	0.341**	0.324**	0.366**	-0.135	0.301*	0.234	1	–	–	–	–	–
Protein	-0.138	-0.012	0.108	0.306*	0.104	0.415**	-0.204	0.314*	0.323**	0.544**	1	–	–	–	–
TPC	-0.150	0.015	0.314*	0.339**	0.455**	0.062	-0.270*	-0.083	0.738**	0.108	0.053	1	–	–	–
TFC	-0.010	0.019	0.371**	0.335**	0.391**	0.104	-0.278*	-0.045	0.770**	0.024	0.185	0.802**	1	–	–
RSA IC_{50}	0.093	-0.038	-0.202	-0.297*	-0.315**	0.020	0.321**	0.195	-0.691**	-0.038	-0.286*	-0.725**	-0.714**	1	–
RP	-0.265*	-0.099	0.294*	0.323**	0.360**	0.093	-0.203	-0.016	0.615**	0.067	0.058	0.665**	0.609**	-0.663**	1

* Correlation is significant at the 0.05 level. EC: electrical conductivity, HMF: Hydroxymethylfurfural, TPC: Total phenolic content, TFC: Total flavonoid content, RSA: DPPH radical scavenging activity, RP: Reducing power. ** Correlation is significant at the 0.01 level.

Total phenolics content

Phenolics are natural compounds known by their high importance in scientific and therapeutic research [Alvarez--Suarez *et al.*, 2010]. Their level in honey affects the profiling of the the antioxidant power and some sensory properties (*e.g.* color) [Tahir *et al.*, 2017]. Total phenolics content (TPC) of Jijelian honeys was obtained in the range of 48.19 to 147.50 mg GAE/100 g (Figure 1) and it differed significantly ($p<0.05$) among the samples. Whereas, it was not significantly correlated with the altitude (Table 3). Bueno-Costa *et al.* [2016] found lower values (61.16–111.37 mg GAE/100 g) for Brazilian honeys, while Flores *et al.* [2015] reported higher values (79.5–187 mg GAE/100 g) for Spanish honeydew honeys. In honey, the content of phenolics is determined by food source, geo-graphical origin, processing, handling, and storage [Flores *et al.*, 2015].

Total flavonoids content

Flavonoids have a substantial contribution to the antioxidant properties of honey, and they are described as the most important functional compounds of honey [da Silva *et al.*, 2016]. The total flavonoids content (TFC) of honeys from Jijel varied between 5.54 and 46.88 mg QE/100 g (Figure 1), and it differed significantly ($p<0.05$). Furthermore, TFC showed no significant correlation with the altitude (Table 3). Chaikham *et al.* [2016] obtained higher TFC values ranging between 31.52 and 60.73 mg QE/100 g for Thai monofloral honeys, whereas Tenore *et al.* [2012] obtained lower values ranging between 6.85 and 23.17 mg QE/100 g for Italian monofloral honeys. On the other hand, Flores *et al.* [2015] reported lower values in the range of 6.6 and 13.1 mg QE/100 g for Spanish honeydew honeys. Several researchers have already reported that the floral source affects the flavonoid content of honey, and the environmental and climatic conditions depict the nectar composition of melliferous flora [Sousa *et al.*, 2016].

DPPH radical scavenging activity

Recently, honeybees and honey products have been utilized as natural antioxidant sources. In addition, to assess the bioactive features of honey, the antioxidant activity is considered among the most valuable methods [Tahir *et al.*, 2017], which is largely evaluated as DPPH radicals scavenging activity [Liu *et al.*, 2013]. Figure 2 shows the DPPH radical scavenging activity (RSA) expressed as IC_{50} of the tested honeys, which differed significantly ($p<0.05$) between 4.20 and 17.92 mg/mL. Lower IC_{50} means better radical scavenging activity. Escuredo *et al.* [2013] reported similar values in the range from 8.6 to 17.8 mg/mL for Spanish honeys. However, Meda *et al.* [2005] and Beretta *et al.* [2005] reported higher RSA with IC_{50} in ranges from 1.63 to 29.13 mg/mL for Burkinabe honeys and from 1.63 to 45.45 mg/mL for Italian honeys, respectively. RSA was not significantly correlated with the altitude (Table 3).

Reducing power

The reducing power is widely known as a strong criterion of antioxidant capacity [Küçük *et al.*, 2007]. The absorbance

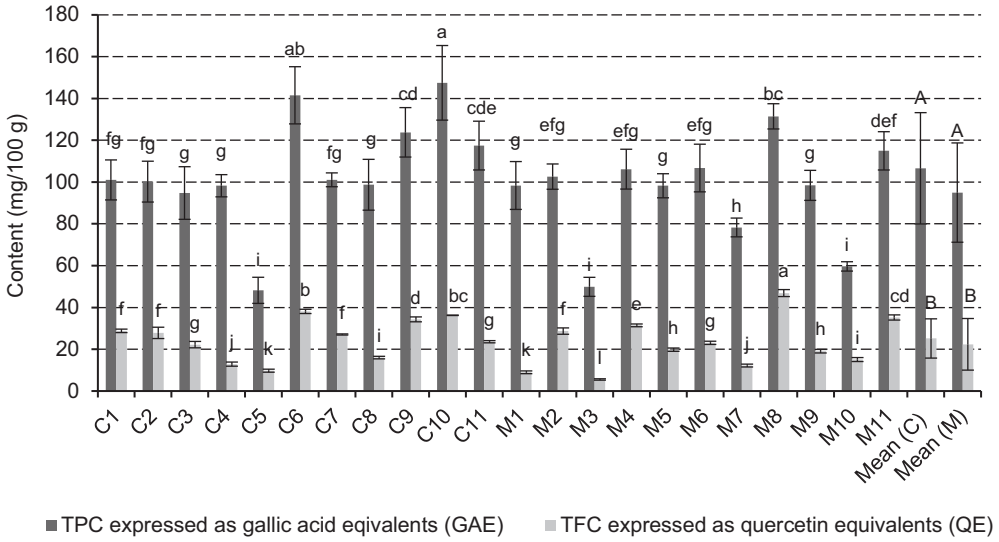

FIGURE 1. Total phenolic content (TPC) and total flavonoid content (TFC) of honeys from coastal (C1-C11) and mountain (M1-M11) Jijel region of Algeria. Bars C and M present mean values for coastal and mountain honeys, respectively. Capital letter (A or B) above these bars indicate no statistically differences between the two group of honeys (t-test, $p \geq 0.05$). Values C1-C11 and M1-M11 with different small letters (a-l) above bars differ significantly (LSD test, $p < 0.05$).

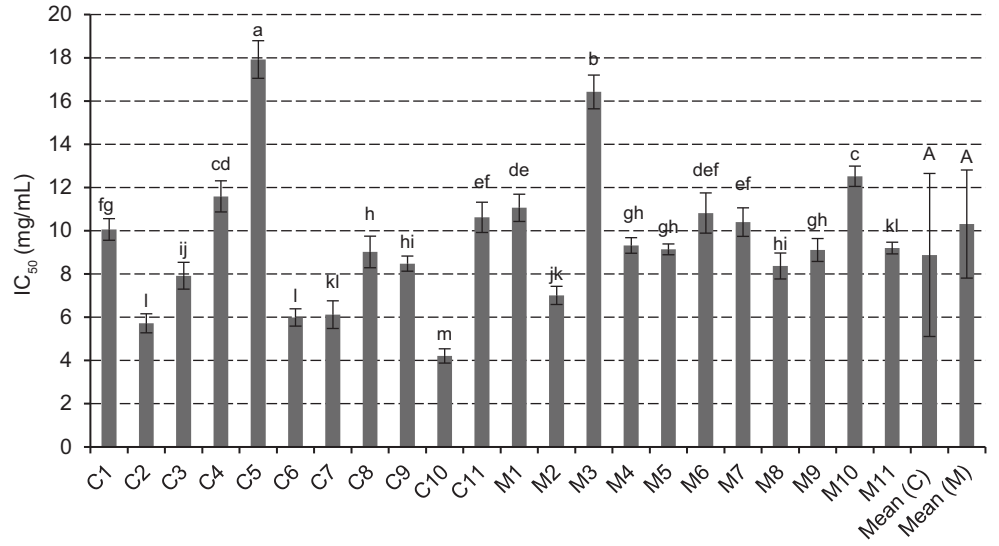

FIGURE 2. DPPH radical scavenging activity (RSA) of honeys from coastal (C1-C11) and mountain (M1-M11) of Jijel region Algeria. Bars C and M present mean values for coastal and mountain honeys, respectively. Capital letter (A) above these bars indicate no statistically significant differences between the two groups (t-test, $p \geq 0.05$). Values C1-C11 and M1-M11 with different small letters (a-m) above bars differ significantly (LSD test, $p < 0.05$).

values of RP assay differed significantly ($p < 0.05$) between 0.11 and 0.47 (Figure 3) and were negatively correlated with the altitude (r=-0.265; $p < 0.05$) (Table 3). Küçük *et al.* [2007] reported that RP varied from 0.11 to 0.78 for three concentrations (1, 5 and 10%) of Turkish honeys, while Saxena *et al.* [2010] reported that it ranged between 0.38 and 0.59 for 10% (v/v) of Indian honeys. Moreover, the coastal honeys presented better reducing power than the mountain honeys (0.35 against 0.29). The reducing power may differ due to the presence of different types of phenolic compounds, non-phenolic compounds (vitamins and amino acids) and other molecules such as enzymes (glucose oxidase and catalase) [Mouhoubi-Tafinine *et al.*, 2016].

Color intensity, TPC, TFC, RSA ($1/IC_{50}$), and RP were highly correlated with each other, with correlation coefficients ranging from r=0.609 to r=0.802 (Table 3) (TFC and RP showed the weakest correlation, while TPC and TFC showed the strongest correlation). Beretta *et al.* [2005] found that the correlation coefficients between color, phenolic content, antiradical activity against DPPH$^{\bullet}$ ($1/IC_{50}$) and ferric reducing antioxidant power (FRAP) of honey from different origin ranged between 0.884 and 0.993. In addition, Alvarez-Suarez *et al.* [2010] found strong correlations between color, TPC, TFC, Trolox equivalent antioxidant capacity (TEAC) and FRAP of monofloral Cuban honeys with r in the range of 0.83–0.97. Furthermore, Ferreira *et al.* [2009] reported

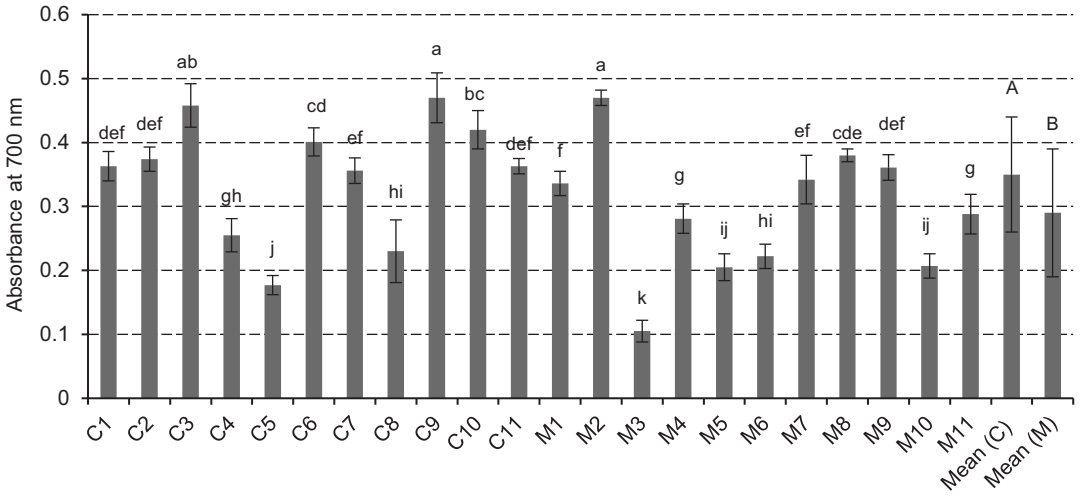

FIGURE 3. Ferric reducing power (RP) of honeys from coastal (C1-C11) and mountain (M1-M11) Jijel region of Algeria. Bars C and M present mean values for coastal and mountain honeys, respectively. Different capital letters (A-B) above these bars indicate significant differences between the two groups (*t*-test, $p<0.05$). Values C1-C11 and M1-M11 with different small letters (a-k) above bars differ significantly (LSD test, $p<0.05$). The absorbance value for 1 mg/mL of ascorbic acid (used as standard) at 700 nm was 1.487.

that dark honey had higher phenolics content, DPPH radical scavenging activity, and reducing power than amber honey and light honey.

Antibacterial activity

The antibacterial activity of honey is mostly depicted by the collective effect of acidity, osmolarity, hydrogen peroxide activity, and phenolic compound content [Molan, 1992]. In this study, 90.90% of Jijelian honeys had an antibacterial activity against *E. coli*, 18.18% against *S. aureus*, and only 9.09% against *P. aeruginosa* (Table 2). Therefore, the Jijelian honeys were more efficient against *E. coli* than *S. aureus* and *P. aeruginosa*. Ten samples of each coastal and mountain honeys presented antibacterial activity against *E. coli* while only C2, C8, M1, and M9 presented antibacterial activity against *S. aureus*. However, only two coastal honeys, C2 and C8, had antibacterial activity against *P. aeruginosa*. Hence, only two coastal samples (C2 and C8) presented antibacterial activity against the three strains of bacteria. Molan [1992] mentioned that honey has antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa*. Our results showed that *E. coli* was the most sensitive microorganism to the Jijelian honeys and *P. aeruginosa* was the most resistant one. On the other hand, Bueno-Costa *et al.* [2016] and Alvarez-Suarez *et al.* [2010] reported that *S. aureus* was the most sensitive microorganism toward Brazilian and Cuban honeys, respectively.

CONCLUSION

The tested honeys from Jijel region of Algeria had a good quality regarding physicochemical parameters, phenolic contents, and bioactive activities and they differed significantly among the samples. In addition, the antibacterial activity analysis showed Jijelian honeys were efficient against *E. coli* and not a good choice against *P. aeruginosa* and *S. aureus*. The coastal samples had higher pH, conductivity, ash and HMF contents, color intensity, and reducing power than the mountainous samples. Whereas, the total acidity was higher in the mountainous honeys. In addition, mountain honeys did not present an antibacterial activity against *P. aeruginosa.* Finally, the altitude was significantly negatively correlated with HMF content, electrical conductivity, ash content, and pH. Further research on the physiochemical properties of honey is recommended and important in order to establish the criteria of assessing the quality of honey.

ACKNOWLEDGEMENTS

The authors thank Pr. Khelili and Pr. Belghobsi for providing the Laboratory of Pharmacology and Phytochemistry (University of Jijel- Algeria) and Pr. Jamie Ellis for providing the Laboratory of Honeybee Research & Extension Lab (University of Florida- USA) to perform some analyses.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Submitted: 5 October 2019. Revised: 8 December 2019 and 9 February 2020. Accepted: 28 February 2020. Published on-line: 08 April 2020.