

Evaluation of Physicochemical and Antioxidant Properties of Raw Honey from Algeria

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Abstract

Honey production in Algeria has very long traditions dating back to ancient times. The purpose of the present work was to study the physicochemical properties and antioxidant capacity of Raw Honey of different botanical sources from Algeria. The study of the physicochemical parameters such as free acidity, pH, moisture, electrical conductivity, Hydroxymethyl furfural (HMF) content, diastase activity, invertase activity, fructose, glucose and disaccharide content were also identified and fructose/glucose ratio was calculated. Different types of honey were assessed for their contents of total phenolics and total flavonoids. The antioxidant capacity of honey was evaluated by Ferric-Reducing/Antioxidant Power Assay (FRAP) and Free Radical-Scavenging Activity (DPPH).

Mean values obtained for physicochemical parameters were: pH 4.17 ± 0.2 ; $16.77 \pm 0.2\%$ moisture; 0.64 ± 0.01 mS/cm electrical conductivity; 17.22 ± 1.05 meq/kg free acidity; 8.46 ± 1.9 unit /kg honey invertase activity 17.44 ± 2.8 Gothe scale diastase activity and 11.65 ± 1.9 mg/kg HMF. The glucose and fructose contents of honey samples are ranged from 21.45 to 28.26 g/100 g and 25.20 to 37.64 g/100 g respectively. The polyphenol and flavonoid contents of four raw honey samples from different origins were found to range from 70.95 to 128.87 mg GAE/100 g and 8.57-21.77 mg QE/100 g respectively. The radical-scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was found to range from 22.70% to 29.76% and the total antioxidant activity as measured by the Ferric-Reducing/Antioxidant Power (FRAP) assay was found to range between 223.19-958.42 $\mu\text{M Fe(II)/kg}$, indicating that raw honey has good antioxidant properties. No significant correlation was found between phenolic contents and antioxidant activity. In general, the raw honeys from Algeria had a good level of quality according to the results obtained for international regulation.

Keywords: Raw honey; Algeria; Physicochemical properties; Antioxidant capacities

Introduction

Honey is a natural sweet substance produced by honey bees (*Apis mellifera*) using nectar that is collected by the bees from the nectar of plants. For centuries, honey has been used for nutrition in different cultures and it has also been used as a traditional medicine due to its healing properties [1]. The physicochemical composition and flavor of honey vary with the floral source used by the honeybees, as well as regional, beekeeping practices and environmental climatic variations [2-4], melissopalynology is the most frequently used method for the determination of honey botanical source in pollen analysis [5]. Honey has been reported to contain about 600 compounds, including a number of carbohydrates, the principal carbohydrate constituents of honey are fructose (38%) and glucose (31%) [6,7]. The concentration of fructose and glucose as well as their ratio are useful indicators for the classification of unifloral honeys [8,9]. In addition, disaccharides, trisaccharides and other oligosaccharides are present in honey in small concentrations. Honey physicochemical quality criteria are well specified by the EC Directive 2001/110 [10]. The major criteria of interest are moisture content, sucrose, pH electrical conductivity, ash content, free acidity, diastase activity and Hydroxymethyl furfural (HMF) content [11]. The physical properties and chemical composition of honey from different sources have been carried out by many researchers [12,13]. These minor constituents include phenolic acids and flavonoids [14], certain enzymes (glucose oxidase, catalase), ascorbic acid, carotenoid-like substances [15]. Also, honey contains roughly 0.5% proteins and free amino acids [16]. The phenolic compounds in the honey under study are the phenolic acids and flavonoids, which are considered potential markers for the botanical origin of honeys [17]. Similarly, polyphenols and flavonoids are

considered as one of the important group of components identified in honey having antioxidant activity. Furthermore, the phenolic profile of honeys and consequently their antioxidant capacity depend on the botanical source, geographical origin, seasonal and climatic conditions [18]. Algeria has a very long tradition of beekeeping. Its favorable climate, good geographical conditions and a variety of botanical species provide great potential for the development of apiculture. However, little information is available on the composition and bioactive properties of honeys from floral sources in Algeria. To date, no data is available on the physicochemical properties of honey samples from western Algeria in the literature. In this article we report, for the first time, the *in vitro* free radical-scavenging and antioxidant capacity of raw honey and its physicochemical composition.

Material and Methods

Honey samples

A total of 4 honey samples collected from different parts of western Algeria. Honey samples weighing 250 g, packed and sealed in glass

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bottles, were purchased from a local market, and stored at 4°C. The samples were analyzed at the earliest in such a way that none of the samples exceeded the storage period beyond six months. The honey samples were kept at ambient temperature (26 ± 2°C) overnight before the analyses were performed.

Botanical origin

The identity of honey samples was based on pollen analysis following the method suggested by Erdtman [19]. Pollen grains were microscopically observed and compared with the reference slides for identification. According to their frequency classes, pollen types were classified as: dominant pollen (D), >45%; secondary pollen (S), 40–10%; pollen of minor importance, <10%, pollen traces (T), <3%. Pollen identification was based on the reference collection from the Laboratory Cari, Louvain-la-Neuve, Belgium.

Physicochemical data

All the physicochemical parameters studied were determined according to the standardized methods proposed by International Honey Commission [20]. All physicochemical tests were performed in triplicate.

pH values: Measurements of pH were performed with a pH meter (Hanna Instruments), in a solution containing 10 g of honey in 75 ml of CO₂ free distilled water.

Moisture content: Moisture in honey was determined with an Abbe refractometer reading at 20°C obtaining the corresponding % moisture from the Chatway Table [21].

Electrical conductivity values: Honey electrical conductivity was measured at 20°C [22] with a Crison Basic 30 conductimeter. Results were expressed in Micro-Siemens per centimeter (µS/cm) [23,24]

Free acidity: Free acidity was determined by potentiometric titration [22]. Honey samples were homogenized in a water bath and filtered through gauze, prior to analysis. Ten grams of honey were then dissolved in 75 mL of distilled water, and alcoholic solution of phenolphthalein added. The solution was titrated with 0.1 N NaOH. Acidity (milliequivalent of acid per kg of honey) was determined, as 10 times the volume of NaOH used in titration.

Sugar composition: Sugar spectra (fructose, glucose) were identified and determined by Bogdanov [25] for di- and Trisaccharides using High-Performance Liquid Chromatography (HPLC).

Diastase and invertase activity: The diastase activity was measured using the Phadebas method for α-amylase. Phadebas is a synthetic reagent which produces a blue color when it is hydrolyzed by the diastase. The absorbance at 620 nm is directly proportional to the diastase activity in the honey sample. Results are expressed in Gothe units per gram of honey, defined as that amount of the enzyme which will convert 0.01 g of starch into the prescribed end point in 1 h at 40°C under test conditions [26]. Invertase activity was spectrophotometrically measured with 4-nitrophenyl-α-D-glucopyranoside and the results are expressed in international units (IU) [27].

Hydroxymethyl furfural (HMF)

HMF content was done using the Winkler method where the solution of the tested honey when reacting with p-toluidin and barbituric acid and in the presence of Hydroxymethyl furfural (HMF) gives a wine-red compound [28].

Determination of total phenolic contents

The total phenolic content was determined by the Folin–Ciocalteu (F-C) method proposed by Singleton [29]. Thirty microlitre of honey solution (0.1 g/ml) was mixed with 2.37 ml of milli Q water and 150 µl of 0.2 N Folin–Ciocalteu reagent. The solution was thoroughly mixed by vortexing and incubated for 2 min at ambient temperature. Four hundred and fifty microlitre of sodium carbonate solution (0.2 g/ml) was added to the reaction mixture and further incubated for 2 hours at ambient temperature. The absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (0–200 mg/l). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

Determination of Total flavonoid contents

The Total Flavonoid Content (TFC) was determined using the aluminium chloride assay according to Amaral et al. [30] A 10 µl volume of a 10% (v/v) honey solution was added to the wells of a 96 well plate; then 30 µl of a 2.5% sodium nitrite, 20 µl of 2.5% aluminium chloride solutions and then 100 µl of a 2% sodium hydroxide solution were sequentially added. The samples were mixed well and absorbance 450 nm was measured. TFC was expressed as mg catechin equivalents (CE)/100 g.

In vitro antioxidant activity

Commonly used antioxidant assays are the FRAP (Ferric Reducing Antioxidant Power) assay, percentage DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging activity.

Determination of antiradical scavenging activity (DPPH)

The DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical scavenging effect (H/e⁻ transferring ability) of honey samples was measured as per the method described by Chen [31]. The DPPH was dissolved in absolute ethanol to a 0.2 mM concentration. A 100 µl aliquot of honey solution (0.1 g/ml) was diluted to 500 µl with 70% ethanol, and vigorously mixed with 400 µl of DPPH solution by vortexing. The mixture was incubated at room temperature for 15 min and the absorbance of the solution (T1) was measured at 517 nm. Sample blank (B1) consisted of 600 µl of 70% ethanol and 400 µl of DPPH whereas DPPH blank (B2) contained 100 µl of honey sample, 500 µl of 70% ethanol and 400 µl of absolute ethanol.

The DPPH scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = [1 - \{(T_1 - B_2)/B_1\}] \times 100$$

where T₁, B₁, and B₂ are the absorbencies of the sample, sample blank and DPPH blank, respectively.

Ferric reducing/antioxidant power (FRAP) assay

The reducing power of the ethanolic extracts of honey was determined according to the method of Oyaizu [32]. A 1 ml aliquot of ethanolic honey extract (10% v/v) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After this, 2.5 ml of 10% trichloroacetic acid was mixed by vortexing. The mixture was centrifuged at 3000 rpm for 10 min. A 2.5 ml aliquot of the supernatant was mixed with an equal amount of milli Q water and 0.5 ml of 0.1% FeCl₃. The absorbance was measured at 700 nm using a spectrophotometer. Precipitation or flocculation was never observed.

Assays were performed in triplicate. Ascorbic acid (1.0 mg/ml) was used as a reference standard. The increase in absorbance provided an indication of higher reducing power of the samples being analyzed.

Statistical analysis

Results were presented as mean values and standard deviations (mean \pm SD). Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($P < 0.05$). All statistical analyses were performed with the Statistica 7.0 software for Windows.

Results and Discussion

Botanical origin

The percentages of the most abundant pollen types in each honey sample, as well as the nectar and pollen character of these plants were taken into account. Following the criteria of Zander [33] when the percentage of the most abundant pollen type was over 45%, the honey sample was classified as unifloral, with an exception of eucalyptus pollen at 70%. Lower percentages were classified as polyfloral (Table 1).

Physicochemical data

Table 2; Table 3; Table 4 summarizes the results obtained (mean, range and standard deviation, SD) from physicochemical analysis of the honey samples.

Moisture content: Moisture content is an important parameter of honey quality and defines the amount of water present in honey [34]. The moisture content of the samples varied from 15.87% to 18.05% as shown in Table 2. These values were similar to those previously reported for other honey samples from Morocco (14.29%-20.20%) [35], South Africa (10.09%-20.37%) [36] and Malaysia (14.86%-17.53%) [34]. Moisture is a physico-chemical parameter that is related to the climatic conditions and degree of maturity of honey [37]. Honey moisture content depends on the environmental conditions and the manipulation from beekeepers at the harvest period, and it can vary from year to year [38]. The moisture content of honey is an important factor contributing to its stability. Higher moisture content could lead to undesirable honey fermentation during storage caused by the action of osmotolerant yeasts and resulting in the formation of ethyl alcohol and carbon dioxide [39]. These results are indicative of good storage ability of these honeys, since high moisture content could lead to fermentation during storage.

pH values: All honey samples analyzed were acidic in nature, with pH values varying from 3.7 to 5 (Table 2). The pH values of honeys were in accordance with AOAC [22]. These results are comparable to 3.42 to 4.68 for Brazilian and 3.2 to 4.5 for Bangladeshi honeys [40,41].

This parameter is of great importance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey [42]. Also, floral and geographic origins can cause great variations in honey pH values, as the nectar pH and soil conditions can greatly influence honey physicochemical characteristics [9].

Free acidity: The free acidity of honey may be explained by taking into account the presence of organic acids, particularly gluconic, pyruvic, malic and citric, in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions, such as phosphate and chloride [43]. The mean value of total acidity was found 17.22 with the range of 10.2 to 26.1 meq/kg (Table 1). According to EU Council directive [10], the upper limit for free acidity is 50.00 meq/kg. Similar results were detected by Costa et al. [44] and Aydin et al. [45]. Differences between the findings obtained from several studies and our findings may be caused to differences in geographical condition, harvesting procedure and storage condition.

Electrical conductivity values: The mean value of electric conductivity of all honey samples was 0.64 mS/cm (0.23-1.52 mS/cm). Electric conductivity of honeydew sample was 1.52 mS/cm (Table 2). The highest electric conductivity was measured in honey sample (ALGH1; 1.057 mS/cm) which did not comply with the requirements of the EU [10] for electric conductivity. The electrical conductivity of honey may be explained by taking into account the ash and acid content of honey, which reflects the presence of ions and organic acids; the higher their content, the higher the resulting conductivity Feás et al. [46]. The electrical conductivity is a good criterion related to botanical origin of honey.

HMF

The HMF and the diastase activity are parameters widely recognized for the evaluation of honey freshness and/or overheating. International regulations set a minimum value of 8 on Gothe's scale for diastase activity, and a maximum HMF content of 40 mg/kg [10]. The HMF content of the honeys analyzed ranged from 3.8 to 21.4 mg/kg (Table 2). The HMF content is indicative of honey freshness [47]. Several factors influence the levels of HMF, such as temperature and time of

Algerian Honey Samples	Density	Predominant pollen $\geq 45\%$	Secondary pollen (10 à 40%)	isolated pollen $<10\%$
ALGH1	High	-	Fabacée (24%). Apiacées (33%). Brassicacées (35%)	Plantain. Renoncule. Rhamnacées. Cistacée. Ronces. Astéracées
ALGH2	Average	-	Apiacées (12%). Lotier (14%). Fabacée (17%). Brassicacées (18%). Orangers (22%)	Chénopodiacées. Poacées. Acacia. Eucalyptus. Lamiacées. Renoncule. Ronces. Rosacées. Cistacée. Plantain. Astéracées
ALGH3	High	Eucalyptus (81%)	Apiacées (13%)	Fabacée. Astéracées. Brassicacées
ALGH4	Average	Astéracées (62%)	Rosacées (11%). Brassicacées (16%)	Lamiacées. Vesce. Apiacées. Chénopodiacées. Eucalyptus. Fabacée. Ronces

Table 1: Pollen analysis of Algerian honey samples.

Parameters	Moisture (%)	pH	Free acidity (mequiv./kg honey)	Electrical conductivity (mS/cm)
ALGH1	18.05 \pm 0.2	5.0 \pm 0.2	26.1 \pm 1.05	1.52 \pm 0.01
ALGH2	17.04 \pm 0.2	3.9 \pm 0.2	10.2 \pm 1.05	0.23 \pm 0.01
ALGH3	16.14 \pm 0.2	4.1 \pm 0.2	15.6 \pm 1.05	0.57 \pm 0.01
ALGH4	15.87 \pm 0.2	3.7 \pm 0.2	17 \pm 1.05	0.27 \pm 0.01
Range \pm SD	(15.87-18.05) \pm 0.2	(3.7-5) \pm 0.2	(10.2-26.1) \pm 1.05	(0.23-1.52) \pm 0.01

Table 2: Physicochemical parameters of selected Algerian honeys (average \pm standard deviation. n=3). Mean values from three repetition \pm standard deviations.

Parameters	Invertase activity	Diastase Activity (Gothe scale)	HMF (mg/kg)
ALGH1	19.6 ± 1.9	25.3 ± 2.8	3.8 ± 1.9
ALGH2	4.3 ± 1.9	7.6 ± 2.8	11.8 ± 1.9
ALGH3	12.6 ± 1.9	26.2 ± 2.8	9.6 ± 1.9
ALGH4	3.2 ± 1.9	16 ± 2.8	21.4 ± 1.9
Mean	8.46	17.44	11.65
Range ± SD	(3.2-19.6) ± 1.9	(7.6-26.2) ± 1.9	(3.8-21.4) ± 1.9

Table 3: Invertase, Diastase, and HMF in fresh honeys. Mean values from three repetition ± standard deviations.

Sugars values	ALGH1	ALGH2	ALGH3	ALGH4
Fructose	25.20 ± 3.32	37.64 ± 3.32	37.15 ± 3.32	36.77 ± 3.32
Glucose	21.45 ± 2.14	28.26 ± 2.14	26.39 ± 2.14	21.61 ± 2.14
F/G: ratio	1.17	1.33	1.41	1.7
Maltose	0.00 ± 1.32	3.54 ± 1.32	5.79 ± 1.32	7.02 ± 1.32
Turanose	8.83 ± 0.64	1.61 ± 0.64	1.66 ± 0.64	2.00 ± 0.64
Mélibiose & isomaltose	2.23 ± 0.38	0.00 ± 0.38	0.00 ± 0.38	0.71 ± 0.38
Sucrose	4.87 ± 0.10	0.31 ± 0.10	0.37 ± 0.10	0.30 ± 0.10
Tréhalose	1.89 ± 0.10	0.00 ± 0.10	0.00 ± 0.10	0.00 ± 0.10
Gentiobiose	ND	ND	ND	ND
Palatinose	0.00 ± 0.08	0.00 ± 0.08	0.00 ± 0.08	0.00 ± 0.08
Raffinose	0.00 ± 0.12	0.00 ± 0.12	0.00 ± 0.12	0.00 ± 0.12
Erlöse	0.00 ± 0.16	0.30 ± 0.16	0.53 ± 0.16	1.19 ± 0.16
Mélézitose	0.22 ± 0.40	0.00 ± 0.40	0.03 ± 0.40	0.00 ± 0.40
Maltotriose	0.00 ± 0.32	0.00 ± 0.32	0.00 ± 0.32	0.00 ± 0.32
Panose	0.00 ± 0.59	0.00 ± 0.59	0.00 ± 0.59	0.00 ± 0.59
Isomaltotriose	0.00 ± 0.09	0.00 ± 0.09	0.00 ± 0.09	0.00 ± 0.09

Table 4: Sugars values (g/100 g) of selected Algerian honeys. Data are expressed as mean ± SD; ND not detected.

heating, storage conditions, pH and floral source, thus it provides an indication of overheating and storage in poor conditions [48].

Diastase and invertase activity

Diastase is a natural enzyme of honey. The diastase activity in honey has been used as a freshness indicator over the years [49]. The legislation has set a minimum level for diastase activity; it should not be less than 8 Diastase Number (DN) units, where 1 DN unit hydrolyses 1 ml of 1% starch using 1 g of honey for 1 h at 37°C. The DN values were between 7.3 and 26.2 Schade units.

Invertase is a natural honey enzyme which is commonly used in Europe as a determinant of freshness. Its content depends on the geographic and floral origins of the product, as well as on its freshness. Table 3 illustrates that invertase activity values ranged from 3.3 to 19.6 unit/kg honey. The invertase is responsible for the conversion of sucrose, maltose, melezitose, raffinose, melibiose and trehalose into glucose and fructose the predominant sugars in bee honey [50].

Sugar composition

Sugar composition has been used to discriminate honey samples by botanical origin [5] or geographical origin [50]. Regarding the sugars, 14 carbohydrates were identified (Table 3). The monosaccharides glucose and fructose are the major constituents of honey. Fructose is always the most important sugar quantitatively followed by glucose. In this study, the glucose and fructose contents of honey samples are ranged from 21.45 to 28.26 g/100 g and 25.20 to 37.64 g/100 g respectively (Table 3).

Fructose/glucose (F/G) ratio of honey samples were determined between 1.17 and 1.70 F/G is a good indicator explaining the structure

and crystallization ratio of honey (Table 3). Also, (F/G) ratio has been recommended to evaluate honey granulation because glucose is less water soluble than fructose [51]. Sucrose, important sugar from a legislative point of view, had low values suggesting an advanced stage of ripening of the honeys, which would encourage the conversion of sucrose into glucose and fructose. Erlöse, sucrose, Mélezitose were present only in some samples ranging from 0.30 % to 1.19 %, 0.3% to 4.87% and 0.03% to 0.22% respectively (Table 3).

Total phenolic and total flavonoid contents

Many authors have studied the phenolic and flavonoid contents of honey to determine their beneficial effect in human health and whether a correlation exists with floral origins [52]. The mean total phenolic contents of the 4 examined honey samples was 96.14 mg GAE/100 g (Table 5). The minimum detected amount was 70.95 mg GAE/100 g, while the maximum one was 128.87 mg GAE/100 g. The total phenolic compound is sensitive to phenol and polyphenol entities and other electron-donating antioxidants such as ascorbic acid and vitamin E.

The total flavonoid contents, it was determined at a mean of 12.79 mg QE/100 g honey, with a minimum of 8.57 mg QE/100 g honey and a maximum of 21.77 mg QE/100 g honey (Table 5). Flavonoids are low-molecular-weight phenolic compounds that affect the aroma and antioxidant properties of honey. The total flavonoid and total phenolic contents vary between different honey sample depending on the geographical location of the different floral sources, such as Malaysia, Slovenia and Tunisia [53-55].

Antioxidant properties

DPPH free radical-scavenging activity: Antioxidant activity measured with the DPPH assay was 22.70% to 29.76% (Table 6). ALGH4 exhibited the highest percentage inhibition (29.76%), again indicating that it has the highest antioxidant potential (Table 6). The percentage of inhibition shown by tualang honey in this study is lower than what was previously reported for tualang honey (41.30%) [56].

The DPPH radical is one of the few stable organic nitrogen free radicals; it has been widely used to determine the free radical scavenging ability of the various samples [57]. The raw honey samples were observed to possess scavenging activities on DPPH free radical tin and this is a significant finding in this present study as it shows that these honey samples could be utilized in the treatment of diseases that have free radical origin.

Parameters	Total phenolic contents (mg GAE/100g honey)		Total flavonoid contents (mg QE/100g honey)	
	Mean	DS	Mean	DS
ALGH1	70.95	2.59	8.57	0.09
ALGH2	101.9	6.72	10.9	0.52
ALGH3	128.87	0.97	21.77	0.46
ALGH4	82.85	14.24	9.94	0.54

Table 5: Total phenolic and flavonoid contents of honey from Algeria. Mean values from three repetition ± standard deviations.

Parameters	Radical scavenging activity (%) DPPH		FRAP (µM Fe(II))	
	Mean	DS	Mean	DS
ALGH1	22.7	5.33	270.78	95.16
ALGH2	24.42	2.68	958.42	51.24
ALGH3	23.17	5.17	281.47	13.46
ALGH4	29.76	5.36	223.19	18.1

Table 6: DPPH radical-scavenging activities and FRAP values of honey from Algeria. Mean values from three repetition ± standard deviations.

Determination of total antioxidant content by FRAP assay

The reducing power test, in which the capacity of breaking radical chain reactions reflected, was considered to be a good indicator of antioxidant capacity [58]. The FRAP assay gives a direct estimation of the antioxidants or reductants present in a sample based on its ability to reduce the Fe^{3+}/Fe^{2+} couple [41]. The mean FRAP value of raw honey samples was $433.46 \pm 2 \mu M Fe(II)/100 g$ (Table 6). The ALGH₂ sample exhibited the highest FRAP values ($958.42 \pm 51.24 \mu M Fe(II)/100 g$), confirming its high antioxidant properties. High FRAP values indicate a greater reduction of ferric ions to ferrous ions.

Our reported FRAP value for raw honey is higher than that reported for Italian honeys (216.57 to 695.64 $\mu M Fe(II)/100 g$) [59].

Correlation between the physicochemical properties of honey

The positive and statistically significant correlation was found between moisture, acidity and pH. As can be seen, we found a positive correlation between the electrical conductivity and diastase activity. The negative correlation between diastase activity and the moisture content can be explained by the increase of the enzyme activity in water.

No significant correlation was found between phenolic contents and antioxidant activity ($r=0.278$), indicating that phenolics are not the only components responsible for the antioxidant effect of honey, but obviously other factors are involved.

In conclusion, this is the first study reporting the melisso palynological study, physicochemical parameters and carbohydrate composition of raw honey from Algeria. Honey samples that are available commercially differ in quality on account of various factors like seasons, packaging and processing conditions, floral source, geographical origin, and storage period. In addition, considerable differences in the physicochemical parameters, sugar composition and palynological parameters between suspected monofloral and multifloral honeys were observed.

References

1. Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH (2011) Phenolic acid composition and antioxidant properties of Malaysian honeys. *J Food Sci* 76: C921-928.
2. Chang X, Wang J, Yang S, Chen S, Song Y (2011) Antioxidative, antibrowning and antibacterial activities of sixteen floral honeys. *Food Funct* 2: 541-546.
3. Brudzynski K, Kim L (2011) Storage-induced chemical changes in active components of honey de-regulate its antibacterial activity. *Food Chem* 126:1155-1163.
4. Manyi-Loh CE, Ndip RN, Clarke AM (2011) Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities. *Int J Mol Sci* 12: 9514-9532.
5. Puusepp L, Koff T (2014) Pollen analysis of honey from the Baltic region, Estonia. *Grana* 53: 54-61.
6. Wang CW, Chen WT, Chang HT (2014) Quantification of Saccharides in Honey Samples Through Surface-Assisted Laser Desorption/Ionization Mass Spectrometry Using HgTe Nanostructures. *J Am Soc Mass Spectrom* 25: 1247-1252.
7. Alvarez-Suarez JM, González-Paramás AM, Santos-Buelga C, Battino M (2010) Antioxidant characterization of native monofloral Cuban honeys. *J Agric Food Chem* 58: 9817-9824.
8. Kaskoniene V, Venskutonis PR (2010) Floral markers in honey of various botanical and geographic origins: A review. *Comp Rev Food Sci Food Saf* 9: 620-634.
9. Wang J, Li QX (2011) Chemical composition, characterization, and differentiation of honey botanical and geographical origins. *Adv Food Nutr Res* 62: 89-137.
10. EU Council Directive 2001/110 relating to honey. Official Journal of the European Communities.
11. Gomes S, Dias LG, Moreira LL, Rodrigues P, Estevinho L (2010) Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food Chem Toxicol* 48: 544-548.
12. Rodríguez I, Salud S, Hortensia G, Jodral M (2010) Characterisation of Sierra Morena citrus blossom honey (Citrus sp). *Int J Food Sci Technol* 45: 2008-2015.
13. Mondragón-Cortez P, Ulloa JA, Rosas-Ulloa P, Rodríguez-Rodríguez R, Resendiz Vázquez JA (2013) Physicochemical characterization of honey from the West region of México. *CyTA-J Food* 11:1; 7-13.
14. Escuredo O, Míguez M, Fernández-González M, Carmen Seijo M (2013) Nutritional value and antioxidant activity of honeys produced in a European Atlantic area. *Food Chem* 138: 851-856.
15. da Silva IA, da Silva TM, Camara CA, Queiroz N, Magnani M, et al. (2013) Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chem* 141: 3552-3558.
16. Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. *J Am Coll Nutr* 27: 677-689.
17. Alvarez-Suarez JM, Giampieri F, Gonzalez-Paramas AM, Damiani E, Astolfi P, et al. (2012a) Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. *Food Chem Toxicol* 50: 1508-1516.
18. Alvarez-Suarez JM, Gasparrini M, Forbes-Hernández TY, Mazzoni L, Giampieri F (2014) The Composition and Biological Activity of Honey: A Focus on Manuka Honey. *Foods* 3: 420-432.
19. Erdtman G (1960) The acetolysis method. A revised description. *Svensk Botanisk Tidskrift* 54: 561-564.
20. Bogdanov S, Martin P, Lullmann C (1997) Harmonized methods of the international honey commission [Extra issue]. *Apidologie* 1-59.
21. Chataway HD (1935) Honey tables, showing the relationship between various hydrometer scales and refractive index to moisture content and weight per gallon. *Can J Res* 6: 532-547.
22. AOAC (1990) Official methods of analysis. (15th edn), Association of official Analytical Chemists, Inc Arlington, VA, USA.
23. Krauze A, Zalewski RL (1992) Classification of honeys by principal component analysis on the basis of chemical and physical parameters. *Zeitschrift Für Lebensmitteluntersuchung und-forschung* 192, 19-23.
24. Mateo R, Bosch-Reig F (1997) Sugar profiles of Spanish unifloral honey. *Food Chem* 60: 33-41.
25. Bogdanov S (1997) Nature and origin of the antibacterial substances in honey. *LWT-Food Science and Technology* 30: 748-753.
26. Bogdanov S (2009) Harmonised method of the International Honey Commission. International Honey Commission.
27. Siegenthaler U (1977) Eine einfache und rasche Methode zur Bestimmung der α -Glucosidase(Sacharase) in Honig. *Mitt Geb Lebensmittelunters Hyg* 68: 251-258.
28. Bogdanov S, Martin P, Lullmann C (2002) Harmonised methods of the international honey commission. FAM, Liebefeld: Swiss Bee Research Centre.
29. Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 299: 152-178.
30. Amaral S, Mira L, Nogueira JMF, Pereira da Silva A, Florêncio MH (2009) Plant extracts with anti-inflammatory properties – A new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. *Bioorg Med Chem* 17: 1876-1883.
31. Chen HY, Lin YC, Hsieh CL (2007) Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. *Food Chem* 104: 1418-1424.
32. Oyaizu M (1986) Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr* 44: 6307-6315.
33. Zander E (1950) The service palynology study of honey. *Annals Spanish Institute Soil Science* 9: 195-209.

34. Moniruzzaman M, Khalil MI, Sulaiman SA, Gan SH (2013) Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. *BMC Complement Altern Med* 13: 43.
35. Chakir A, Romane A, Marcazzan GL, Ferrazzic P (2011) Physicochemical properties of some honeys produced from different plants in Morocco. *Arab J Chem*.
36. Serem JC, Bester MJ (2012) Physicochemical properties, antioxidant activity and cellular protective effects of honeys from southern Africa. *Food Chem*. 133: 1544–1550.
37. Baroni MV, Arrua C, Nores ML, Fayéc P, Pilar Díaz MD, et al. (2009) Composition of honey from Cordoba (Argentina: Assessment of North/South provenance by chemometrics. *Food Chem* 114: 727-733.
38. Acquarone C, Buera P, Elizalde B (2007) Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. *Food Chem* 101: 695-703.
39. Saxena S, Gautam S, Sharma A. (2010) Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chem* 118: 391-397.
40. Sant'Ana LD, Sousa JP, Salgueiro FB, Lorenzon MC, Castro RN (2012) Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. *J Food Sci* 77: C135-140.
41. Islam A, Khalil I, Islam N, Moniruzzaman M, Mottalib A, et al. (2012) Physicochemical and antioxidant properties of Bangladeshi honeys stored for more than one year. *BMC Complement Altern Med* 12: 177.
42. Terrab A, Escudero ML, Gonzalez-Miret ML, Heredia FJ (2004) Colour characteristics of honeys as influenced by pollen grain content: a multivariate study. *J Sci Food Agric* 84: 380-386.
43. Al-Khalifa AS, Al-Arify IA (1999) Physicochemical characteristics and pollen spectrum of some Saudi honeys. *Food Chem* 67: 21-25.
44. Costa LSM, Albuquerque MLS, Trugob LC, Quintero LMC, Barth OM, et al. (1999) Determination of non-volatile compounds of different botanical origin Brazilian honeys. *Food Chem* 65: 347–352.
45. Duman Aydin B, Sezer C (2008) Oral NB. *ars'ta Satisa Sunulan Süzme Ballarin Kalite Niteliklerinin Arastirilmasi*. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 14: 89–94.
46. Feás X, Pires J, Estevinho ML, Iglesias A, De Araujo JPP (2010) Palynological and physicochemical data characterization of honeys produced in the Entre-Douro e Minho region of Portugal. *International Int J Food Sci Technol* 45: 1255-1262.
47. Terrab A, Diez MJ, Heredia FJ (2002) Characterization of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chem* 79: 373–379.
48. Fallico B, Arena E, Verzera A, Zappalà M (2006) The European Food Legislation and its impact on honey sector. *Accred Qual Assur* 11: 49–54.
49. Ivanov T (1978) Study of invertase, acid and alkaline phosphatase and esterase activities in bee honey. *Zhivonovud Na uk* 15: 103-112.
50. Gómez Báñez JA, García-Villanova RJ, Elvira García S, Rivas Palá T, González Paramás AM, et al. (2000) Geographical discrimination of honeys through the employment of sugar patterns and common chemical quality parameters. *Eur food res technol* 210: 437–444.
51. Ojeda de Rodríguez G, Sulbarán de Ferrer B, Ferrer A, Rodri'guez B (2004) Characterization of honey produced in Venezuela. *Food Chem* 84: 499–502.
52. Lachman J, Hejtmankova A, Sykora J, Karban J, Orsak M, et al. (2010) Content of major phenolic and flavonoid antioxidants in selected Czech honey. *Czech J Food Sci* 28: 412-426.
53. Khalil MI, Sulaiman SA, Alam N, Moniruzzaman M, Bai'e S, et al. (2012) Gamma irradiation increases the antioxidant properties of Tualang honey stored under different conditions. *Molecules* 17: 674-687.
54. Bertonecelj J, Doberšek U, Jamnik M, Golob T (2007) Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chem* 105: 822–828.
55. Boussaid A, Chouaibi M, Rezig L, Hellal R, Donsi F, et al. (2014) Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arab J Chem*.
56. Mohamed M, Sirajudeen K, Swamy M, Yaacob NS, Sulaiman SA (2009) Studies on the antioxidant properties of Tualang honey of Malaysia. *Afr J Tradit Complement Altern Med* 7: 59-63.
57. Morais M, Moreira L, Feás X, Estevinho LM (2011) Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity. *Food Chem Toxicol* 49: 1096-1101.
58. Dong R, Zheng YN, Xu BJ (2011) Floral origin identification and amino acid profiles of Chinese unifloral honeys. *Int J Food Prop*.
59. Perna A, Simonetti A, Intaglietta I, Gambacorta E (2013) Antioxidant Properties, Polyphenol Content and Colorimetric Characteristics of Different Floral Origin Honeys from Different Areas of Southern Italy. *Life Sci* 7: 428-436.

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