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ORIGINAL RESEARCH PAPER

PHYSICOCHEMICAL AND BIOCHEMICAL PROPERTIES OF HONEY BEE PRODUCTS IN SOUTH ALGERIA

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Abstract: The aim of the present study was to characterize the physical, biochemical and antioxidant properties of south Algeria honey samples (n = 5). Physical parameters, such as *p*H, moisture content, electrical conductivity, total dissolved solids, color intensity, total sugar content were measured. Several biochemical and antioxidant tests were performed to determine the antioxidant properties of the honey samples. The mean *p*H was 4.54, and moisture content was 14.88 %. The mean electrical conductivity was 0.597 $mS \cdot cm^{-1}$, and the mean total dissolved solid was 0.14 % and the mean color was 163.6 mm Pfund. The mean total sugar contents was 82.76 %. High mean values of phenolic and flavonoid contents were respectively 697.22 mg $GAE \cdot kg^{-1}$ and 290.70 $mgREE \cdot kg^{-1}$. Antioxidant activity was also measured using DPPH assays, value of 26.19 $mg \cdot mL^{-1}$ was detected for south Algeria (El-Oued) honey.

Keywords: *antioxidant, honey, phenols, physicochemical, DPPH*

INTRODUCTION

Honey is produced by honeybees from nectar of plants, as well as from honeydew. This latter is a sugar-containing substance excreted by some plant-sucking insects [1]. Honey contains at least 181 substances. Chemically, honey comprises sugars (70 – 80 %), water (10 – 20 %) and other minor constituents such as organic acids, mineral salts, vitamins, proteins, phenolic compounds and free amino acids. The monosaccharides, fructose and glucose, are the main sugars found in honey. Amino acids account for 1 % and proline is the major contributor with 50 – 80 % of the total amino acids [2].
Honey is generally evaluated by physico-chemical analysis of its constituents [3]. The manipulation of honey and its possible adulteration is reflected in many of its physico-chemical properties such as HMF and sugar. Therefore, to ensure the authenticity, it is necessary to analyze honey samples in detail [4].
The studies of the physico-chemical properties of honey are important for the certification process that determines honey quality. The aim of this study was to evaluate and compare the quality of some honey samples from the south east region of Algeria to the international standards.

MATERIAL AND METHODS

Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH), 3, 4, 5-Trihydroxybenzoic acid (Gallic acid; GA) (99 %), butylated hydroxyanisole (BHA) (96 %), Ascorbic acid (AA) (99 %), were procured from Alfa Aesar (Etats-Unis). Sodium phosphate (Na_3PO_4, anhydrous, powder, extra pure), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4) (98 %), aluminium chloride ($AlCl_3$) and sodium carbonate (Na_2CO_3) were purchased from Prolabo (Etats-Unis). The Folin-Ciocalteu reagent (FCR), methanol (MeOH) and hexane were obtained from BIOCHEM chemopharma Co (FRANCE). High purity water was used in all experiments. All other reagents used were of analytical grade.

Instrumentation and software

Spectrophotometric measurements were performed on an UV-1800 Schimadzu Spectrophotometer (double-beam) equipped with 1 cm quartz cuvettes (JAPAN).
*p*H meter PHM 210 (Radiometry, FRANCE), Electrical conductivity meter CDM 210 (Radiometry, FRANCE), Digital abbe refractometer WAY-2S (Only Laboratory Inc, China).

Honey samples

Five samples of honeys produced in various regions of El-Oued (south east of Algeria) were collected from beekeepers in 2012. The samples were stored in a refrigerator in airtight plastic containers until analysis. The regions from which the samples of honey were collected are indicated in Figure 1.

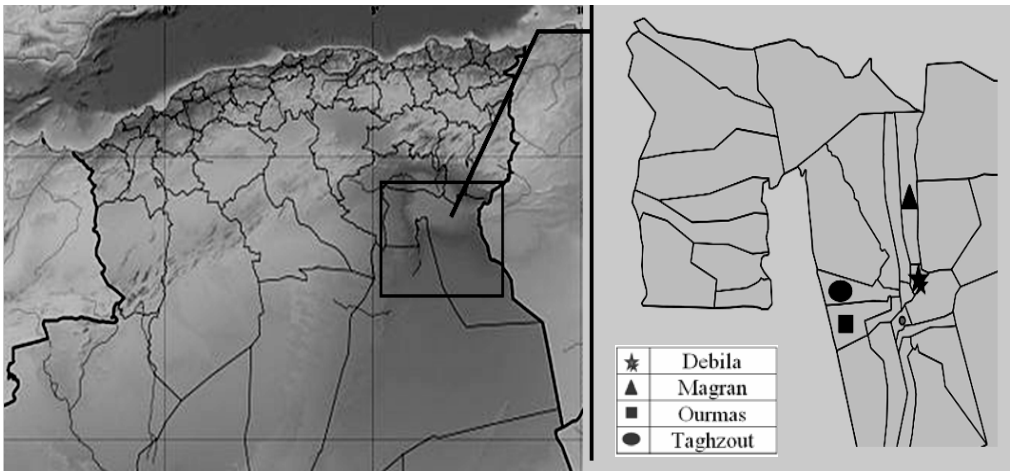

Figure 1. *Distribution of the samples in the El-Oued region (southeast of Algeria)*

Methods

Physico-chemical determinations

The determination of physical and chemical parameters such as content of water, conductivity and *p*H and was carried out in accordance with the methods described in Harmonized methods of the European Honey Commission [5].

Specific gravity

The specific gravity of honey (density) was determined by dividing the weight of specific gravity bottle (50 mL) filled with honey by the weight of the same bottle, filled with water [6].

Determination of pH

A *p*H meter was used to measure the *p*H of a 10 % (w/v) solution of honey prepared in ultrapure water [7].

The free acidity was determined as follows, by the titrimetric method: the addition of 0.05 N NaOH, is stopped at *p*H 8.50 (free acidity). Results were expressed as meq·kg $^{-1}$ [5].

Determination of electrical conductivity

Electrical conductivity EC was measured using a conductivity meter for a 20 % (w/v) solution of honey suspended in milli-Q water [5].

Determination of moisture content

The moisture content was determined using a refractometric method. In general, the refractive index increases with increases in the solid content of a sample. The refractive indices of honey samples were measured at ambient temperature using a refractometer, and measurements were further corrected for the standard temperature of 20 °C by the addition of the 0.00023/°C correction factor. The moisture content was measured in triplicate, and the percentage of moisture content, which corresponds to the corrected refractive index, was calculated using Wedmore's table [5].

Determination of total solids

Percentage total solids of each sample was determined using the following formula: Total solids (%) = 100 – Moisture content [8].

Honey color analysis

Honey samples were heated to 50 °C to dissolve sugar crystals, and the colour was determined by spectrophotometric measurement of the absorbance of a 50 % honey solution (w/v) at 635 nm. The honeys were classified accordingto the Pfund scale after conversion of the absorbance values Eq. (1) [9]:

$$mm \text{ Pfund} = -38.70 + 371.39 \times Abs \qquad (1)$$

where *mm* Pfund is the intensity of honey colour in the Pfund scale; *Abs* is the absorption of honey solution.

Analysis of antioxidant properties

Determination of total phenolics content (TPC)

Total phenolic content was determined using Folin - Ciocalteu reagents according to the method of Beretta [10-11], briefly described as 0.5 mL of Folin and Ciocalteu's phenol reagent was mixed with 100 µL honey extract. After 3 min, 2 mL of 20 % aqueous sodium carbonate solution was added to the mixture. The reaction was kept in the dark for 30 min, after which the absorbance was read at $\lambda = 760$ nm.

Gallic acid was used as the standard to produce the calibration curve (0.03-0.3 $mg \cdot mL^{-1}$). The mean of three readings was used and the total phenolic content expressed in mg of gallic acid equivalents (GAEs) ($mg \cdot kg^{-1}$).

Determination of total flavonoids content (TFC)

Flavonoids are the most common group of phenolic compounds in the human diet and exist ubiquitously in natural products. They are most commonly known because of their antioxidant activity. The total flavonoid content in bee honey was estimated by a colorimetric assay based on the procedure of LIANDA Regina [12]. Total flavonoids in bee honey were determined as follows: one milliliter (1 mL) of sample (1 $mg \cdot mL^{-1}$) was mixed with (1 mL) of (2 %) aluminium chloride. After incubation for 30 min at room temperature, the absorbance was measured at 430 nm.

Distilled water was used as a blank and control. A calibration curve of rutin was prepared, and flavonoid contents were determined from the linear regression equation of the calibration curve. The results were expressed as mg of rutin equivalents (REs) per g of extract. All the samples and the standards were analyzed in triplicate.

DPPH free radical-scavenging activity

The antioxidant properties of each honey sample were also studied by evaluating the free radical-scavenging activity of the DPPH radical, which was based on the method proposed by Ferreira [13]. Briefly, honey extract (1 mL) was mixed with methanolic solution containing DPPH radicals (0.25 mM, 1 mL). The mixture was vigorously shaken and left to stand for 30 min in the dark (until their absorbance remained unchanged). The reduction of the DPPH radical was determined by measuring the absorbance of the mixture at 517 nm [14].

The radical-scavenging activity (RSA) was calculated as the percentage of DPPH discoloration using the following equation:

$$\% \text{ RSA} = ([A_{DPPH} - A_S]/A_{DPPH}) \times 100$$

where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

Biochemical analyses

Protein content

The protein content of honey was measured according to Lowry's method [15]. Briefly, BSA solutions were prepared by diluting a stock BSA solution (1 mg·mL^{-1}) to 5 mL. BSA concentrations ranged from 0.05 to 1.00 mg·mL^{-1}. Based on these different dilutions, 0.2 mL of protein solution was placed in different test tubes and 2 mL of alkaline copper sulfate reagent (analytical reagent) was added. After the resulting solution was mixed properly, it was incubated at room temperature for 10 min. Then, 0.2 mL of reagent Folin-Ciocalteau solution was added to each tube and incubated for 30 min. The colorimeter was calibrated with a blank, and the absorbance was measured at 760 nm.

RESULTS

Physico-chemical determinations

The results of physico-chemical analyses of honey from different sources are summarized in Table 1 and Table 2.

Table 1. *Physicochemical characteristic of EL-Oued honey*

	Origin					**Mean±SD**	**Limits of internation al standards**
	Debila	**Magrane**	**Ourmas**	**Taghzout**			
Samples code	**H01**	**H02**	**H03**	**H04**	**H05**		
Density	1.50	1.43	1.48	1.53	1.59	1.50±0.05	/
Water content [%]	15.5	16.5	16.4	13.1	12.9	14.88±1.76	not more than 200 g·kg^{-1}
Total solids [%]	84.5	83.5	83.6	86.9	87.1	85.12±1.76	/
Water insoluble solids content [%]	0.19	0.17	0.1	0.11	0.15	0.14±0.03	not more than 5 g·kg^{-1}
*p*H	4.54	4.66	4.29	4.74	4.5	4.54±0.17	/
Free acidity [meq·kg^{-1}]	49.8	63.2	29.2	26.1	30.8	39.82±16.03	not more than 50 meq·kg^{-1}
Electrical conductivity [mS·cm^{-1}]	0.830	0.407	0.281	0.972	0.497	0.597±0.291	not more than 0.8 mS·cm^{-1}
Color [Pfund index]	287	88	102	117	224	163.6±87.4	no fixed limit

Table 2. *The variation of total phenolic content for different types of honey samples*

Sample	Total phenolic content [mg GAE·kg^{-1}]	Total flavonoids [mgREE·kg^{-1}]	DPPH, IC_{50} [mg·mL^{-1}]
H01	1831.8	497.56	6.74
H02	394.4	249.42	16.85
H03	179.2	159.42	43.98
H04	567.5	277.15	30.82
H05	513.2	269.99	32.56
Mean±SD	697.22±651.53	290.70±124.84	26.19±14.52

SD: Standard deviation

DISCUSSION

Physico-chemical determinations

Density values depend on water content, pure honey has a higher density compared with the other adulterated samples except molasses.

The water content depends on various factors such as harvesting season, degree of maturity reached in the hive and climatic factors [16]. All the values obtained were below 17 % (Table 1) indicating a good degree of maturity are included in the water range limits approved by the European Commission [17] and the Codex Alimentarius [1]. Higher water content could lead to undesirable honey fermentation during storage [18]. According to this result, lower water content is highly important for the shelf-life of the honey during storage.

The electrical conductivity of the honey is closely related to the concentration of mineral salts, organic acids and proteins; it is a parameter that shows great variability according to the floral origin and is considered one of the best parameters for differentiating between blossom honeys and honeydews [19]. The electrical conductivity of the samples varied between 0.281- 0.972 mS·cm^{-1}. According to Codex Alimentarius [1] and European Commission [17] value for the nectar honey should be less than 0.8 mS·cm^{-1} (with few exceptions). Moreover, the values of EC of our samples are typical of blossom honeys (H02, H03, H05) and other samples. honeydew honey (H01, H04).

These differences in mineral content are dependent on the type of soil in which the original nectar bearing plant was located [20].

The *p*H of 5 honeys analyzed is less than 4.7; these are typical *p*Hs in floral honeys. The *p*H values are of great importance during the extraction and storage of honey as they influence the texture, stability and shelf life [17].

The acidity of honey developed due to the presence of organic acids. A high total acidity may mean that the honey had fermented at some time, and that the resulting alcohol was converted into organic acid [21]. In our samples, the values of total acidity ranged between 26.1–63.2 meq·kg^{-1}; these values are similar to some locally produced honey in Algeria as reported by Chefrour [22]. The total acidity was below the limit proved satisfactory in international trade (50 meq·kg^{-1} of honey), indicating absence of undesirable fermentation in our samples.

Color is based on the classification of Aubert and Gonnet [23], four honey samples are dark. The color of honey was related to its mineral content and the color of pollen [24].

On the other hand, the color intensity is supposed to be related to pigments (carotenoids, flavonoids, etc.), which are also known to have antioxidant properties [25].
The total solids of honey samples were presented in Table 1. The mean total solids ranged from 83.5 % - 87.1 % with a mean value of 85.1 %. Total solids were least in Magrane and highest in Tghzout. The total solids were within acceptable international range of 80-90 %.

Analysis of antioxidant properties

Total phenolic content (TPC)

Honeys include a great number of phenolic compounds, the nature and the quantity of which change widely according to the botanic origin [2, 26]. Total phenolic compounds ranged from 179.2 to 1831.8 mg·kg^{-1} for our samples. Many researchers found that honeys with dark color have a higher amount of total phenolic compounds [27]. In the present study, the coefficient of correlation between phenolic compounds and color is R^2 = 0.693 (Figure 2). Gheldof and Meda have demonstrated a correlation between antioxidant activity and the total phenolic content [27, 28]. Al-Mamary indicates that the determination of total phenolic content of honey is a good parameter for the assessment of its quality and possible therapeutic potential [29].

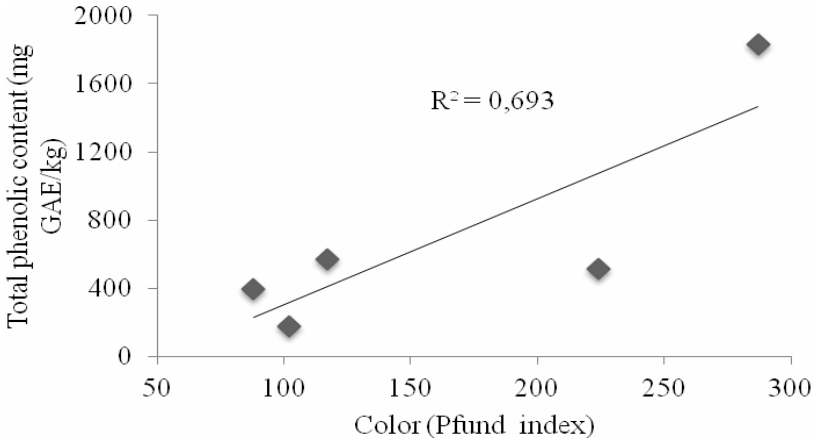

Figure 2. *The correlation between phenolic content and color of honey samples*

Determination of total flavonoids content (TFC)

All of the Algeria honey samples had a higher content of flavonoids (mean = 290.70 mgREE·kg^{-1}) (Table 2). The total flavonoid content in these samples was also slightly higher than that reported for Algeria honey (65.85 mg Catechin·kg^{-1}) [26]. The range of Algeria honey flavonoid concentrations was between 159.42 and 497.56 mg REE·kg^{-1}, which is similar to that of Malaysian honey samples [30], but higher than that of samples from Cuba [31]. The highest flavonoid content was again found in sample H01 (497.56 mg REE·kg^{-1}).

DPPH free radical-scavenging activity

The DPPH radical scavenging test is one of the shortest available to investigate the overall hydrogen/electron-donating activity of single antioxidants and health promoting dietary antioxidant supplements. Table 2 shows the scavenging ability expressed as the

IC50 on the DPPH radical. A large variation was found in the antiradical power of honey samples used in the experiment (Table 2). The antiradical activity of H01 honey (6.74 mg·mL^{-1}) was significantly higher than those of others. The antiradical activity of honey samples was increased in response to increasing phenolic contents. Generally, antiradical activity varied between 6.74 (H01) and 43.98 (H03). Results indicate that the antiradical activity is related to phenolic contents of honeys. The significant variation in their total phenolic contents and antiradical activity is due to the variation in their plant sources. In the present study, the total antiradical activity of honey samples was found significantly high. A correlation (Figure 3) was established between the phenolic content and antiradical activity of honeys R^2 = 0.637 and indicated that antiradical activity of honeys is mainly due to its phenolic constituents. As a result, these honeys contain several antioxidant constituents; the interaction between them shows honeys to be an important natural antioxidant source that have an effect at several cell tissues. The quantity of these components varies widely according to the floral and geographical origin of honey. In addition, the processing, handling and storage of honey may effect its composition [32].

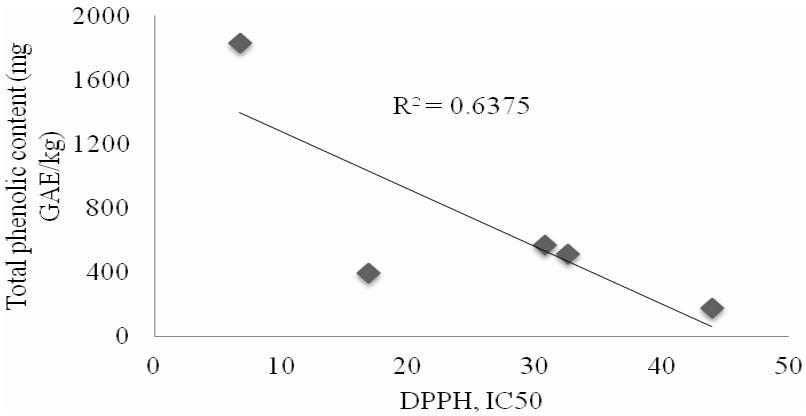

Figure 3. *The correlation between phenolic content and antioxidant activity of honey samples*

CONCLUSIONS

This is the first study to investigate the physicochemical, biochemical and antioxidant properties of south Algeria honey more elaborately. This study showed that south Algeria honey samples have high antioxidant potential, as indicated by their high phenolic and flavonoid contents. Our results indicate that Algerian honey is a good source of antioxidants.

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