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Quality of Some Algerian Honey: Study of Botanical and Some Physicochemical Parameters

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Abstract: The aim of this study was to investigate some properties of various honeybee samples collected from different Algerian regions by using different honey analysis tests as refractive index, ash content, electrical conducting, protein content, acidity, pH and finally HMF content. These determinations indicate the quality of 32 Algerian honey samples from *Apis mellifera* which is needed for international trade. 32 Honey samples were collected from beekeepers from the different regions of Algeria between 2012 and 2014 for the investigation of physicochemical composition. Each sample weighed 150 g and clearly identified by date of harvest and floral and geographical origin. Honey samples were analyzed for pH, moisture, total acidity, ash, electrical conductivity, total sugars, sucrose, hydroxymethylfurfural (HMF). All of these analyses were done following the European Honey Commission and International Honey Commission Methods. The study revealed the following results: Moisture content had a value of 13.00% (minimum) and 20.13% (maximum) indicating optimum harvesting and good degree of maturity. All honeys analysed in this work had water contents less than ranged 21%; pH 3.74 (minimum) et 5.55 (maximum); ashes 0.006 % (minimum) and 0.371 % (maximum) ; Electrical conductivities ranging between 0.097 mS / cm and 1.110 mS / cm); hydroxymethylfurfural 0.00 mg kg^{-1} (minimum) to 1380 mg kg^{-1}(maximum); free acidity 6.87 (minimum) and 58.75 mg g-1 (maximum) and protein 0.09% et 0.81%. To summarize, the results of physic-chemical properties of honey from Algeria indicate good quality of 72% of honeys and that the remaining quality parameters agree in general, with international regulations.

Key words: Polyfloral honey · HMF · Protein Content · EC · Acidity · Pollen Grains

INTRODUCTION

Since times immemorial; Algerian considered honey as a symbol of prosperity, sanctity and is widely used in traditional medicine. Honey is a sweet and viscous fluid produced by honeybees (And some other species) and derived from the nectar of flowers [1]. It is an important food which is not only supplies energy, but also containing various nutrients [2]. Honey has a content of 80-85 % carbohydrates, 15-17 % water, 0.3 % proteins, 0.2 % ashes and minor quantities of amino-acids and vitamins as well as other components in low levels of concentration [3].

Honey was found to be a suitable alternative for healing wounds, burns and various skin conditions and also to have potential role in cancer care. The intrinsic properties of honey have been reported to affect the growth and survival of microorganisms by bacteriostatic or bactericidal actions [4]. For this raison honey is a product with minimal types of microorganisms [5].

According to the current standards of the *Codex Alimentarius* and the European Union (EU-Council Directive [6, 7], several physicochemical parameters such water content, pH, acidity, electrical conductivity, protein and HMF are important indicators of the quality of honey.

The change in the composition and properties of honey depends on the floral and honeydew sources collected by honeybees, as well as on regional and climatic conditions [8-11]. Therefore, the aim of this work was to evaluate some properties of various samples collected from different regions in Algeria by using different honey analysis tests, refractive index, moisture,

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electrical conductivity, ash, pH, acidity, protein and HMF. These determinations are highly useful for determining the quality of Algerian honey.

MATERIAL AND METHODS

Sampling: In this research, thirty two honey samples were collected from the Algerian beekeepers (Table 1). Sample collection is accompanied by an interview with the beekeeper for information on these honeys as:

- Location of the apiary
- Date of harvest
- The mode of extraction
- The main flowers pollinated

This information allows us to have an idea about the production of these honeys: origin, date of collection and the different modes of extraction. Each sample (100 g) is placed in a glass jar and stored away from light and moisture and kept at 4±0.5°C. These samples (Fig. 1) will be used for pollen analysis and physicochemical analyses.

Quantitative Melissopalynological Analysis: Melissopalynology is the study of pollen contained in honey and, in particular, the pollen's source [12]. The quantification of pollen in honey was performed according to Maurizio's method cited by Louveaux *et al.* [13]; The examination under the microscope was carried out in order to counting microscopically the number of pollen present in the honey sediment after centrifuging a honey solution (400 to 1000x). The results were based on the average number in the 400 fields of view and expressed as the number of pollen grains in 10 g honey. The honeys were placed into one of the five pollen representativity classes as distinguished by Louveaux *et al.* [13].

Table 1: Geographical and botanical origins of Algerian honey samples

Samples	Geographic origin	Year collected	Botanical origin
E 01	Rmal-hssan	2013	polyfloral
E 02	Ain-sennour	2013	polyfloral
E 03	El-mechroha	2012	polyfloral
E04	Souk-ahras	2013	polyfloral
E 05	Djbel-dekma	2012	polyfloral
E 06	Sidi-fredj	2014	polyfloral
E07	El-henancha	2013	polyfloral
E 08	Tifeche	2013	polyfloral
E 09	Ouled-idriss	2013	polyfloral
E10	Djbal-bni-saleh (Guelma)	2014	polyfloral
E11	Oued-el-djedra	2013	polyfloral
E12	El mashroha	2013	polyfloral
E13	Ain-ouessara (El djelfa)	2012	polyfloral
E14	Collo (Skikda)	2012	polyfloral
E15	El-mdéa	2012	polyfloral
E16	Tanga(El taref)	2012	polyfloral
E17	Khenchela	2012	polyfloral
E18	Tamalouss(Skikda)	2012	polyfloral
E19	El mdea	2012	polyfloral
E20	El mdea	2012	polyfloral
E21	Collo (Skikda)	2012	polyfloral
E22	Souk ahras	2012	polyfloral
E23	Tébessa	2012	polyfloral
E24	Tamalouss (Skikda)	2012	polyfloral
E25	Khenchela	2012	polyfloral
E26	El taref	2012	polyfloral
E27	Metidja (boufarikblida)	2012	polyfloral
E28	Souk ahras	2012	polyfloral
E29	Jnanlshouk(Souk ahras)	2012	polyfloral
E30	Metidja (Alger)	2012	polyfloral
E31	Elhammamat (Tébessa)	2012	polyfloral
E32	El zitouna (El taref)	2013	polyfloral

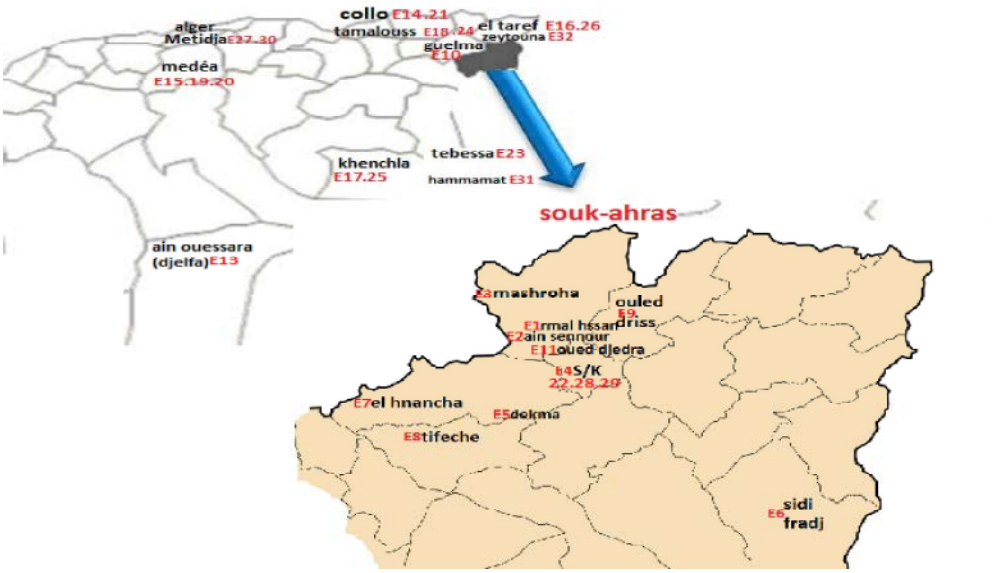

Fig 1: Distribution of the regions samples analysed in the studies

Physico-Chemical Analysis: All physicochemical determinations were carried out according to the European Honey Commission and International Honey Commission Methods [14] and compared with that of International Honey Standards. The honey samples were analysed using the following methods:

- Water content was determined with a refractometer Abbe-type and the refractive index was correlated using Chataway Charts [15].
- Determinations of total solids (ash) were obtained by ashing in a muffle furnace at 550°C for 5 hours to obtain constant weight. A percentage total solid of each sample was determined using the following formula:

  Total solids (%) = 100 - Moisture content

- Electrical conductivity (EC, Ms.cm-1) was obtained in a 20% honey dry matter solution at 20°C, using AD 3000 conductivity meter to obtain the data.

Free Acidity: Acidic components were neutralized with a standard solution of sodium hydroxide in aqueous honey solution (10 g in 75 ml of double distilled water).

Determination of pH: The pH of honey samples were determined by measuring out 10 ml of each honey sample into a clean beaker and its pH was determined using a pH meter (AD1030).

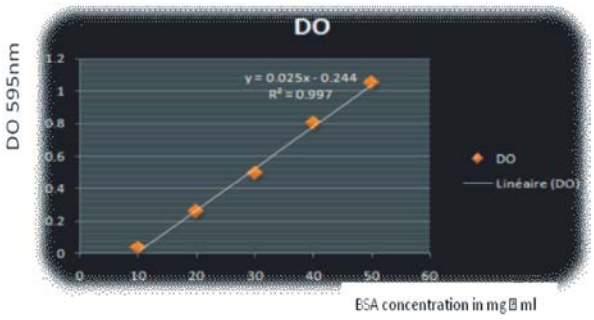

Fig 2. BSA Calibration curve for Bradford Method

- The protein concentration in honeys was determined by using Bradford [16]. Bovine Serum Albumin (BSA) at different concentrations of 0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg/ml were used for the standard curve. Honey samples were serially diluted. A sample of 5.0 ml was mixed with 50.0 ml of Bradford solution and incubated at room temperature for five minutes. The absorbance was measured at 595nm. Assays were performed in triplicate. The protein contents were calculated from the standard curve (Fig. 2).
- Hydroxymethylfurfural (HMF) was determined by Winkler's method [17] using barbituric acid and p-toluidine solution.

Statistical Analysis: The collected data were processed using a combination of statistical methods. Results are expressed as mean ± SD. The differences between

experimental groups were compared by one-way analysis of variance (ANOVA). The results were considered statistically significant when $P < .05$.

RESULTS AND DISCUSSION

Quantitative Melissopalynological Analysis: The indicate the richness of the samples in pollen sediments [18]. The number of pollen grains in 10 g of honey ranged between 0 and 311400 pollen grains.

Following Louveaux *et al.* [13] Five Groups Were Considered: Group I (honey low in pollen < 20,000/10 g), Group II (normal honey 20,000-100,000/10 g), Group III (honey rich in pollen 100,000-500,000/10 g), Group IV (honey extremely rich in pollen 500,000-1,000,000/10 g), Group V (pressed honeys >1,000,000/10 g). According to the total number of plant elements, the honey samples were distributed into five classes (Fig. 3).

The results from the pollen analysis (pollen spectrum), show that all honeys were polyfloral, because any pollen type had been access 45 percent of the total pollen grain number found in honey samples. Presence of Secondary pollen (16 to 45%): Fabaceae type, *Vicia sativa*, Boraginaceae type, *Acacia sp*, Myrtaceae type, *Eucalyptus sp*, Fabaceae type, *Hedysarum coronarium*, *Trifolium repens*,Poaceae type,Rutaceae type, Oalidacea type, Labiaceae type.

Minor pollen (3 to 15%): *Trifolium alexandrium*, Oxalidacea type, Brassicaceae type, *Erica Salicaceae,* Apiaceae type, *Daucus carota* and Poaceae type.

Pollen very minor or isolated (<3%): *Malva sp*, Apiaceae type, Poaceae type, *Opuntia ficus-indica*, Convolvulaceae type, *Zizyphus lotus*, *Echium plantagineum*, *Pimpinella anisum, Citrus sp*, Malvaceae type, Cactaseae type, *Erica arborea*, Convolvulaceae type, *Convolvus arvensis,* Asteraceae type, *Sinapis arvensis*, *Thymus vulgaris.*

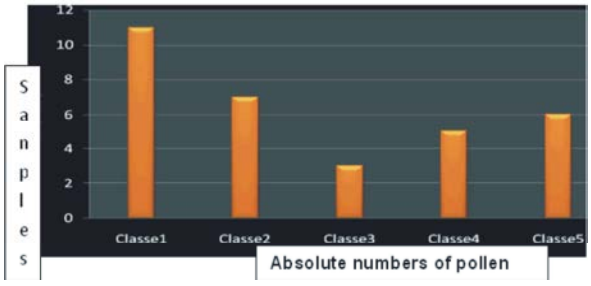

Fig 3: Classification of honey samples according to the absolute numbers of pollen grains

Physicochemical Parameters: Fig. 4 shows results of the chemical analysis in natural honey samples, with median, mean, standard deviation and range. The results showed that the water content (Fig. 4a) or moisture percentage of our honey samples analysed ranged between 13.00% and 20.13%, with a mean of 16.20 ± 1.56. This results match refractive indices between 1.4863 to 1.5082, with an average of 1.5. These values were within the limit (21%) recommended by the *Codex Alimentarius* Commission [4].

These results were similar to the results observed by Ouchmoukh *et al*. [19]; Chefrour *et al*. [20]; Makhloufi *et al*. [21]; Benaziza-Bouchema *et al*. [22]; Bendeddouche and Dahmani [23]; Amri and Ladjama [24]; Zerrouk *et al*. [25].

However in this study we observed that values of our honeys samples E5, E11, E 22 which have 19.53%, 20.30% and 19% respectively exceed value of 18% of water contan. Therefore, uniformly high water content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow [26] and lead to undesirable honey fermentation during storage [27].

Honey moisture content, a critical variable influencing product quality, granulation and texture, is significantly affected by conditions under which honey is stored following its extraction from the hive. Moreover, the water content depends upon the environmental factors during production such as weather and humidity inside the hive, but also on nectar conditions and treatment of honey during extraction and storage. It can be reduced before or after extraction by special techniques [28]. Electrical conductivities ranging between 0.097 mS / cm ± 0.005 (17 E) and 1.110 mS / cm ± 0.035 (E 3) with an average of 0.45 mS / cm ± 0.29. The electrical conductivity is a good parameter for distinguishing between honeydew and nectar honey. According to the Codex *Alimentarius* standards, honeydew honeys have a conductivity greater than 0.8 mS / cm (Except for chestnut honey) and nectar honeys have a conductivity of less than 0.8 mS / cm (Except for mixing nectar / honeydew). Then after observing (Fig. 4b) we can say that 84%of samples are nectar honeys and 16 % of samples are probably honeydew or mixtures of nectar and honeydew.

The ash content in honey is generally small and depends on nectar composition of predominant plants in their formation [28,29]. The ash content in the honey samples varied between 0.006% (31 E) and 0.371% (E-9) 0.09 - 0.18 % (Fig. 4c) is in the acceptable range and do not exceed 0.6% described by Codex. These values

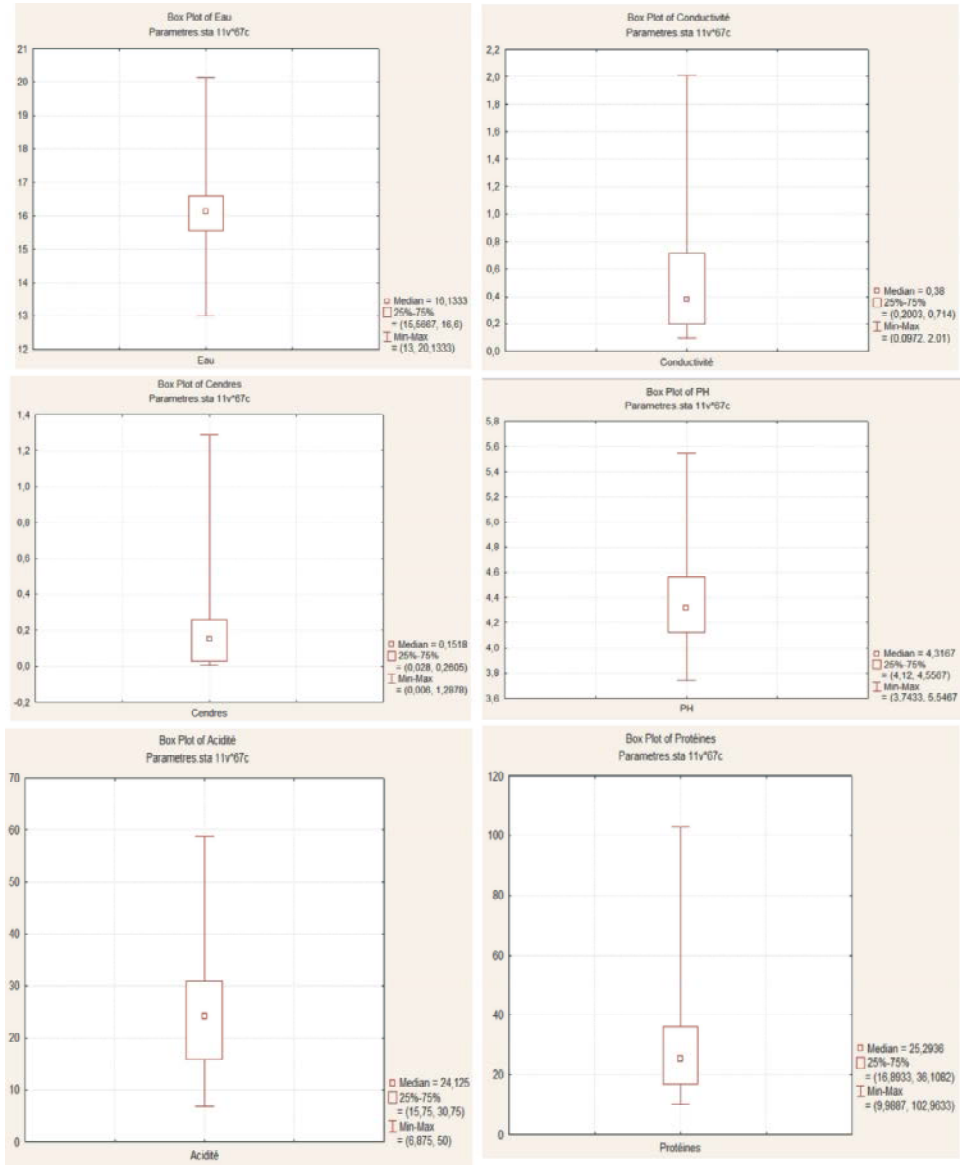

Fig 4: Box plot of physicochemical parametres of honey (a. box plot of moisture content, b. box plot of electrical conductivity, c. box plot of ash content, d. box plot of pH, e. box plot of acidity content, f. box plot of protein content)

showed good agreement with the reported values by various workers from Algeria 0.019 to 0.518% [24]. Similar values were observed by Zerouk *et al*. [30].

The ash content is associated with botanical and geographical origins of honey samples. The variability in the ash content of honeys could be due to harvesting processes, beekeeping techniques and the material collected by the bees during the foraging on the flora [31].

pH value of honey has a great importance during extraction and storage as they influence texture, stability and shelf-life [32,33]. All of the investigated Algerian honey samples were acidic in character irrespective of their variable geographical origin. 72% of honey samples have pH values ranged between 3.74 (E 32) and 4.5(E 13) (Fig. 4d) and 28% of the samples ranged between 4.5 and 5.5. These samples could be honeydew or honeydew / nectar mixture. These values were similar to those previously reported for other honey samples from Brazil, India, Algeria and Turkey, which were reported to have pHs between 3.49 and 4.70 [27, 34-38]. The results of Makhloufi et *al*. [21] found that pH values were ranged from (3.40 to 6.23) for 66 Algerian honeys.

This parameter may be used as an indication of the botanical origin [39]. In another hand, the pH strongly influences the rate of degradation of sugars and has demonstrated to have a strong influence on the activity of acid phosphatase. The higher the pH, the greater the acid phosphatase activity. However, Honeys that ferment more easily have shown higher acid phosphatase activities than unfermented honeys [40]. Thus the majority of our samples can be fragile honey and can't be stored for long time.

Acidity ranging between 6.87 meq/kg (E 20) and 58.75 (E 22) meq/kg with an average of 24.84 meq/kg ± 12.16 (Fig.4e). All the values obtained were in agreement with the current legislation for *Apis mellifera* honey. The Acidity of honey may be explained by taking into account the presence of organic acids in equilibrium with their corresponding lactones, or internal esters and some inorganic ions, such as phosphate and chloride [41, 42]. Variation in acidity among different honeys can be attributed to floral origin or to variation because of the harvest season. When the acidity becomes high, the honey becomes sour. High acidity can be indicative of fermentation of sugars into organic acids [26, 34, 43], which is responsible for two important characteristics of honey: flavor and stability against microbial spoilage [44].

The values obtained in this study shows that the protein contents of honey samples from some of Algerian samples ranged between 0.09% et 0.81% (Fig.4f). The honey proteins are mainly in the form of enzymes [45]. The honey bees add different enzymes during the process of honey ripening. The enzymes added include diastase (Amylase), which digest starch to maltose and is relatively stable to heat and storage and invertase (Saccharase or α-glucosidase), which catalyses the conversion of sucrose to glucose and fructose. The invertase also catalyses many other sugar conversions and is mainly responsible for the sugar patterns of honey. Glucose oxidase and catalase are two other enzymes added by the honey bee, which regulate the production of hydrogen peroxide H_2O_2; it serve as one of the anti-bacterial factor in honey [46]. The level of protein is dependent on the type of flora and thus it is variable [26].

The most commonly monitored parameters for determining honey freshness include HMF levels and diastase activity [47].The results obtained (Fig. 5) showed that, in 5 samples, the content of HMF was higher than the maximum allowed, which is 40 ppm [48]. The HMF occurs naturally in honey. It is present only in trace amounts [49]. its When detected in high amounts,

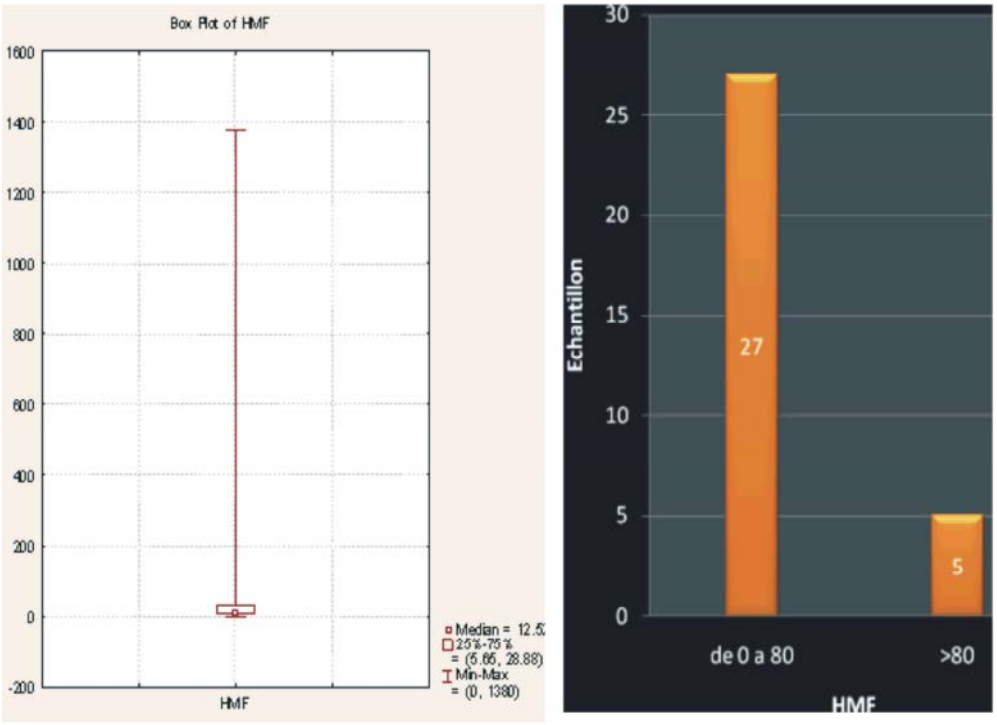

Fig 5: Result of content of HMF

Table 2: Correlation matrix of physico chemical components of honey samples

	Correlations (parametres)							
Variable	Acidity	Ash	Protein	Moisture	Nbre of pollen	HMF	pH	C.E
Acidity	1.00							
Ash	0. 26	1.00						
Protein	0.36	0.19	1.00					
Moisture	0.43	0.06	0.07	1.00				
Nbre of pollen	-0.09	0.21	0.30	0.24	1.00			
HMF	-0.33	-0.27	-0.39	-0.13	0.08	1.00		
pH	-0.35	-0.08	0.10	-0.34	-0.03	0.17	1.00	
C.E	0.47	0. 7593	0.39	0.19	0.18	-0.39	-0.18	1.00

it indicates the adulteration of honey wether with the addition of commercial sugar or undue heating procedure. The high levels in the five honey samples could be as a result of heating during processing or increase in temperature during storage or while being Transported [50]. The content of HMF can increase with age and pH changes, during storage, indicating the deterioration of product quality. All these changes affect the nutritional value of the product. The HMF is therefore an important parameter used to indicate the purity and freshness of honey [51].

The results of the proximate analysis of honey samples obtained from different region of Algeria are presented on Fig. 3. The results showed no significant differences (P>.05) between the samples for HMF contents (p=.42) as well as the energy values of the honey samples from all the states. However, significant differences (P<.05) in moisture contents, ash, protein contents, free acidity, pH, Electrical conductivities were observed between the honey samples.

From the table of correlation matrix (Table 2), the the ash was positively well correlated with electrical conductivity (correlation coef?cient, r = 0.7593), the conductivity (C.E) is positively correlated with the acidity (r = 0.47) and low correlation is obtained between the electrical conductivity and protein content (r = 0.39).

Observing correlation table shows that the pH was negatively correlated with the acidity (r = - 0.35).

Thus, there are negative correlation between water content the acidity (r = -0.43). However on the HMF setting it is negatively correlated with protein and the electrical conductivity (r = -0.39).

CONCLUSION

The aim of present study was to evaluate and compare the physico-chemical properties of some honey samples from Algeria. The results showed that 72% of samples coincide with those specified by the international honey regulations and have good quality. This study would be helpful to understand local honey properties and very important towards the commercialization of regional honey.

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