

Article

Study of Physicochemical Characteristics of Some Honey Samples Collected from Setif in Algeria

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Abstract: The research was carried out to determine the physicochemical quality aspects of three samples of honey that were collected from different regions of Setif and one imported honey sample was purchased in store. The statistical design adopted in this study is one way completely randomized with three repetitions. Water content, total sugar, refractive index, Density, Total soluble solids, Potential of hydrogen (pH), Conductivity, Ash, and free acidity were assessed for evaluating honey samples. This study aims to compare the quality parameters between the local products (three samples) and imported honey (one sample). The values of all physic-chemical parameters of both local and imported honey samples are within the quality limits set in world legislation and the ANOVA analysis proved a significant difference ($p < 0.05$) among the samples tested for all parameters except free acidity. The Water content and total sugar are the important parameters for honey quality evaluation. The values of water content varied from 16.5 to 18 %, the lowest value registered by the local sample Sidr honey, low water content indicates a good quality honey. Water content and Relative density (specific gravity) are correlated and are used as a measure of adulteration in honey. Electrical conductivity is used as an indicator for quality control to distinguish floral honey from honeydew honey. The values of conductivity varied from 180 to 314.66 $\mu\text{S}/\text{cm}$, this difference could be due to the dependency of electrical conductivity on ash, organic acids, and some complex sugars and varies with botanical origin ($< 800 \mu\text{S}/\text{cm}$). The content of Ash ranged from 0.033 to 0.155 %. Both electrical conductivity and crude ash analyses are frequently used in honey quality inspections. In conclusion, the result of this study indicated that honey samples collected from eastern Algeria, Setif, were mostly of good quality.

Keywords: Algerian honey; free acidity; pH; sugar content; water content.

1. Introduction

The Indonesian National Standard (SNI) 3545: 2013 defines honey as a natural liquid that generally has a sweet taste produced by honey bees (*Apis* sp.) from the nectar of flowers (flora nectar) or other parts of plants. Honey is characterized by high nutritional value and fast absorption of its carbohydrates upon consumption [1].

Honey is a very complex natural product that contains sugars, organic acids, amino acids, enzymes, minerals, vitamins, lipids, phenols, flavonoids, pigments, waxes, pollen grains, and other phytochemicals [2]. Honey has been known since ancient times for its nutritional and therapeutic properties [3].

Interestingly, honey has been cited in the Quran, a Holy book for Muslims (Surah An-Nahl, Verse 68-69) ("And your Lord inspired to the bee, take for yourself among the mountains, houses, and among the trees and [in] that which they construct, then eat from all the fruits and follow the ways of your Lord laid down [for you], there emerges from their bellies a drink, varying in colors, in which there is healing for people, indeed in that is a sign for a people who give thought."), about its medicinal properties.

Honey has attracted great attention as a natural sweetener that promotes health, honey is also associated with decreases in inflammation *in vitro* through the simultaneous effects of its antioxidant and antibacterial properties [4].

It's generally evaluated by physicochemical analysis of its constituents, many authors propose the determination of physicochemical parameters as useful markers to define the geographical origin and the quality of different honeys [5], including water content, pH, sugar content, acidity, ash and electrical conductivity [6].

In addition, [7] provides that the quality control evaluation can be done by assessing physicochemical parameters such as the pH, moisture, ash value, hydroxyl methyl furfural (HMF) and color, etc., to evaluate and test the purity of marketed honey.

Honey has various bioactive compounds such as vitamins (E, A, K, B₁, B₂, B₆, C), phenolics, flavonoids, amino acids, and fatty acids which contribute to pharmacologic properties such as wound healing, antimicrobial activity, anti-inflammatory, antidiabetic, antioxidant, and antitumoral effects [8].

Honey is also associated with decreases in inflammation *in vitro* through the simultaneous effects of its antioxidant and antibacterial properties [4]. According to a study conducted by [9], the antiviral activity of honey may be beneficial for patients with COVID-19 as it boosts the host immune system and improves comorbid conditions.

It has been also proven effective against several viruses such as HIV, influenza virus, herpes simplex, and varicella-zoster virus. Honey helps in the treatment of cardiovascular diseases, cancer, cataracts, and several inflammatory diseases [10].

The objective of this study is to compare the quality of four different samples of honey based on their physico-chemical properties such as water content, sugar content, acidity, electric conductivity, density, Total soluble solids, and ash.

2. Materials and Methods

Honey samples

Three samples of honey were collected in different regions of Setif from professional apiaries, during the seasons of the year 2022 according to floral sources, and the imported honey sample is purchased in store and stored at 4–6°C. The experimental design adopted during this study is a one-way completely randomised design with three replications.

Water content, total sugar, refractive index, Density, and Total soluble solids

All parameters cited below are determined using an ATC Refractometer, from the reading recorded by the equipment upon placing a couple of drops of the honey sample on the prism. According to the harmonized method for honey developed by the International Honey Commission, the measured values of water content, total sugar, and refractive index were corrected according to the temperature of the laboratory [11].

The Baume degree is an indirect unit of measurement of concentration, via density, invented by Antoine Baume. It is denoted by °B, °Be or °Bé at 20°C using the refractometer method, The Baume degree can be used to determine the density based on the formula: $d = 140 / (B^\circ + 130)$, d: density and B°: Baume degree. In addition, the formula $TSS (\%) = 100 - M$, Was used to calculate the total solids expressed as a percentage. Where: TSS = Total soluble Solids (%); M = sample water content (%) [12].

Potential of hydrogen (pH) and Conductivity

The pH and electrical conductivity are measured by the methods of Sereia et al. [13]. The pH of aqueous honey solutions (10 g/75 mL) was measured with a combined electrode, equipped with a pH meter, and the Conductivity was measured at 20 °C using a conductivity meter (HI-98194 MULTIPARAMETER, Hanna instruments); the results were expressed in micro Siemens per centimeter ($\mu\text{S}/\text{cm}$).

Ash and free acidity

The ashes of the honey samples were calculated from the weight of the inorganic residue remaining after carbonizing at 600 °C to constant weight; the results were expressed in grams of total ash per 100 grams of honey (%) [14].

Free acidity was estimated from an acid-base titration. The aqueous honey solution was titrated with 0.1 N NaOH in the presence of phenolphthalein as an indicator; the results were expressed in milliequivalents of acid/kg of honey (meq/kg) [15].

Statistical methods

All analyses were carried out in triplicate. Costas 6.1 statistical program was used for statistical analysis.

3. Results and Discussions

3.1. Water content, total sugar, and density

As illustrated in Table 1, the water content and total sugar are significantly affected by the honey flora types. The values of water content varied from 16.5 to 18 %, the lowest value registered by the local sample Sidr honey, low water content indicates a good quality honey. Low values of moisture content prevent the fermentation of honey and increase shelf life and also the storage time [15].

In the present study, the water content of all samples analyzed was within the range of acceptable international standards (<20%), water content is an important quality parameter to evaluate honey quality, and the susceptibility to honey increases towards microorganisms with water contents above 17% [16]. Sugar is the principal component of honey. Sugar composition and moisture percentages are considered as ripeness parameters of honey.

The total sugar content in honey samples analyzed averaged 79.87 % with a range of 79.0–80.5 % (Table 1). [17] reported a range of 76–81.3% for different types of honey from a local market in Mexico; sugars contribute to the antibacterial properties of honey and its preservation because they provide high osmolarity and low water activity.

Relative density (specific gravity) and moisture content are correlated and are used as a measure of adulteration in honey. As water content in honey increases, relative density decreases. The mean relative density analyzed was 1.411 with a range of 1.405–1.416. These values are similar to those reported by [18].

Table 1. Water, total sugar, and density content of honey sample tested.

Name of honey	Water content (%)	Total sugar (%)	Density (g/cm³)
Sidr honey	16.5 ^b	80 ^a	1.413 ^a
Mountain honey	17.83 ^a	79 ^b	1.405 ^b
Imported honey	18 ^a	80 ^a	1.413 ^a
Multifloral honey	18 ^a	80.5 ^a	1.416 ^a
Mean	17.583	79.875	1.411
Max	18	80.5	1.416
Min	16.5	79	1.405
SD	0.668	0.678	0.0015
Sig-level	***	*	*
LSD (5%)	0.271	0.815	0.05
Codex Alimentarius	max. 20 %	70-83 %	1.38-1.5

*, *** significant effects at 5, 0.1 %, respectively. In each column, different letters mean significant differences ($p < 0.05$).

2. Total soluble solids and Refractive index

The results of total soluble solids (%), may reflect the total sugar contents of honey. As shown in Figure 1, the total soluble solids are significantly affected by the honey flora types, the highest value is registered by the local sample of Sider honey; the ANOVA analysis proved that there is no significant difference between Mountain, Multifloral, and Imported honey. The values of total soluble solids are varied from 82 to 83.5%. For all the honey samples, total soluble solids were generally more than 80% and can be considered high-grade and highly stable upon storage. The total soluble solids are closely connected to the amount of sugars existing in honey, making it an essential marker of conceivable adulteration [18].

According to [12] Albu et al. (2021) honey with total soluble solids greater or equal to 81.4% is considered of higher grade (A and B), while the values ranged between 80-81.3% are considered to be of lower grade C. Thus, the honey investigated in this study can be considered stable concerning fermentation upon storage and thus of high grade. Refractive index (RI) was determined in all samples, there is a significant difference among different honeys ($p < 0.05$). The RI values ranged between 1.488 and 1.4915.

The values of the Refractive index are correlated to and dependent on the values of other parameters, such as water content, solids substances, total soluble solids, and specific gravity, the higher values of the refractive index in correlated with the lower the moisture content. Low values of moisture content prevent the fermentation of honey and increase shelf life and storage time [12]. Our results follow the finding of [12] which indicates that the values of RI are ranged from 1.485 to 1.499.

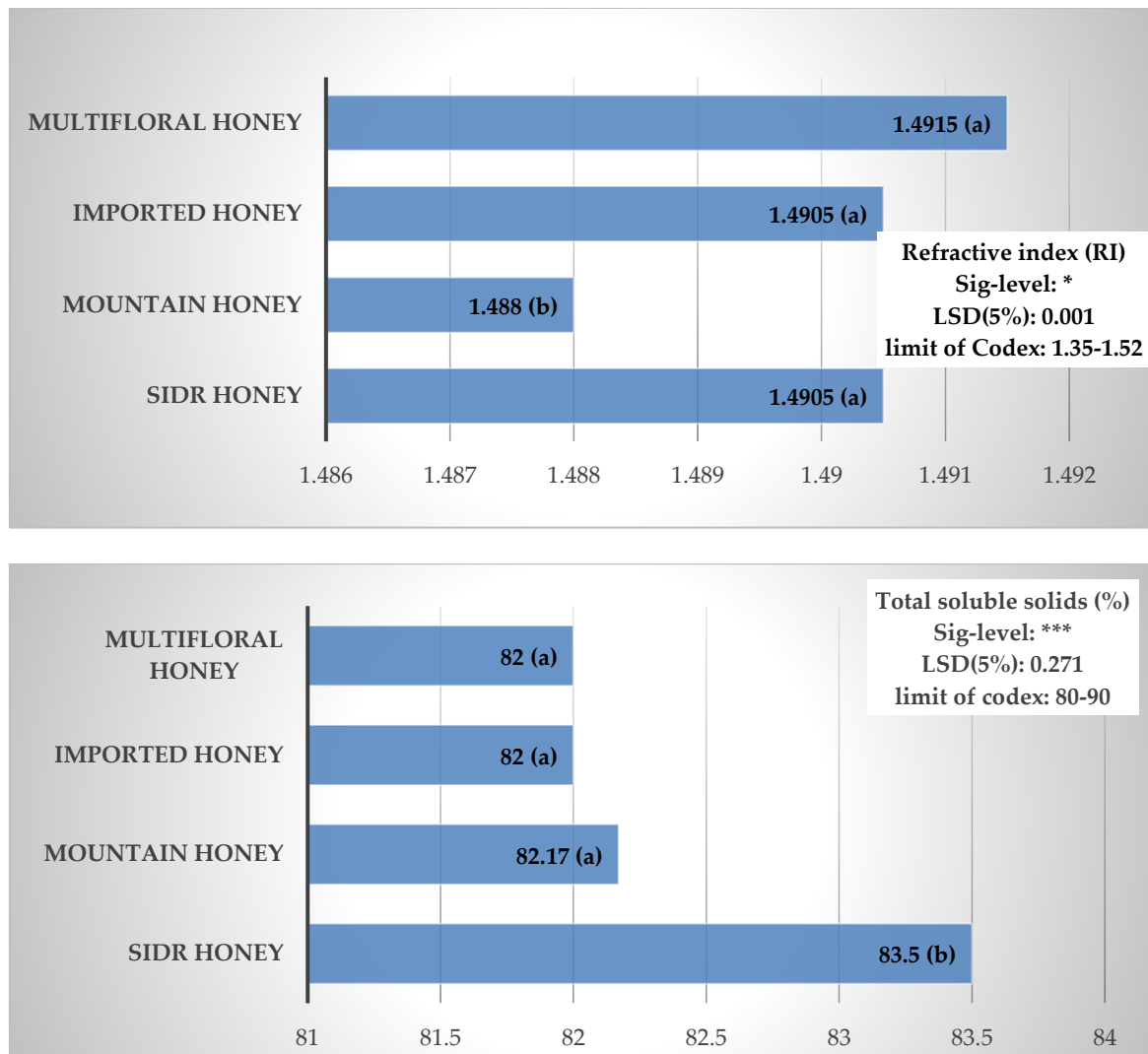


Figure 1. Variation in the Total soluble solids and Refractive index for honey samples.

3.3. Conductivity (mS/cm) and Ash content (%)

Electrical conductivity is used as an indicator for quality control of honey that can be used to distinguish floral honey from honeydew honey [19]. The mean values of conductivity as in Table 2, varied from 180 to 314.66 $\mu\text{S}/\text{cm}$, a high significance difference ($p < 0.001$) was observed among the honey sample. This difference could be due to the dependency of electrical conductivity on ash, organic acids, and some complex sugars and varies with botanical origin.

According to [20] the electrical conductivity of floral honey must not exceed 800 $\mu\text{S}/\text{cm}$. Our results proved that all samples of honey tested originated from floral honey. As illustrated in Table 2, there are significant effects of floral types of honey on the content of Ash, the values ranged from 0.033 to 0.155 %.

Rysha et al. [21] reported that the conductivity of honey depends on the acid as well as the ash contents, where higher ash and acid contents show higher conductivity.

The percentage of Ash content for the honey from all floral types was within the guidelines stipulated by the Codex Alimentarius [22] of less or equal to 0.6% and is lower than the value reported by Salim [23], who reported the ash content of between 0.075 and 0.330 % for honey harvested from central Algeria. Both electrical conductivity and crude ash analyses are frequently used in honey quality inspections. Those traits, especially electrical conductivity, are considered very good criteria for the assessment of the botanical origin and purity of honey [24].

In addition, Finola et al. [25] reported that the variability in the ash content of honey could be due to harvesting, beekeeping techniques, and the material collected by the bees during foraging on flora.

Table 2. Mean values of Conductivity and Ash content in tested honey.

Name of honey	Conductivity ($\mu\text{S}/\text{cm}$)	Ash content (%)
Sidr honey	262.00 ^b	0.155 ^a
Mountain honey	314.66 ^a	0.140 ^a
Imported honey	185.33 ^c	0.049 ^b
Multifloral honey	180.00 ^c	0.033 ^b
Mean	235.50	0.094
Max	314.66	0.155
Min	180.00	0.033
SD	58.89	0.064
Sig-level	***	**
LSD	14.142	0.071
Codex Alimentarius	max. 800	max. 0.6

, * significant effects at 1, 0.1 %, respectively. In each column, different letters mean significant differences ($p < 0.05$).

3.4. Potential of hydrogen (pH) and Free acidity

The pH of Honey is naturally acidic due to the presence of many organic acids and some inorganic ions, such as phosphate and chloride that contribute to its flavor and stability against microbial spoilage [26].

In addition, Al Hadhramie et al. [27] indicate that the pH of honey is important because it affects the texture of honey as well as its stability and shelf life. The data in Table 3 shows the pH values for four honey samples. Where the pH values of the samples showed that they were acidic, the pH ranged from (4.28-4.72), and within the standard limit (pH of 3.40 to 6.10) [22] (Codex Alimentarius, 2001). There is a significant difference ($p < 0.01$) between the different honey samples tested.

Based on the results of Chefrour and Coworkers [28], our honey samples are considered to be blossom honey (pH ranged from 3.5 to 5), while honey with a pH above 5 is of low quality. Free acidity measurement is useful for the evaluation of honey fermentation, authentication of unifloral honey, and differentiating nectar from honeydew honey [29]. The free acidity of all samples tested fell within the permitted range proposed by Codex Alimentarius [22] of no more than 50 illiquid acid/kg.

The free acidity of honey samples in this study ranged from 9 to 17 illiquid acid/kg (Table 3). There was no significant difference in the free acidity content of honey samples ($p > 0.05$). According to Baltac and Candan [30], a lower value of acid indicates the absence of undesirable fermentations.

Table 3. Mean values of Potential of hydrogen (pH) and free acidity.

Name of honey	Potential of hydrogen (pH)	Free acidity (meq/kg)
Sidr honey	4.723 ^a	14 ^a
Mountain honey	4.4 ^{bc}	17 ^a
Imported honey	4.506 ^b	9 ^a
Multifloral honey	4.283 ^c	12.666 ^a
Mean	4.478	13.166
Max	4.723	17
Min	4.283	9
SD	0.190	8.74
Sig-level	**	ns
LSD	0.195	18.141
Codex Alimentarius	3.24-6.1	max. 50

**, ns significant effects at 1, no significant, respectively. In each column, different letters mean significant differences ($p < 0.05$).

4. Conclusions

As a conclusion from this research, we can say that the values of all physicochemical parameters of both local and imported honey samples are within the quality limits set in world legislation. The physio-chemical properties such as moisture content, total sugar, and density recorded in this study showed that honey from the various samples is of high quality. In addition, both electrical conductivity and crude ash analyses are frequently used in honey quality inspections. The results of our study proved that all samples tested are of good quality. Those traits, especially electrical conductivity, are considered very good criteria for assessment of the botanical origin and purity of honey, mountain and Sidr honey are very good values of electrical conductivity. A low pH indicates a good environment that inhibits microorganism growth, while the free acidity level is a biochemical marker for honey samples freshness based on this idea all samples honey tested have a good quality. Overall, the result of this study indicated that honey samples tested from eastern Algeria -Setif-, were mostly of good quality. In the future, we need to study many samples from different regions to evaluate the effect of climatic conditions on the quality of honey.

Conflicts of Interest: The authors declare no conflict of interest.

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