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Physicochemical properties and antioxidant activities of honeys from diverse regions in northern Algeria

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Abstract
The quality of honey can vary due to several factors. This research aimed to assess the physicochemical properties, including moisture content, pH, ash content, free acidity, electrical conductivity, Brix degree, and hydroxymethylfurfural concentrations, as well as the antioxidant activities of nine varieties of Algerian honey in 2022. The objective was to evaluate the overall quality of these honey samples. The results indicated that the physicochemical attributes of the honey samples generally complied with the Codex Alimentarius guidelines, except for the sample from Chaffia (H3), which exceeded the recommended hydroxymethylfurfural (HMF) threshold. This deviation suggests potential issues related to overheating or inadequate storage conditions. Furthermore, the study identified variability in antioxidant activity among the honeys, with IC50 values ranging from 12.7 mg/ml to 26.52 mg/ml, indicating differences in their antioxidant efficacy. These findings enhance the understanding of Algerian honey and can guide future efforts to ensure consistent quality and maximize its health benefits.

Keywords: Antioxidant Activities; Honeys qualities; Northern Algeria; Physicochemical properties.

Introduction

In the scientific investigation of honey quality across various Algerian regions, it is essential to recognize its historical significance as a global sweetener. Archaeological and textual evidence indicates its use for over a millennium (Biswas et al., 2023) and highlights its widespread applications across cultures Tafere (2021). The composition of honey primarily consists of carbohydrates, predominantly monosaccharides such as fructose and glucose, along with disaccharides and minor trisaccharide components. It is further enhanced by the presence of enzymes, minerals, vitamins, phenolic acids, amino acids, volatile compounds, flavonoids, carotenoids, and organic acids, all of which contribute to its health benefits (Brar et al., 2023). The intricate chemical composition of honey is significantly influenced by its botanical and geographical origins, which encompass factors like meteorological conditions, soil composition, floral diversity, and nectar availability (Anjos et al., 2015). Beekeeping practices, as well as storage and packaging methodologies, also affect honey's composition (Thrasyvoulou et al., 2018).

Despite its natural origins, honey is vulnerable to adulteration, particularly through the addition of industrial syrups, which compromise its quality (Brar et al., 2023; Moskric et al., 2021). Quality assessment of honey involves sensory, microbiological, and physicochemical analyses, based on criteria established by the EU Directive 2001/110/EC. These criteria include measurements of electrical conductivity, diastase activity, moisture content, sugar levels, ash content, pH, acidity, density, and hydroxymethylfurfural (HMF) levels (Bogdanovet al., 2002).

Monofloral honey, which must contain at least 45% total pollen from a single botanical source, is noted for its unique aromatic profiles that may reflect specific volatile compound compositions (Soares et al., 2017). The link between the phenolic content in honey and its antioxidant and antibacterial properties has been well-established through various studies (Hernanz et al., 2023; Can et al., 2015; Doukani et al., 2014), underscoring the significant role of both enzymatic and non-enzymatic antioxidants in honey in combating oxidative processes associated with numerous diseases (Al et al., 2009; Aljadi and Kamaruddin 2004).

Recent years have witnessed a burgeoning interest in beekeeping in Algeria, driven byvarious incentivization measures (M.A.D.R. 2015). Our research aims to elucidate the physicochemical properties and antioxidant capacities (assessed via the DPPH assay) ofhoney samples from different northern regions of Algeria, seeking to provide valuable insights into regional quality variations and potential health implications.

Materials and methods

Collection of Honey Samples

Nine distinct honey samples were sourced from various locales across the northern regions of Algeria in 2022. To ensure the reliability of the analysis, each sample was subdivided into three separate aliquots, culminating in a comprehensive examination of 27 individual samples. Prior to analysis, these samples were meticulously stored in conditions described as cool and dry to safeguard their integrity. The geographic origins of the samples are detailed inTable 1.

Physicochemical Analysis

The physicochemical properties of the honey samples were assessed through several determinations:
Moisture Content and Brix Degree: Moisture content and Brix degree were measured using an Abbe refractometer (AR4) at 20°C. The Brix degree of the honey was determined using the Chataway table based on the refractive index (Bogdanov et al., 1999).

Electrical Conductivity: The method for determining electrical conductivity was carried out as described by Bogdanov et al. (1997). The electrical conductivity (EC) values of the honey are reported in milli Siemens per centimeter (mS/cm) at 20°C.

Ash Content: Ash content was determined by igniting a honey solution at 630°C to a constant weight, following the procedure described by Bogdanov et al. (2002).

pH and Free Acidity: The pH and free acidity of the honey samples were measured according to the guidelines of the International Honey Commission (Bogdanov, 2009). These measurements were conducted using a HI902 potentiometric titrator (Hanna Instruments, pH 211 microprocessor pH meter)

Density Measurement: The density of the honey samples was determined according to the protocol established by Bogdanov et al., (1999).

Hydroxymethylfurfural (HMF): The quantification of HMF was performed using spectrophotometry in the UV range, employing the method known as White's method (White, 1979), as specified in the Algerian Standard NA 19410 (2018).

Evaluation of Antioxidant Activity

The antioxidant activity of the honey samples was evaluated by their ability to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. This assessment was conducted following the procedure described by Brand-Williams et al., (1995). Specifically, 1 ml of the honey sample was mixed with 2 ml of a 0.06 mM DPPH solution in methanol. After an incubation period of 15 minutes, the absorbance was measured at 517 nm. The percentage of DPPH radical scavenging activity was calculated using the formula:

Percent Inhibition (%) = $\frac{AC-AS}{AC} \times 100$

Where AC represents the absorbance of the control, and AS denotes the absorbance of the honey sample.

Table 1. Geographical origins of honeys samples from Algerian regions

honeys samples	Sites	Geographic coordinates
H1	Seraidi (Annaba)	36° 55′ 00″ N; 7° 40′ 00″ E
H2	Besbes (Annaba)	36° 42′ 08″ N;7° 50′ 50″ E
H3	Chaffia (El-Taref)	36° 36′ 40″ N ; 8° 02′ 20″ E
H4	Hoceinia (Ain defla)	36° 19′ 24″ N ; 2° 24′ 14″ E
H5	Mekhatria (Ain defla)	36° 18′ 09″ N ; 1° 57′ 35″ E
H6	El main (Ain defla)	36° 03′ 03″ N ; 1° 45′ 30″ E
H7	Boufarik (Blida)	36° 34′ 00″ N ; 2° 55′ 00″ E
H8	Oued alleug (Blida)	36° 33′ 11″ N ; 2° 47′ 27″ E
H9	Chrea (Blida)	36° 25′ 32″ N ; 2° 52′ 36″ E

Statistical Analysis

Each honey sample underwent measurements in triplicate, and the results are reported as the mean ± standard deviation (SD). Significant differences, denoted by different letters, were identified using a one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test, with a significance level set at $p < 0.05$. The correlation between the Brix degree and moisture contents was examined by the Pearson correlation test; all statistical analyses were performed using Minitab 16 statistical software

Results and discussion

The results of physicochemical parameters and antioxidant activity of nine Algerian honey samples are detailed in Table 2.

The moisture content of the samples ranged from 18.367±0.513% to 21.583±0.381%. These findings are consistent with those reported by Hailu Reta and Dassalegn Daraje (2023), who documented similar moisture content ranges in honey samples from various locations in northern Algeria in 2020. A one-way ANOVA showed that region significantly affectsmoisture content ($F = 17.63$; $p < 0.001$). These results are in accordance with the moisture content standards established by the Codex Alimentarius in 2001, thus confirming that the sample moisture levels adhere to recognized international benchmarks. Chirife et al., (2006) noted that elevated moisture levels can predispose honey to fermentation and acetic acid production during storage. In contrast, Terrab et al., (2003) identified the beneficial aspects oflow moisture content in extending the shelf life of honey. Several factors, including the harvesting season, hive maturity, botanical origin, and meteorological conditions, significantly influence the moisture content of honey (Finola et al., 2007; Kahraman et al., 2010).

The pH values observed in this study ranged from 3.670 ± 0.028 to 4.910 ± 0.090, which are consistent with findings from diverse geographical regions including northen Algeria Laouar and Tahar (2018), West Shoa (Gela et al., 2021), and Brazil (Azevedo et al., 2021). The inherent acidity of honey, primarily due to the presence of organic acids, not only enhancesits flavor but also contributes to its microbial stability (Ibrahim et al., 2011). AV1 indicated a significant effect of region on pH. (F=103.65; p< 0.001). The pH values reported in this study comply with the standards set by the Codex Alimentarius in 2001, confirming that the sample pH values fall within established international guidelines.

The amount of ash in the samples analyzed fluctuated between 0.063±0.014 and 0.685±0.043 g/100g. Nandaa et al., (2003) established a maximum threshold of 0.6% for nectar honeys. Ash content serves as an indicator for determining the botanical origin of honey (floral,honey or mixed) Terrab (2004), and the observed values are consistent with the limits set by the Codex Alimentarius (2001) for nectar-derived honeys. AV1 revealed a significant Variations in ash content can be attributed to differences in harvesting methods, beekeeping practices, and foraging materials, as noted by Ouchemoukh et al., (2007) and Finola et al., (2007).

Measurements of free acidity revealed no signs of fermentation, with values ranging from 20.33±1.528 meq/kg to 45.330±2.50 meq/kg. Notably, the free acidity levels observed in our study were higher than those reported by Hailu and Dassalegn (2023), highlighting variability in organic acid composition across studies (Bogdanov et al., 2004; Gomes et al., 2010). AV1 indicated that region significantly influenced these levels. (F = 49.18; p< 0.001). Moreover, these results align with the free acidity guidelines established by the Codex Alimentarius in 2001, confirming that the free acidity levels in our samples meet international standards (50 meq / 100g honey).

Hydroxymethylfurfural (HMF) concentrations, which are indicative of honey quality and freshness, ranged from 7.463±0.474 to 32.417±2.504 mg/kg across most samples. However, the HMF level in the honey sample from H3 was significantly higher at 76.34 mg/kg,surpassing the Codex Alimentarius standard of 60 mg/kg. This marked increase suggests potential overheating or suboptimal storage conditions, consistent with findings from other regional studies (Rodríguez Flores et al., 2019; Ajlouni and Sujirapinyokul 2010; Mohammedet al., 2013). AV1 confirmed a significant effect of geographic region on HMF levels (F = 614.21; p< 0.001).

Density measurements of honey were observed to range from 1.34 ± 0.04 to 1.58 ± 0.030 g/ml. Consistent with these findings, Laouar and Tahar (2017) reported density values for some Algerian honeys ranging from 1.32 to 1.55 g/ml. The density of honey is influenced by factors such as water content and practices related to harvesting and storage Prost (1987); Louveaux (1985). Statistical tests employing one-way ANOVA demonstrated a significant impact of geographic area on the density of honey (F = 19.43; p< 0.001). These results conform to the density specifications established by the Codex Alimentarius guidelines of 2001, thus validating the honey samples against recognized international standards.

Table 2. Assessment of physicochemical parameters (mean ± sd) and antioxidant activities (ic50) in honey varieties from different Algerianregions. (means for each site (the columns): values followed by different lowercase letters are significantly different at $p \leq 0.05$.)

Sites	PH	Free acidity meq/Kg	Density g/ml	Moisture content g/100g	Ash content g/100g	HMF content mg/kg	Brix degree g/100g	Electrical Conductivity Ms/cm	Antioxidant Activity IC50 mg/ml
H1	3.920 ± 0.020^{bc}	30 ± 1^{bcd}	1.346 ± 0.046^{d}	21.583 ± 0.381^{a}	$0,685\pm0.043^{a}$	7.463 ± 0.474^{g}	78.417 ± 0.382^{e}	0.8 ± 0.01^{a}	19,99
H2	3.690 ± 0.078^{d}	32.500 ± 2.50^{b}	1.40 ± 0.045^{cd}	18.367 ± 0.513^{cde}	$0,063\pm0.014^{b}$	32.417 ± 2.504^{b}	81.633 ± 0.513^{abc}	0.23 ± 0.01^{d}	26,52
H3	3.670 ± 0.026^{d}	34.333 ± 1.528^{b}	1.370 ± 0.07^{cd}	19.633 ± 0.777^{abc}	$0,653\pm0.031^{a}$	63.667 ± 2.517^{a}	80.367 ± 0.777^{cde}	0.33 ± 0.02^{c}	23,18
H4	4.916 ± 0.092^{a}	31.667 ± 0.577^{bc}	1.580 ± 0.03^{a}	16.850 ± 0.999^{e}	$0,545\pm0.057^{a}$	23.343 ± 0.179^{c}	83.150 ± 0.999^{a}	0.77 ± 0.07^{ab}	26,42
H5	3.853 ± 0.049^{bcd}	25.333 ± 1.528^{def}	1.366 ± 0.015^{d}	19.210 ± 1.391^{bcd}	$0,403\pm0.105^{a}$	17.607 ± 0.085^{c}	80.790 ± 1.391^{bcd}	0.68 ± 0.02^{b}	22,56
H6	3.836 ± 0.041^{bcd}	45.333 ± 3.055^{a}	1.533 ± 0.02^{ab}	17.367 ± 0.153^{de}	$0,512\pm0.061^{a}$	9.910 ± 0.056^{fg}	82.633 ± 0.153^{ab}	0.76 ± 0.04^{ab}	20,43
H7	3.713 ± 0.060^{d}	20.333 ± 1.528^{f}	1.356 ± 0.015^{d}	20.440 ± 0.233^{ab}	$0,110\pm0.011^{b}$	12.703 ± 0.078^{ef}	79.560 ± 0.233^{de}	0.32 ± 0.02^{cd}	12,7
H8	3.796 ± 0.035^{cd}	22.667 ± 1.528^{ef}	1.355 ± 0.030^{d}	21.583 ± 0.144^{a}	$0,090\pm0.012^{b}$	15.657 ± 0.025^{de}	78.417 ± 0.144^{e}	0.3 ± 0.01^{cd}	20,04
H9	4.016 ± 0.117^{bc}	27.000 ± 2^{cde}	1.473 ± 0.015^{bc}	18.817 ± 0.548^{bcde}	$0,489\pm0.089^{a}$	23.193 ± 0.055^{c}	81.183 ± 0.548^{abcd}	0.74 ± 0.01^{ab}	22,54

Electrical conductivity measurements, which reflect the concentrations of minerals, organic acids, and proteins, ranged from 0.23 ± 0.01 ms/cm to 0.80 ± 0.01 ms/cm. These values are consistent with those reported in previous studies (Machado et al., 2022; Homrani et al., 2020; Ghorab et al., 2021; Alves et al., 2013). AV1 demonstrated a significant regional effect($F = 164.70$; $p < 0.001$). The electrical conductivity values of these samples conform to the standards outlined in the Codex Alimentarius (2001), thereby aligning with established international benchmarks.

The degree Brix, quantified as the percentage of dry matter, serves as an indicator of sucrose concentration in honey (Silva et al., 2017). It has been observed that the dry matter content of honey inversely correlates with its water content, evidenced by a strong negative correlation between Brix values and moisture content ($r = -0.997$). Brix values ranged from 78% to 83.80%, aligning with findings reported by Dahmani et al., (2020). AV1 revealed a

significant regional influence on these values ($F = 17.62$; $p < 0.001$). Notably, the Brix values of these samples comply with the standards outlined in the Codex Alimentarius (2001), thereby adhering to established international benchmarks

The antioxidant capacity of the samples was evaluated using DPPH assay, yielding IC50 values ranging from 12.7 mg/ml to 26.52 mg/ml. These findings align well with globally reported data (Al-Farsi et al.,2018); Gül and Pehlivan (2018), highlighting the samples substantial antioxidative potential. This evidence underscores the health-promoting propertiesof honey, further establishing its significance as a natural product.

Conclusion

In conclusion, this investigation into the physicochemical and antioxidant characteristics of honey collected from nine regions in Northern Algeria confirms the high quality of these products. Detailed attention was directed towards a comprehensive analysis of physicochemical properties; however, samples from region H3 were noted to have elevated levels of hydroxymethylfurfural (HMF). There was significant statistical variation in physicochemical parameters across regions. All analyzed parameters conformed to the standards prescribed by the Codex Alimentarius for honey. This study not only reinforces the superior quality of Algerian honey but also emphasizes the profound impact of environmental conditions and handling practices on its health-promoting attributes. These insights are crucial for consumers, producers, and researchers focused on the nutritional and therapeutic values of honey.

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