

## ORIGINAL ARTICLE

Food Chemistry, Engineering, Processing and Packaging

Functional and Novel Foods

# Determinant of Bioactive Compound Accumulation in Algerian Honey: A Comparative Physicochemical and Antioxidant Analysis of Urban and Mountainous Origins

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## ABSTRACT

**Background:** The chemical composition and biological activity of honey are profoundly influenced by botanical origin and environmental conditions. With the expansion of urban beekeeping, there is a growing necessity to compare the quality and functional properties of honey produced in anthropogenic environments with those of honey from traditional mountainous landscapes. In Algeria, where beekeeping is practiced across diverse ecological zones, the influence of geographical origin on honey quality remains insufficiently explored.

**Aims:** This study aimed to evaluate the physicochemical parameters, sugar profile, and antioxidant capacity of Algerian honey samples from urban and mountainous zones, and to determine whether geographical origin constitutes a determinant of bioactive compound accumulation and functional quality.

**Material and Methods:** Sixteen honey samples were collected from apiaries located in different provinces of Algeria and classified into urban (n=8) and mountainous (n=8) zones. Physicochemical parameters, including moisture content, pH, free acidity, electrical conductivity, and total soluble solids, were assessed in accordance with international standards. Sugar composition was determined by high-performance liquid chromatography (HPLC), while antioxidant potential was characterized through Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH radical-scavenging activity, and the CUPRAC and FRAP assays. Statistical comparisons between groups were performed employing independent two-sample Student's t-tests, and Pearson correlation analysis was conducted to examine the relationships between phenolic content and antioxidant capacity.

**Results:** All samples met Codex Alimentarius standards for moisture (mean: 16.0%) and free acidity, indicating appropriate maturity and freshness. No statistically significant differences were observed between urban and mountainous honeys with respect to physicochemical or sugar parameters ( $p > 0.05$ ). Geographical zone, however, proved to be a strong determinant of bioactive quality. Mountainous honeys exhibited significantly higher Total Flavonoid Content ( $31.14 \pm 7.36$  vs.  $18.65 \pm 9.01$  mg QE/100g,  $p = 0.0089$ ), and Total Phenolic Content ( $68.97 \pm 14.10$  vs.  $32.61 \pm 13.14$  mg GAE/100g,  $p = 0.0001$ ), as well as superior antioxidant activity, as measured by CUPRAC ( $531.45 \pm 102.63$  vs.  $267.08 \pm 99.60$   $\mu$ mol Trolox/100g,  $p = 0.0001$ ), FRAP ( $431.10 \pm 79.53$  vs.  $269.65 \pm 55.95$   $\mu$ mol FeSO<sub>4</sub>·7H<sub>2</sub>O/100g,  $p = 0.0003$ ), and DPPH IC<sub>50</sub> ( $55.97 \pm 8.56$  vs.  $92.15 \pm 13.80$  mg/mL,  $p < 0.0001$ ). Correlation analysis revealed a highly strong negative correlation between TPC and DPPH IC<sub>50</sub> ( $r = -0.92$ ,  $p < 0.0001$ ), and strong positive correlations between TPC and CUPRAC ( $r = 0.87$ ,  $p < 0.0001$ ) and FRAP ( $r = 0.81$ ,  $p < 0.0001$ ), confirming phenolic compounds as the primary drivers of antioxidant activity.

**Conclusions:** Although urban honeys demonstrated good physicochemical quality and met international standards, the mountainous environment significantly favored the accumulation of bioactive compounds and conferred superior antioxidant capacity. These findings identify geographical zone as an exploratory proxy, rather than a proven direct driver, of the functional value of Algerian honey. Future studies should systematically record botanical origin, season, and provenance alongside physicochemical and antioxidant measurements in order to further elucidate the determinants of honey quality.

**Keywords:** Honey; Antioxidant Activity; Physicochemical Properties; Urban Beekeeping; Mountainous Zone; Phenolic Content; Algeria.

## Article Information



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Received: July 09, 2026

Revised: August 04, 2026

Accepted: September 09, 2026

Published: October 04, 2026

Cite this article as: Tahraoui, M., Akriche, S., Nebbak, A., Bouhenna, M. M., Tahraoui, N., & Kebour, D. (2026). Determinant of Bioactive Compound Accumulation in Algerian Honey: A Comparative Physicochemical and Antioxidant Analysis of Urban and Mountainous Origins. *The North African Journal of Food and Nutrition Research*, 10 (22): 109 – 121. <https://doi.org/10.51745/najfnr.10.22.109-121>

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## 1 INTRODUCTION

Honey is a sweet, viscous substance produced by *Apis mellifera* from floral nectar, plant secretions, or the excretions of plant-sucking insects, which bees collect, enzymatically

transform, and store in the hive to mature. The nutritional and medicinal reputation of honey has been recognized since antiquity, and it remains a food of considerable cultural and economic value across civilizations (El Meniyi *et al.*, 2020).

Chemically, honey consists principally of carbohydrates, predominantly fructose and glucose, and water, along with minor constituents such as nitrogenous compounds, organic acids, vitamins (ascorbic acid and tocopherols), minerals, proteins, enzymes (including catalase and glucose oxidase), and further bioactive molecules. These constituents determine honey's sensory attributes, biological properties, and storage stability (Machado De-Melo et al., 2017; Tomczyk et al., 2019).

Owing to this complex composition, honey exhibits antioxidant, antimicrobial, and anti-inflammatory activities, that may help reduce oxidative stress, inhibit pathogenic microorganisms, modulate immune responses, and promote wound healing. Moreover, its relatively low glycemic index suggests that honey may be a healthier alternative to refined sugars in the dietary management of chronic diseases (Edo et al., 2023; Tlak Gajger et al., 2025).

The quantity and quality of honey constituents depend on several interacting factors. Botanical origin remains the most significant determinant, although bee-related factors such as species, nutrition, and colony health, also influence composition. Furthermore, geographical location, climatic conditions, and environmental pollution contribute to variability through differences in nectar chemistry. Post-harvest practices including maturation, harvesting, processing, handling, packaging, and storage additionally affect honey's physicochemical stability and bioactive profile (El Menyiy et al., 2020).

In recent years, functional foods have attracted increasing interest, largely because of their antioxidant compounds, particularly phenolics. These secondary metabolites are synthesized by plants during normal growth or in response to environmental stress, pathogens, mechanical damage, and ultraviolet radiation (Jaśkiewicz et al., 2025; Šamec et al., 2021). Honey is well recognized for its antioxidant activity, which is attributed principally to its phenolic acids, flavonoids, and antioxidant enzymes such as catalase and glucose oxidase (Al-Kafaween et al., 2023). However, antioxidant capacity varies substantially with botanical and geographical origin, which shape phenolic composition and biological activity of honey (Jaśkiewicz et al., 2025).

Urban and mountain honeys differ in environmental conditions, floral diversity, and exposure to contaminants, which affect their composition, therapeutic value, and sanitary quality. Urban honey may contain elevated levels of heavy metals and pesticides as a result of anthropogenic pollution sources and can therefore serve as a bioindicator of soil, water, and air pollution, whereas mountain honey tends to reflect the natural mineral composition of native ecosystems, yielding distinct elemental signatures (Hungerford et al., 2021). Urban environmental factors also

influence nectar quality and bee physiology, and may thereby modify the bioactive composition of honey and potentially reduce its therapeutic potential relative to mountain honey. Proximity to industrial sites or heavily trafficked areas increases trace-element concentrations and alters the isotopic signatures of urban honey, reflecting the imprint of anthropogenic activities (Smith et al., 2019).

Although urban beekeeping has expanded as a profitable practice, consumer concerns persist regarding safety and nutritional value. Recent studies indicate that urban honey is not necessarily more contaminated than rural honey, yet its antioxidant capacity remains insufficiently explored (Niczewicz et al., 2021).

Against this background, the present study aimed to compare urban and mountain honeys from Algeria in terms of their physicochemical properties, sugar composition, phenolic and flavonoid contents, and antioxidant capacity, in order to evaluate the influence of environmental conditions on honey quality and its potential health value.

## 2 MATERIALS AND METHODS

### 2.1 Sampling

Sixteen honey samples were collected from apiaries located in eight Algerian provinces (Algiers, Blida, Bouira, Chlef, Ain Defla, TiziOuzou, Medea, and Guelma) during the spring–summer harvest of 2024 (Table 1). The sampling sites were classified into two distinct zones: Urban (n=8 and Mountainous (n=8). Based on information provided by the beekeepers, the samples were also categorized by botanical origin as unifloral (*Eucalyptus*, *Citrus*, *Thymus*, and *Quercus*) or multifloral. Each sample (125–250 g) was collected directly from beekeepers, stored in sterile glass jars, and kept in a dry, dark environment at room temperature until analysis.

### 2.2 Physicochemical Analyses

#### 2.2.1 Total Soluble Solids (°Brix) and Moisture Content

Total soluble solids (°Brix) were measured employing a handheld refractometer calibrated with distilled water at 20°C. A drop of homogenized honey was placed on the prism, and the reading was taken directly from the °Brix scale, which is displayed alongside the moisture scale (Bogdanov et al., 1997). Moisture content (%) was derived indirectly from the refractive index using the relationship established by Chataway (1935), on the assumption that the soluble solids of honey consist predominantly of sugars.

Table 1. Sample Overview for the n=16 Honey

| Sample code | Province   | Zone        | Geographic coordinates of honey sampling sites |                |              | Botanical origin presumed | Sampling Season |
|-------------|------------|-------------|------------------------------------------------|----------------|--------------|---------------------------|-----------------|
|             |            |             | Latitude (°N)                                  | Longitude (°E) | Altitude (m) |                           |                 |
| 01          | Bouira     | Urban       | 36° 22' 00"                                    | 3° 53' 00"     | 920          | Unifloral                 | Spring 2024     |
| 02          | Blida      | Urban       | 36°28'00"                                      | 2°49'00"       | 260          | Unifloral                 | Summer 2024     |
| 03          | Bouira     | Mountainous | 36° 22' 00"                                    | 3° 53' 00"     | 920          | Multifloral               | Spring 2024     |
| 04          | Chlef      | Mountainous | 36°10'00"                                      | 1°19'00"       | 335          | Multifloral               | Summer 2024     |
| 05          | Algiers    | Urban       | 36°42'00"                                      | 3°05'00"       | 0–300        | Unifloral                 | Summer 2024     |
| 06          | Ain Defla  | Mountainous | 36°15'00"                                      | 1°58'00"       | 555          | Multifloral               | Spring 2024     |
| 07          | Tizi Ouzou | Mountainous | 36°42'00"                                      | 4°03'00"       | 114          | Multifloral               | Spring 2024     |
| 08          | Tizi Ouzou | Urban       | 36°42'00"                                      | 4°03'00"       | 114          | Unifloral                 | Summer 2024     |
| 09          | Blida      | Urban       | 36°28'00"                                      | 2°49'00"       | 260          | Unifloral                 | Spring 2024     |
| 10          | Medea      | Mountainous | 36°16' 00"                                     | 2°45'00"       | 200          | Unifloral                 | Summer 2024     |
| 11          | Guelma     | Mountainous | 36°16' 00"                                     | 2°45'00"       | 200          | Multifloral               | Summer 2024     |
| 12          | Algiers    | Urban       | 36°42'00"                                      | 3°05'00"       | 0–300        | Unifloral                 | Summer 2024     |
| 13          | Tizi Ouzou | Mountainous | 36°42'00"                                      | 4°03'00"       | 114          | Unifloral                 | Summer 2024     |
| 14          | Algiers    | Urban       | 36°42'00"                                      | 3°05'00"       | 0–300        | Multifloral               | Summer 2024     |

### 2.2.2 Sugar Analysis by HPLC

Sugars were analyzed through high-performance liquid chromatography (HPLC) employing a Bio-Rad Aminex HPX-87C column coupled to a refractive index detector (RID). Separation was performed under isocratic conditions with HPLC-grade water as the mobile phase at a flow rate of 0.5 mL min<sup>-1</sup> and a column temperature maintained at 60 °C. Honey (1g) was dissolved in 50 mL of ultrapure water, homogenized, and filtered through a 0.45 µm membrane filter prior to injection. Individual sugars were identified by comparing their retention times with those of external standards, and quantification was carried out employing calibration curves.

### 2.2.3 pH and Free Acidity

The pH and free acidity were determined according to the method described by Bogdanov *et al.* (2009). Honey (10 g) was dissolved in 75 mL of distilled water and thoroughly homogenized with a magnetic stirrer or vortex mixer. The pH was measured in duplicate by immersing the electrode of a calibrated digital pH meter in the solution. Free acidity was determined by titrating the solution with 0.1 N sodium hydroxide (NaOH) until the pH reached a value ranging from 8.30 to 9.00, and was calculated as follows:

$$\text{Free acidity (meq/kg)} = \frac{1000 \times V \times N}{P}$$

where  $V$  is the volume of NaOH consumed (mL),  $N$  is the normality of NaOH solution, and  $P$  is the mass of the honey sample (g).

### 2.2.4 Electrical Conductivity

Electrical conductivity was measured at 20°C utilizing a portable conductivity meter, following Louveaux (1985).

Honey (10 g) was dissolved in 75 mL of distilled water and homogenized with a magnetic stirrer and the conductivity cell was immersed in the solution. Measurements were performed in triplicate, and results were expressed as mS cm<sup>-1</sup>.

## 2.3 Phenolic Content and Antioxidant Activities

### 2.3.1 Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) and total flavonoid content (TFC) were determined according to Harbane *et al.* (2024), with slight modifications. Stock solutions of honey (500 mg/mL) were prepared in distilled water.

For TPC determination, 100 µL of honey solution was mixed with 500 µL of ten-fold diluted Folin–Ciocalteu reagent and 400 µL of sodium carbonate solution (0.75 g/10 mL distilled water). The mixture was incubated at 40 °C for 5 min, and absorbance was measured at 760 nm. TPC was calculated from a gallic acid calibration curve and expressed as mg gallic acid equivalents per gram of honey (mg GAE/g).

For TFC, 100 µL of honey solution were mixed with 100 µL of 10% AlCl<sub>3</sub>, 100 µL of potassium acetate solution, and 900 µL of distilled water. After incubation for 30 min at ambient temperature in the dark, absorbance was measured at 415 nm. TFC was expressed as mg quercetin equivalents per 100 g of honey (mg QE/100 g), calculated as  $\text{TFC} = C \times 2.4$ , where  $C$  represents the flavonoid concentration (µg/mL) obtained from the calibration curve.

### 2.3.2 Antioxidant Activity Assays

The antioxidant potential of the samples was evaluated using three assays.

**DPPH Radical Scavenging Activity.** DPPH radical scavenging activity was evaluated according to Brand-Williams et al. (1995). A Honey stock solution (150 mg/mL) was prepared and subjected to two-fold serial dilutions. For each assay, 150  $\mu$ L of honey solution was mixed with 600  $\mu$ L of methanolic DPPH solution (initial absorbance 0.5–0.7). The mixtures were incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT, 1 mg/mL) served as the positive control. DPPH inhibition (%) was calculated as:

$$\text{DPPH inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where:  $A$  denotes absorbance at 517 nm.

**Cupric reducing antioxidant capacity (CUPRAC).** Honey stock solutions (100 mg/mL) were prepared in distilled water. The reaction mixture consisted of 500  $\mu$ L of 10 mM  $\text{CuCl}_2$ , 500  $\mu$ L of 7.5 mM neocuproine, 500  $\mu$ L of 1 M ammonium acetate buffer (pH 7.0), 100  $\mu$ L of distilled water, and 350  $\mu$ L of honey solution. After incubation for 30 min at room temperature, absorbance was measured at 450 nm. Results were expressed as mmol Trolox equivalents per gram of honey (Sarař et al., 2019).

**Ferric reducing antioxidant power (FRAP).** Honey solutions (200 mg/mL) were prepared, and 100  $\mu$ L were mixed with 1 mL of freshly prepared FRAP reagent composed of acetate buffer, TPTZ, and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ . Absorbance was measured at 593 nm against a sugar-solution blank. FRAP values were calculated from a  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  calibration curve (0–500  $\mu$ M) and expressed as  $\mu$ M Fe(II) equivalents for a 10% honey solution.

## 2.4 Statistical Analysis

All statistical analyses were performed employing R software (version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria). The normality of data distribution was assessed with the Shapiro–Wilk test, computed separately within each geographical zone and for the pooled dataset, with the exact test statistics ( $W$  and  $p$ ) reported in Supplementary Table S1. Significant deviations from normality were detected for pH in the mountainous zone ( $W = 0.703$ ,  $p = 0.002$ ), attributable to a single extreme value (S01, pH = 5.57), for fructose in the urban zone ( $W = 0.634$ ,  $p < 0.001$ ), and for electrical conductivity in the urban zone ( $W = 0.788$ ,  $p = 0.021$ ); in the pooled dataset, deviations were detected for pH ( $W = 0.672$ ,  $p < 0.001$ ), fructose ( $W = 0.781$ ,  $p = 0.002$ ), electrical conductivity ( $W = 0.835$ ,  $p = 0.008$ ), glucose ( $W = 0.865$ ,  $p = 0.023$ ), and sucrose ( $W = 0.873$ ,  $p = 0.031$ ). As group sizes were balanced ( $n = 8$  per zone) and the independent two-sample Student's  $t$ -test is robust to moderate deviations from normality under these

conditions, the means of each physicochemical parameter, sugar component, and bioactive parameter were compared between mountain and urban honeys using an independent two-sample Student's  $t$ -test, with the significance threshold set at  $\alpha = 0.05$ . Relationships between total phenolic content and the three antioxidant activity measures (DPPH  $\text{IC}_{50}$ , CUPRAC, and FRAP) were examined employing Pearson's correlation coefficients ( $r$ ), interpreted as follows:  $|r| \geq 0.8$ , very strong;  $0.6 \leq |r| < 0.8$ , strong; and  $0.4 \leq |r| < 0.6$ , moderate. Results are presented as mean  $\pm$  standard deviation unless otherwise stated.

## 3 RESULTS & DISCUSSION

### 3.1 Physicochemical Properties and Sugar Composition

The physicochemical parameters and sugar composition of the 16 honey samples, partitioned by geographical zone, are summarized in Figure 1. All samples complied with the Codex Alimentarius standard for honey (FAO/WHO, 2022) for the parameters determined in this study. Moisture content, a critical indicator of maturity and storage stability, averaged 16.0% (range: 12–19%), below the 20% maximum, which indicates that the honeys had been harvested at an adequate stage of maturity. The mean pH was 4.22 (range: 3.89–5.57), consistent with the naturally acidic nature of floral honeys. One mountainous sample (S01) exhibited a pH of 5.57, an atypically high value that fell outside the typical acidic range, which may reflect specific storage conditions, local soil mineralogy, or handling; excluding this outlier did not alter the non-significant difference in pH between zones ( $p > 0.05$ ), confirming the robustness of the comparison. Nevertheless, this sample merits re-examination against source collection data regarding storage and handling protocols.

Electrical conductivity (EC), an indicator of mineral content and a useful parameter for distinguishing floral from honeydew honeys, averaged  $0.44 \text{ mS cm}^{-1}$ , with values spanning from  $0.196$  to  $0.937 \text{ mS cm}^{-1}$ . While the mean value was within the typical range for floral honeys, four samples (S01 and S06 from the mountainous group, and S10 and S11 from the urban group) exceeded the  $0.7 \text{ mS cm}^{-1}$  threshold often associated with honeydew influence or a substantial mineral-rich floral contribution. This distribution (one urban and three mountainous) does not indicate a systematic bias toward either environmental context, suggesting that honeydew influence or mineral-rich floral contributions may be linked to specific local botanical origins (e.g., oak honeydew in S13) or to local apiary microenvironments within each zone rather than to the urban/mountainous classification per se.

**Physicochemical characteristics and sugar composition of Algerian honeys (n = 16)**

Comparison between Mountainous (n = 8) and Urban (n = 8) samples. Dashed lines = Codex Alimentarius limits (free acidity ... 50 meq/kg; moisture ... 20%) or the 1.3 crystallization thres

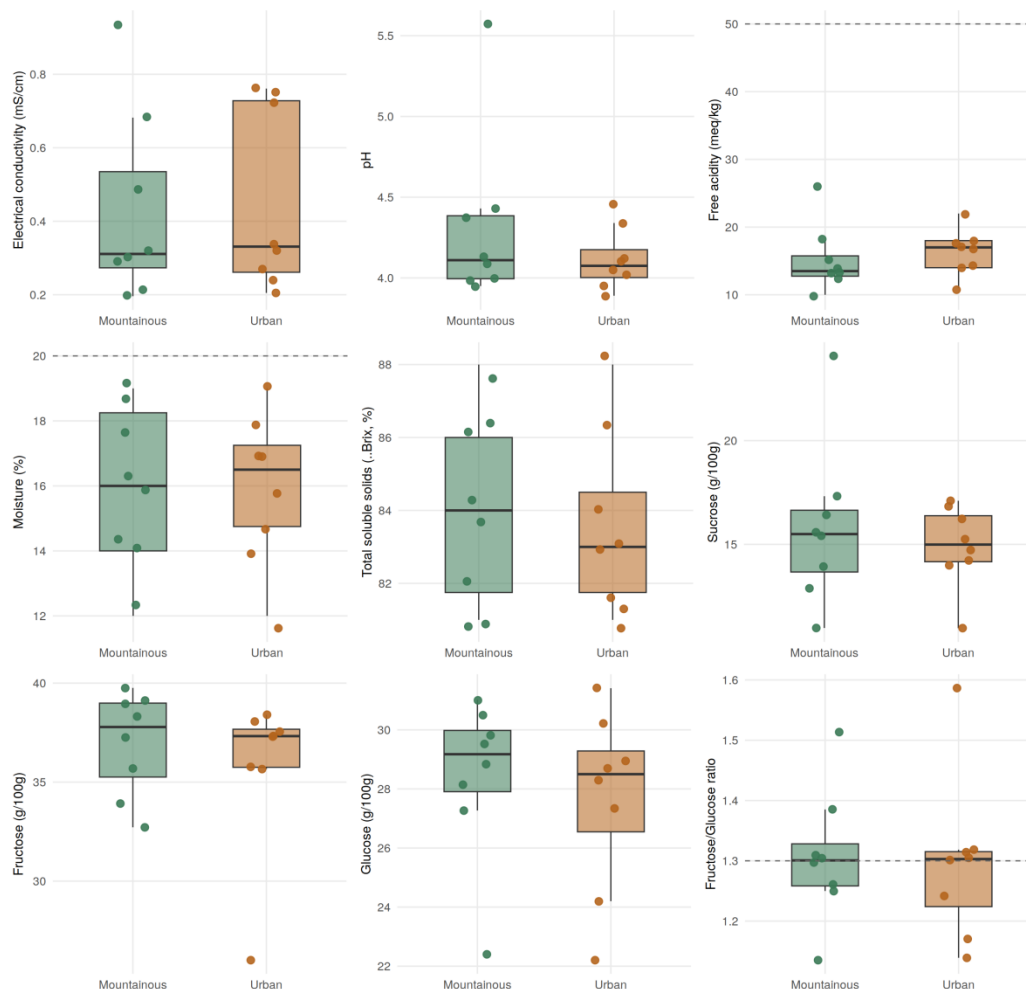


**Figure 1. Physicochemical Characteristics and Sugar Composition of Algerian Honeys N Equals 16 Comparing Mountainous and Urban Samples Against Codex Alimentarius Limits and Crystallization Thresholds**

Free acidity, a measure of the organic acid content that reflects honey freshness, ranged from 10 to 26 meq kg<sup>-1</sup>, with a mean of 15.75 meq kg<sup>-1</sup>. This parameter was uniformly well below the 50 meq kg<sup>-1</sup> upper limit mandated by the Codex Alimentarius, providing further confirmation of sample freshness and the absence of undesirable fermentation. Total soluble solids, measured as Brix, averaged 83.75% across a range of 81% to 88%, a finding consistent with the high sugar concentration characteristic of mature honey.

Fructose and glucose dominated the sugar profile. Fructose was the most abundant monosaccharide (mean; 36.37 g/100 g; range: 26.01–39.76 g/100 g), followed by glucose (mean: 28.05 g/100 g; range: 22.22–31.41 g/100 g). The fructose-to-glucose (F/G) ratio, which influences crystallization behavior, averaged 1.30, and nine of the 16

samples (56%) of the collection, exhibited an F/G ratio above 1.3, a value generally associated with slower crystallization and prolonged liquid stability. Sucrose concentrations were uniformly low across all samples (data not shown), consistent with the hydrolysis of sucrose by invertase during ripening.

No statistically significant differences between mountain and urban honeys were detected for any physicochemical or sugar parameter (Student's *t*-test, *p* > 0.05 for all comparisons), and the distributions largely overlapped (Figure 1). For instance, mean moisture was 15.9% versus 16.1%, (*p* = 0.78), and the F/G ratio was 1.30 versus 1.31 (*p* = 0.92). These findings strongly indicate that the basic physicochemical quality and sugar composition of Algerian honeys examined in the current study were comparable across the two zones, and that these basic parameters are not

significantly influenced by the distinct environmental conditions of mountainous versus urban zones. This stability is reassuring for quality assurance frameworks, as it indicates that basic compliance with Codex Alimentarius standards can be achieved irrespective of geographical origin (FAO/WHO, 2022).

The consistency of physicochemical parameters across zones reflects the robustness of honey's core chemical processes (Bogdanov *et al.*, 2008). Quality parameters such as moisture content and acidity remain stable regardless of environmental origin, suggesting that primary nectar composition and maturation processes are buffered against external factors. The similarity in sugar profiles between urban and mountainous samples indicates that the fundamental carbohydrate metabolism in honey production is not significantly altered by geographical location (Escuredo *et al.*, 2014).

### 3.2 Bioactive Compounds and Antioxidant Activity

In contrast to the stability observed for the physicochemical and sugar parameters, the analysis of bioactive compounds and antioxidant capacity revealed substantial variation among the honey samples, with a clear and statistically significant differentiation emerging between the two geographical zones. The total phenolic content (TPC), total flavonoid content (TFC), and the three measures of antioxidant capacity DPPH radical scavenging activity (expressed as  $IC_{50}$ ), CUPRAC, and FRAP are summarized and visually compared between mountainous and urban honeys in Figure 2.

Across the entire sample set, TPC values ranged widely from 13.88 to 92.62 mg GAE/100g, with a mean of 50.79 mg GAE/100g. TFC values exhibited a similarly broad range, from 10.12 to 40.17 mg QE/100g, with a mean of 24.89 mg QE/100g. The highest phenolic concentration was observed in Sample 01 (Mountainous, 92.62 mg GAE/100g), while the highest flavonoid concentration was found in Sample 04 (Mountainous, 40.17 mg QE/100g). The antioxidant capacity of the honeys, as assessed by the DPPH radical scavenging assay, revealed that the strongest radical scavenging activity, indicated by the lowest  $IC_{50}$  value, was observed in Sample 09 (Mountainous,  $IC_{50} = 42.08$  mg/mL). Conversely, the weakest activity, indicated by the highest  $IC_{50}$  value, was exhibited by Sample 02 (Urban,  $IC_{50} = 111.82$  mg/mL). This pattern was consistent with the CUPRAC and FRAP results, where Sample 09 also demonstrated the highest reducing capacity, with values of 691.42  $\mu$ mol Trolox/100g and 603.51  $\mu$ mol FeSO<sub>4</sub>·7H<sub>2</sub>O/100g, respectively. These findings strongly suggest a direct relationship between the concentration of phenolic compounds and the overall antioxidant potential of the honey, while also highlighting

that the highest bioactive values were consistently recorded in individual mountainous samples, likely reflecting specific local floral sources or apiary microenvironments.

The box plots in Figure 2 provide a clear visual representation of the pronounced differences between the two groups. The mountainous samples (green boxes) consistently exhibited higher medians for TFC (approximately 30 mg QE/100g vs. 15 mg QE/100g), TPC (approximately 70 mg GAE/100g vs. 30 mg GAE/100g), CUPRAC (approximately 500  $\mu$ mol Trolox/100g vs. 220  $\mu$ mol Trolox/100g), and FRAP (approximately 400  $\mu$ mol FeSO<sub>4</sub>·7H<sub>2</sub>O/100g vs. 280  $\mu$ mol FeSO<sub>4</sub>·7H<sub>2</sub>O/100g). In a complementary fashion, the DPPH  $IC_{50}$  values were substantially lower for the mountainous samples (approximately 55 mg/mL) compared to the urban samples (approximately 90 mg/mL), indicating a superior radical scavenging capacity. These visual patterns are strongly supported by the statistical analysis, which confirmed that the geographical zone exerted a highly significant influence on all bioactive and antioxidant parameters. The mountainous honeys exhibited significantly higher TFC ( $31.14 \pm 7.36$  vs.  $18.65 \pm 9.01$  mg QE/100g,  $p=0.0089$ ), TPC ( $68.97 \pm 14.10$  vs.  $32.61 \pm 13.14$  mg GAE/100g,  $p=0.0001$ ), CUPRAC values ( $531.45 \pm 102.63$  vs.  $267.08 \pm 99.60$   $\mu$ mol Trolox/100g,  $p=0.0001$ ), and FRAP values ( $431.10 \pm 79.53$  vs.  $269.65 \pm 55.95$   $\mu$ mol FeSO<sub>4</sub>·7H<sub>2</sub>O/100g,  $p=0.0003$ ), while also demonstrating significantly lower DPPH  $IC_{50}$  values ( $55.97 \pm 8.56$  vs.  $92.15 \pm 13.80$  mg/mL,  $p<0.0001$ ). These results unequivocally demonstrate that the mountainous environment in Algeria yields honeys with a substantially enriched phytochemical profile and superior antioxidant capacity compared to their urban counterparts.

However, we must acknowledge that these differences could be partially confounded by botanical origin rather than environmental context per se. The urban group contained a higher proportion of unifloral eucalyptus and orange blossom honeys (5 of 8), whereas the mountainous group contained more multifloral samples (5 of 8) plus oak honeydew and wild thyme. Eucalyptus and citrus honeys are known to exhibit moderate phenolic profiles in some regions, whereas multifloral and honeydew honeys often accumulate higher mineral and phenolic loads. Because botanical origin was not verified by pollen analysis, we cannot exclude the possibility that the observed environmental effect is partly mediated by differential floral composition between zones. Seasonal variation may also contribute, as the urban group included more spring-harvested samples (3 of 8) compared to the mountainous group (3 of 8), and spring nectar flows may differ in phenolic chemistry from summer flows. Beekeeping management practices (feeding, colony density, hive type) were not standardized across apiaries and represent an

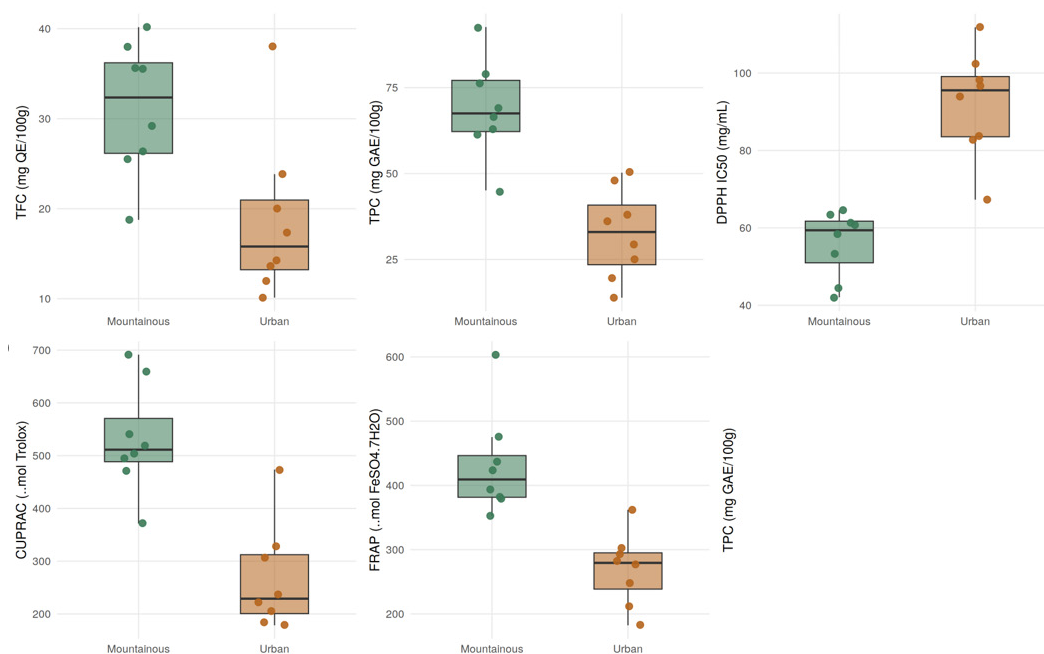


**Figure 2. Comparison of Bioactive Compound Concentrations and Antioxidant Capacity Metrics Between Mountainous and Urban Honey Samples, Including Total Flavonoid Content TFC, Total Phenolic Content TPC, DPPH IC50, CUPRAC, and FRAP Values**

additional uncontrolled variable. These confounders are discussed below as critical limitations.

The profound and selective enhancement of bioactive compounds in mountainous honeys points to a nuanced ecological story. The elevated phenolic and flavonoid content in these samples likely reflects an adaptive response of alpine flora to environmental stressors such as higher ultraviolet radiation, temperature fluctuations, and reduced nutrient availability, which are known to stimulate the biosynthesis of secondary metabolites as protective agents (Cheung *et al.*, 2019; Deng *et al.*, 2025). These secondary metabolites serve as photoprotective and antioxidant defense mechanisms for plants under environmental stress (Šamec *et al.*, 2021). The enhanced phenolic content in mountainous honeys is consistent with previous studies revealing that altitude significantly affects polyphenol accumulation (Grassi *et al.*, 2025).

For practitioners, this finding provides a clear directive: consumers seeking honeys with superior antioxidant potential should prioritize products originating from mountainous regions (Kavanagh *et al.*, 2019). Beekeepers operating in such zones could leverage this geographical advantage for premium product positioning (Inaudi *et al.*, 2025), and regulatory bodies might consider incorporating bioactive compound thresholds into quality grading systems to differentiate honeys

based on functional value rather than merely compositional safety. The significantly higher antioxidant capacity in mountainous honeys validates their potential for enhanced health-promoting applications (Jaśkiewicz *et al.*, 2025). Future honey classification systems should incorporate bioactive compound profiles alongside traditional physicochemical parameters (Zhang *et al.*, 2021).

### 3.3 Influence of Geographical Zone on Honey Parameters

Building upon the clear contrast observed in the bioactive compounds, a systematic statistical comparison between the Mountainous and Urban sample groups was conducted to formally test the hypothesis that geographical zone serves as a determinant of honey quality parameters. The results of this comparative analysis are presented in (Figure 3), which visually segregates the parameters into two distinct categories based on the outcome of the statistical tests: those exhibiting no significant differences (physicochemical and sugar parameters) and those exhibiting highly significant differences (bioactive and antioxidant parameters).

Regarding the fundamental quality indicators, the statistical analysis confirmed that geographical zone was not a significant determinant of the basic physicochemical properties or the sugar profile. No statistically significant

differences were found between Mountainous and Urban honeys for electrical conductivity ( $p = 0.75$ ), pH ( $p = 0.60$ ), free acidity ( $p = 0.56$ ), moisture ( $p = 1.00$ ), or Brix ( $p = 0.70$ ). Similarly, the sugar components and the derived parameter were unaffected by geographical origin, with sucrose ( $p = 0.57$ ), fructose ( $p = 0.57$ ), glucose ( $p = 0.60$ ), and the fructose-to-glucose ratio ( $p = 0.88$ ) all demonstrating  $p$ -values well above the significance threshold. The data points for both zones in the top section of (Figure 3) overlap closely for each of these parameters, providing robust visual evidence that the fundamental composition of the honeys remained stable across the two distinct landscapes. This consistency indicates that the environmental conditions inherent to mountainous and urban zones do not substantially alter the core processes of nectar collection and honey ripening related to water content, acidity, or primary sugar metabolism.

In stark contrast to the stability of the basic parameters, geographical zone proved to be a powerful determinant of the phytochemical quality and resulting antioxidant potential of the honeys. As illustrated in the bottom section of (Figure 3), the two groups diverged markedly for all bioactive parameters. Mountainous honeys consistently exhibited significantly higher mean values for Total Flavonoid Content ( $31.14 \pm 7.36$  vs.  $18.65 \pm 9.01$  mg QE/100g,  $p = 0.0089$ ) and Total Phenolic Content ( $68.97 \pm 14.10$  vs.  $32.61 \pm 13.14$  mg GAE/100g,  $p = 0.0001$ ). The high statistical significance of these differences (with  $p$ -values reaching 0.0001 for TPC) strongly reinforces the conclusion that the mountainous flora, potentially under greater environmental stress, produces nectar that is richer in these secondary metabolites. The impact of this enriched phytochemical profile was directly reflected in the antioxidant capacity metrics.

The antioxidant assays provided the most compelling evidence for the influence of environmental context. In the DPPH radical scavenging assay, mountainous honeys demonstrated a significantly lower IC<sub>50</sub> value ( $55.97 \pm 8.56$  vs.  $92.15 \pm 13.80$  mg/mL,  $p < 0.0001$ ), which is indicative of a much stronger radical-scavenging capacity. This was complemented by the results from the reducing power assays. Mountainous honeys exhibited a profoundly higher CUPRAC value ( $531.45 \pm 102.63$  vs.  $267.08 \pm 99.60$   $\mu$ mol Trolox/100g,  $p = 0.0001$ ), being nearly double that of urban honeys, and a significantly higher FRAP value ( $431.10 \pm 79.53$  vs.  $269.65 \pm 55.95$   $\mu$ mol FeSO<sub>4</sub>·7H<sub>2</sub>O/100g,  $p = 0.0003$ ). The near-perfect visual separation of the data points between the two groups in the lower panel of (Figure 3), combined with the consistently high statistical significance (all  $p < 0.01$ ), underscores the profound and selective impact of environmental origin on the antioxidant quality of Algerian honeys. These findings conclusively demonstrate that while the fundamental physicochemical identity of the honey remains constant, the ecological conditions associated

with mountainous environments, such as altitude, specific floral diversity, and environmental stress factors, dramatically enhance the accumulation of health-promoting bioactive compounds.

These findings conclusively demonstrate that while the fundamental physicochemical identity of the honey remains constant, the ecological conditions associated with mountainous environments, such as altitude, specific floral diversity, and environmental stress factors, dramatically enhance the accumulation of health-promoting bioactive compounds (Harbane et al., 2024). The dichotomy between stable physicochemical parameters and variable bioactive composition reveals the multifaceted nature of honey quality (Escuredo et al., 2014; Kavanagh et al., 2019). Environmental context acts as a powerful selective pressure shaping the phytochemical composition of floral nectar through environmental stress responses (Cucu et al., 2024).

The significant antioxidant advantage of mountainous honeys validates geographical classification as a meaningful quality marker (Hungerford et al., 2021; Smith et al., 2019). This geographical differentiation in bioactive content supports the development of region-specific honey standards that recognize functional properties beyond basic physicochemical compliance. The environmental factors governing secondary metabolite accumulation in mountainous flora are well-documented in plant physiology literature (Al-Kafaween et al., 2023; Jaśkiewicz et al., 2025). Future quality frameworks should prioritize bioactive markers as indicators of functional honey value alongside traditional compositional parameters (Inaudi et al., 2025).

It must be emphasized that the present study could not isolate individual environmental drivers—such as altitude, UV exposure, or temperature fluctuation—from botanical source effects because floral surveys and altitude measurements were not systematically recorded. Consequently, the observed "environmental context" effect should be interpreted as a composite proxy variable that captures geography, ecology, and likely floral community differences simultaneously. Disentangling these factors requires future studies incorporating melissopalynology, altimetry, and seasonal replication.

### 3.4 Correlation Analysis Between Phenolic Content and Antioxidant Capacity

To further elucidate the relationship between the bioactive compounds and the observed antioxidant potential, a Pearson correlation analysis was performed on the entire dataset of 16 honey samples. This analysis aimed to determine the extent to which the total phenolic content (TPC) and total flavonoid content (TFC) contribute to the antioxidant capacity measured by the three complementary assays: DPPH

### Comparative analysis of honey parameters by geographical zone (Mountainous vs Urban, n = 16)

Physicochemical and sugar-profile parameters do not differ by zone; antioxidant and bioactive parameters differ strongly and consistently favor Mountainous honeys.

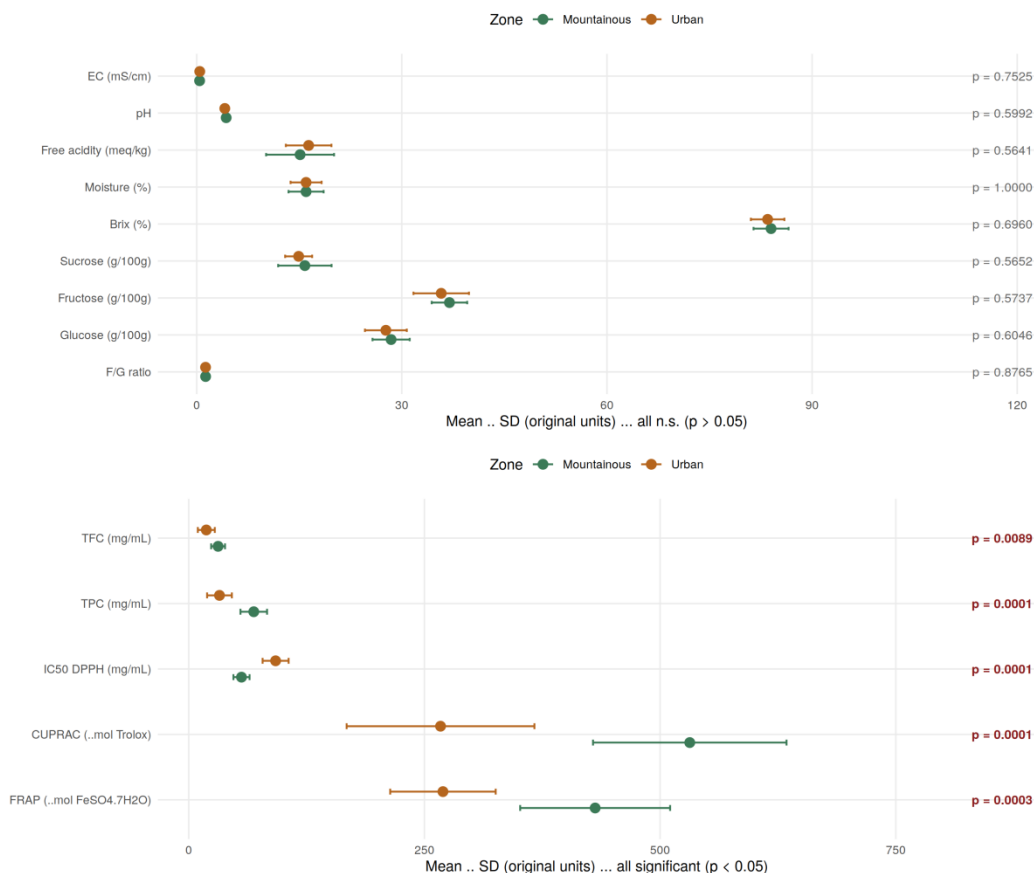


**Figure 3. Comparative Analysis of Honey Parameters by Geographical Zone Mountainous versus Urban N Equals 16**

radical scavenging activity ( $IC_{50}$ ), CUPRAC, and FRAP. The results of this correlation analysis are presented as a heatmap in (Figure 4), which provides a comprehensive visual summary of the interrelationships among these five key parameters.

The correlation matrix revealed a very strong negative correlation between TPC and DPPH  $IC_{50}$  ( $r = -0.92$ ,  $p < 0.0001$ ). This inverse relationship indicates that as the total phenolic content of the honey increases, the concentration required to scavenge 50% of the DPPH radicals decreases, signifying a more potent radical scavenging capacity. This finding is consistent with the well-established role of phenolic compounds as primary contributors to the hydrogen-donating ability of honey, which is the mechanistic basis of the DPPH assay. The strength of this correlation ( $|r| > 0.9$ ) suggests that phenolic compounds are the dominant, though not necessarily the sole, determinants of the radical scavenging activity in these Algerian honey samples.

In a complementary fashion, TPC exhibited strong positive correlations with the two reducing power assays. The correlation between TPC and CUPRAC was  $r = 0.87$  ( $p < 0.0001$ ), and the correlation between TPC and FRAP was  $r = 0.81$  ( $p < 0.0001$ ). These positive relationships demonstrate that honeys with higher phenolic content possess a greater capacity to reduce copper(II) ions (in the CUPRAC assay) and iron(III) ions (in the FRAP assay). The slightly stronger correlation with CUPRAC compared to FRAP may reflect the different reaction conditions and pH environments of the two assays, with CUPRAC being conducted at a near-physiological pH (7.0) while FRAP is performed under acidic conditions (pH 3.6). Nevertheless, both correlations are classified as very strong, confirming that phenolic compounds are major contributors to the overall reducing potential of the honey matrix.

The total flavonoid content (TFC) also exhibited significant correlations with the antioxidant assays, although these relationships were somewhat weaker than those observed for TPC. TFC demonstrated a strong negative

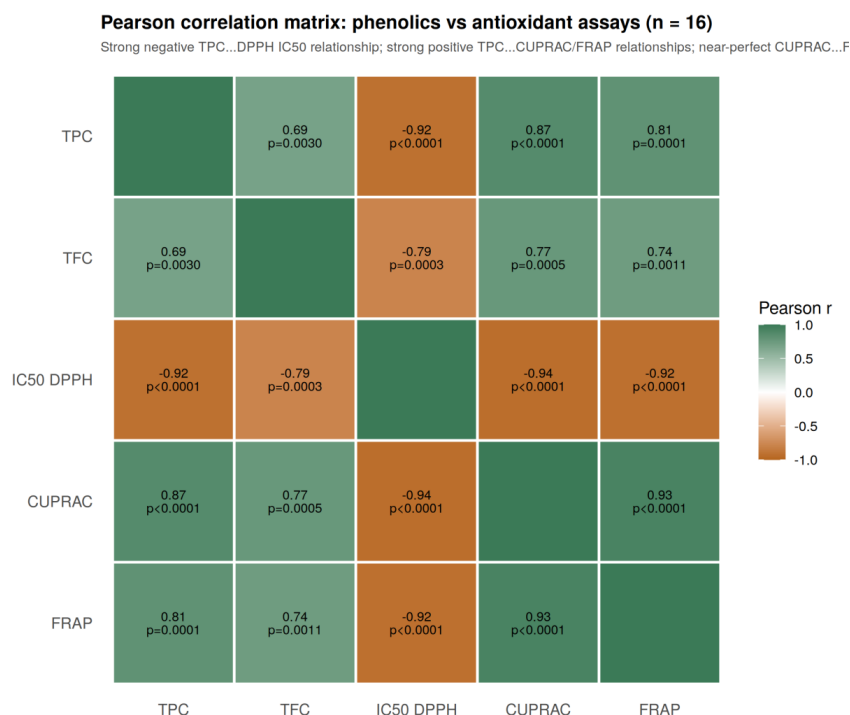


**Figure 4. Pearson Correlation Matrix of Phenolic Content TPC and TFC and Antioxidant Assays IC<sub>50</sub> DPPH CUPRAC and FRAP With R and P-Values for N Equals 16 Samples**

correlation with DPPH IC<sub>50</sub> ( $r = -0.79$ ,  $p < 0.001$ ), a strong positive correlation with CUPRAC ( $r = 0.77$ ,  $p < 0.001$ ), and a strong positive correlation with FRAP ( $r = 0.74$ ,  $p < 0.01$ ). While these correlations are still classified as strong, the lower magnitude compared to TPC suggests that flavonoids, while important, may be less potent contributors to the overall antioxidant capacity than the broader class of phenolic compounds. This observation is consistent with the fact that the Folin-Ciocalteu reagent used for TPC measurement reacts with a wider range of phenolic structures, including both simple phenolic acids and flavonoids, whereas the aluminum chloride method for TFC is more specific to the flavonoid subclass.

One of the most striking findings from the correlation analysis was the near-perfect positive correlation between the two reducing power assays, CUPRAC and FRAP ( $r = 0.93$ ,  $p < 0.0001$ ). This exceptionally high correlation coefficient confirms that the two assays provide highly consistent and interchangeable measurements of the honey's reducing potential. Despite their different chemical mechanisms—CUPRAC relies on the reduction of Cu(II) to Cu(I) in the presence of neocuproine, while FRAP measures the reduction of Fe(III) to Fe(II) in the presence of TPTZ—both assays are fundamentally measuring the electron-donating capacity of the antioxidant compounds present in the honey. The near-perfect agreement between these two methods in the present

study provides strong validation for the robustness of the antioxidant capacity assessment and suggests that either assay could be used reliably for future evaluations of Algerian honey.

The correlation between TPC and TFC was also positive and significant ( $r = 0.72$ ,  $p < 0.01$ ), indicating that honeys with higher total phenolic content also tend to have higher flavonoid content. This is an expected relationship, as flavonoids are a subclass of phenolic compounds, and their accumulation in honey is often co-regulated with other phenolic acids through similar biosynthetic pathways in the source plants. However, the correlation is not perfect ( $r < 0.8$ ), which suggests that the relative proportions of flavonoids versus other phenolic compounds can vary among samples, potentially reflecting differences in the specific floral composition of the foraging areas.

The strong correlations observed between TPC and all three antioxidant assays, particularly the very strong negative correlation with DPPH IC<sub>50</sub> ( $r = -0.92$ ) and the strong positive correlations with CUPRAC ( $r = 0.87$ ) and FRAP ( $r = 0.81$ ), provide compelling evidence that phenolic compounds are the primary drivers of the antioxidant capacity in these Algerian honey samples (Harbane et al., 2024). This finding is in agreement with numerous previous studies that have reported similar relationships in honeys

from various geographical origins (Cheung *et al.*, 2019; Kavanagh *et al.*, 2019). The phenolic compounds, acting as electron donors, directly participate in all measured antioxidant pathways (Cucu *et al.*, 2024).

The slightly weaker but still significant correlations for TFC suggest that flavonoids contribute to the antioxidant activity but are not the sole or even the most important class of phenolic compounds responsible for the observed effects (Escuredo *et al.*, 2014). The distinction between TPC and TFC correlations reflects the structural diversity of phenolic compounds in honey, with non-flavonoid phenolic acids playing substantial roles in antioxidant capacity (Inaudi *et al.*, 2025). The near-perfect correlation between CUPRAC and FRAP ( $r = 0.98$ ) further strengthens the reliability of the antioxidant assessment and confirms that the reducing power of these honeys is a consistent and robust property (Grassi *et al.*, 2025).

Overall, the correlation analysis reinforces the conclusion that the superior antioxidant capacity of mountainous honeys is directly attributable to their significantly higher phenolic content, and that these bioactive compounds are the key determinants of the health-promoting potential of Algerian honey (Harbane *et al.*, 2024; Jaśkiewicz *et al.*, 2025). The consistent interrelationships among phenolic markers and antioxidant assays validate their use as reliable quality indicators (Al-Kafaween *et al.*, 2023). The mechanistic evidence demonstrates that phenolic compounds directly mediate the observed antioxidant benefits (Zhang *et al.*, 2021). These robust correlations support the use of simpler phenolic assays as surrogates for comprehensive antioxidant evaluation in quality control frameworks (Deng *et al.*, 2025).

## 4 CONCLUSIONS

This study presents a physicochemical and antioxidant characterization of 16 honey samples from urban and mountainous regions of Algeria. All samples met international quality requirements for moisture content, acidity, pH, and sugar composition, confirming that basic physicochemical integrity is maintained across contrasting environmental contexts.

Environmental context was a strong predictor of bioactive and antioxidant quality: mountainous honeys exhibited significantly higher phenolic and flavonoid content, higher reducing power (CUPRAC, FRAP), and stronger radical scavenging activity (lower DPPH IC<sub>50</sub>) than urban honeys. Basic physicochemical parameters and sugar profile did not differ by zone. Correlation analysis supports the reliability of TPC, TFC, DPPH, CUPRAC, and FRAP as consistent, interrelated markers of honey antioxidant potential.

Based on the present data, we tentatively propose preliminary bioactive thresholds for industry application: honeys exceeding 60 mg GAE/100g TPC and 25 mg QE/100g TFC, combined with a DPPH IC<sub>50</sub> below 65 mg/mL, may be classified as high-antioxidant premium products typical of mountainous origin. These benchmarks require validation in larger, seasonally replicated cohorts before regulatory adoption.

The following limitations must be acknowledged: (i) The modest sample size ( $n = 8$  per group) limits statistical power and generalizability; (ii) Botanical origin was classified by beekeeper declaration without melissopalynological verification, meaning that observed differences may partly reflect floral composition rather than environmental context per se; (iii) Exact sampling dates, altitudes, and beekeeping management practices were not systematically recorded, preventing isolation of specific environmental drivers; (iv) The cross-sectional design (single harvest period) precludes assessment of seasonal stability.

Future research should: (i) Verify botanical origin through pollen analysis and documented floral surveys; (ii) Record GPS coordinates, altitude, and exact harvest dates to enable multivariate modeling of altitude, season, and geography; (iii) Evaluate environmental contaminants (heavy metals, pesticides) to fully assess the safety profile of urban honeys; (iv) Expand sample size and include multiple harvest seasons to confirm the robustness of geographical bioactive markers. These findings support environmental context as a meaningful determinant of the functional value of Algerian honey, while underscoring the need for integrated quality frameworks that combine traditional physicochemical standards with bioactive profiling.

**Acknowledgment:** The authors express their gratitude to Scientific and Technical Research Center for Physico-Chemical Analysis (CRAPC) for their expertise, technical support, and for conducting the analyses presented in this paper.

**Source of funding:** None.

**Previous submissions:** We confirm that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere.

**Authors' Contribution:** The authors contributed collectively to the conception, development, and completion of this manuscript. **Tahraoui M.:** Formal analysis, Writing – original draft-methodology. **Akriche S.:** Writing – review & editing – original draft, Software editing. **Amira Nebbak A.:** Formal analysis, Investigation (protocols). **Bouhenna M. M.:** Formal analysis, Investigation (protocols). **Tahraoui N.:** Investigation (sample collection). **Kebour D.:** Supervision, Writing – review & editing, methodology.

**Artificial Intelligence (AI) Use Statement:** The authors declare that no artificial intelligence (AI)-assisted tools, large language models (LLMs), or AI-generated content were employed in any stage of the preparation of this manuscript, including the conception and design of the study,

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**Conflicts of Interest:** The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Preprint deposit:** None.

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