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Research Article

Evaluation of Polyphenols, Antioxidant Activity, and Mineral Content in Honey Produced by *Apis mellifera* L. According to Floral Origin

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Abstract

Background/Objectives: Honey is in high demand because of its nutritional value, therapeutic properties, and sensory characteristics. Honey varieties were evaluated according to their floral origin and physicochemical composition, and total polyphenols, antioxidants, and minerals were analyzed for conformity and preference. Methods: Seven honey samples were analyzed for physicochemical composition using the Official Methods of Analysis method, total polyphenols using the Folin–Ciocalteu method, flavonoids using the ferric trichloride method, antioxidant activity and inhibitory concentration using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and ferric reducing capacity using the ferric-reducing antioxidant power (FRAP) method. Inductively coupled plasma optical emission spectroscopy was used to determine the mineral composition of the honey varieties. Results: The physicochemical compositions of the honey samples did not show significant differences and complied with national regulations. Total phenolic compound content was 336.9–1064.9 µg EAG/g, whereas flavonoids were between 0–151.9 µg EQ/g, with eucalyptus honey having the highest concentration. The antioxidant capacity measured using DPPH and FRAP was 0.095–0.186 and 0.168–0.654 mM TEAC/g, respectively. Variability was observed in the calcium (161.55 mg/kg in eucalyptus), magnesium (35.20 mg/kg in eucalyptus), potassium (901.17 mg/kg in eucalyptus), and sodium (172.18 mg/kg in pecan aroma) levels. Heavy metals did not exhibit significant values. The correlation between total flavonoid content and antioxidant capacity was weak; a strong correlation was observed between total phenolic compounds and antioxidant activity. Conclusions: The antioxidant activity, bioactive compounds, and minerals in honey vary according to its floral origin, which could be helpful in the pharmaceutical industry and gastronomy.

Keywords

Honey, Antioxidant Activity, Polyphenols, Minerals

1. Introduction

Honey obtained from the nectar of various flowers in the Ica province of Peru is a complex mixture of natural sweet substances. Its nutritional and medicinal properties have been well-appreciated for millennia [1]. Different cultures have used honey as a food and for therapeutic purposes because of its vitamin content, antioxidant capacity, and mineral richness,

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which act as micronutrients [2].

Advanced analytical methods are used to investigate the physicochemical properties of honey collected in various locations in the Ica province. The natural characteristics of the region, such as soil type, climate, and organic farming practices, contribute to obtaining honey varieties of high nutritional quality. The quality of honey from Quito and Imbabura, Ecuador [3] was analyzed, and significant variability was observed in its physicochemical characteristics [4]. The chemical composition of honey highlights its bioactive components and antioxidant properties [5].

Recently, different honey varieties have been investigated to prevent oxidation during storage and as food stabilizers in edible packaging and coatings. The safety and authenticity of honey are essential for guaranteeing its quality and preventing adulteration. Humidity, pH, and hydroxymethylfurfural (HMF) content change in stored honey [6]. Furthermore, pollen samples correlated with the quality of honey in an evaluation of pine flora in the Oxapampa Valley [7]. The maximum humidity allowed in honey varieties is 20%, and these present a low susceptibility to fermentation compared to honey varieties produced in Spain [8, 9].

Free radicals play a central role in the pathogenesis of various diseases, and the associated reactive species maintain the homeostatic balance of the organism [10]. The mechanism of antioxidant action includes the ability to bind free radicals, catalyze electron transfer, and eliminate free radicals and Fe^{2+}, Cu^{2+}, and Zn^{2+} [11]. Antioxidants neutralize or eliminate free radicals during oxidative activity in the body [12]. However, high antiradical activity does not necessarily correlate with high antioxidant activity; some synthetic phenolic compounds have high reactivity towards free radicals but show moderate antioxidant activity [13]. An antioxidant is any substance that significantly delays or prevents the oxidation of the substrate by acting as an electron donor at low concentrations [14].

In Peru, especially in the Ica province, the growing demand for agro-export has increased the biodiversity of plant species, including flowers and plants with antioxidant properties. Various metabolites are formed by plants [15]. Bees transport the metabolites responsible for these properties for honey production, highlighting their biological benefits. The interest in honey produced by *Apis mellifera* L. is because of its bioactive compounds and polyphenols, which act as antioxidants in biological systems. The predominant pollen varies depending on the botanical source among the seven types of honey produced from *A. mellifera* L. This study aimed to evaluate different honey varieties based on their floral origin by analyzing their physicochemical composition, total polyphenol content, antioxidant activity, and mineral profile to assess their conformity and consumer preference.

2. Materials and Methods

2.1. Obtaining Honey Samples Produced by *A. mellifera* L.

Honey samples produced by *A. mellifera* L. from different plant sources were collected by a beekeeper in the Ica province (at 409 masl), following appropriate protocols regarding location, time, season, and storage method (glass jars with lids and labeling). The honey varieties were certified by the beekeeper based on their floral origins. The samples were sent to the laboratory of the Research Institute of the University, San Luis, Gonzaga, where they were kept at room temperature away from direct light until analyses were performed.

2.2. Physicochemical Characterization

The physicochemical characterization of the seven honey varieties was evaluated using the analytical methods of the Association of Official Methods of Analysis (AOAC). Density: The weight of water and the sample were compared in a constant volume using a pycnometer and a thermal bath at 27 ℃. Humidity: The refractive index of the sample was measured using the Karl Fischer method until it reached a value expressed in g/100 g, according to the floral origin of honey. Acidity: Acidity was determined by titration with sodium hydroxide and 0.05 N hydrochloric acid. Sugar content: The sample was diluted with Fehling reagents A and B for hot titration with sodium thiosulfate solution until the color changed to brick red. In addition, sucrose hydrolyzed with concentrated hydrochloric acid was used to observe a more intense color change, which was neutralized with NaOH. Insoluble solids: The amount of insoluble solids remaining on the paper after filtering was determined. Ash: The weight of the residue of the sample calcined in a muffle furnace at 600 ℃ until white ash was obtained was measured. Hydroxymethylfurfural (spectrophotometric method): The sample was read at a wavelength of 284–336 nm and compared with sodium bisulfate [16].

2.3. Total Polyphenol Measurement

The Folin–Ciocalteu method was used with gallic acid as the standard to determine the total polyphenol content. Honey solutions were dissolved in water containing different gallic acid concentrations. Distilled H_2O (1800 µL), 200 µL of undiluted Folin–Ciocalteau reagent, and 500 µL of 20% (m/v) Na_2CO_3 were added to the samples. The mixture was stirred and homogenized, and the final volume was adjusted to 3 mL with water. The mixture was then incubated for 60 min in the dark. Absorbance was measured at 760 nm using water as a blank. Different concentrations of gallic acid (0–1000 ppm) were prepared and used to draw the calibration curve, which was expressed as EAG/g [17].

2.4. Flavonoid Measurement

The method proposed by Zhishen et al. [18] was used with some modifications to determine the total flavonoid content [19, 20]. Stock dilution (200 µL) was mixed in 1000 µL of water, and 75 µL of 5% $NaNO_2$ was added. The mixture was allowed to react for 5 min, then 75 µL of 10% $AlCl_3$ was added and incubated for 6 min. Then, 500 µL of 1 M NaOH was added, allowing it to stand for 5 min, and the optical density was read at 510 nm. The total flavonoid content is indicated in mg eq. of quercetin per gram of sample. The absorbance averages obtained were 700 µg/mL (0.625), 500 µg/mL (0.456), and 250 µg/mL (0.234).

2.5. Antioxidant Activity

DPPH and FRAP were used to evaluate antioxidant activity as previously described with some modifications [21]. The antioxidant capacity was measured by dissolving 3.1 mg of DPPH in 100 mL of absolute ethanol, and the absorbance was determined at 517 nm, with an inhibitory capacity that varied between 0.095 and 0.186 mg TEAC/g. The absorbance for the FRAP radical was measured at 593 nm, using Trolox as the standard, with values ranging from 0.168 to 0.654 mM TEAC/g [11].

2.6. Mineral Measurement

The minerals were analyzed using the atomic emission method with inductively coupled plasma optical emission spectroscopy. First, dry digestion of honey samples was performed. One gram of each sample was weighed, which was turned into ash in a muffle furnace at approximately 550 ℃. The ash was dissolved in 10% hydrochloric acid and filtered, and the final volume was adjusted to 25 mL using distilled water. Standard solutions were prepared for each mineral to generate calibration curves. To analyze Ca, Na, and K, 10 mL of 1% lanthanum solution was added to 2 mL of the sample and standard solution. The reading was then performed using the atomic emission equipment to obtain significant values expressed in mg/kg or ppm.

3. Results

3.1. Physicochemical Characteristics of Honey

The physicochemical characteristics of the different honey varieties are presented in Table 1. "Sierra" honey showed a pH of 3.45, indicating moderate acidity. In athe study by Ramos et al. [6] indicated that the pH remained constant between 4.05 and 4.06 during honey storage, suggesting that acidity was not considerably altered over time. "Eucalyptus" honey had a moisture content of 14.5%, a relatively high value that could affect crystallization and durability. The humidity ranged from 14.7% to 21% in another study [22]. "Aroma-Pecan" honey showed a conductivity of 0.03 µS/cm, indicating good quality in terms of minerals and salts, with values of 0.05–0.15 S/m that correspond to reported values [23]. Eucalyptus honey had a sugar content of 82.0 °Brix, attributable to climatic conditions and the degree of maturity, reflecting the concentration of sugars due to reduced humidity. The soluble solid content was between 78.83 and 83.80 °Brix [6]. Huarango and Eucalyptus honey varieties had ash contents of 0.09% and 0.13%, respectively, owing to the climatic and soil conditions in the Ica province. However, these values differ significantly from those of another study, where a range of 0.1% to 0.4% was reported [23]. Huarango and Eucalyptus honey had HMF values of 0.7 and 0.8 mg/100 g, respectively, which indicates freshness and good quality. However, another study reported HMF values of 0.2–1.8 mg/100 g, which may vary depending on factors related to the nectar source [24]. In summary, a comparison between these studies highlights the variations in the physicochemical composition of different honey samples and how specific parameters change over time.

Table 1. *Physicochemical characteristics of honey varieties according to floral origin.*

Samples	pH	Humidity (%)	Conductivity (µS/cm)	Ash (%)	°Brix	HMF mg/100
Orange	2.64	18.24	0.5	0.30	70	2.7
Tangerine	2.89	18.76	0.4	0.42	74	2.0
Huarango	2.70	15.20	0.4	0.09	80	0.7
Tamarix	2.90	17,12	0.5	0.30	70	2.8
Aroma-Pecan	3.15	16.45	0.3	0.23	76	2.0
Saw	3.45	16,24	0.4	0.28	75	1.7
Eucalyptus	3.32	14.50	0.2	0.13	82	0.8

HMF, hydroxymethylfurfural

3.2. Determination of Phenolic Compounds

Flavonoids were initially considered substances without benefits but later demonstrated numerous positive effects in eliminating free radicals [18, 25]. Phenolic compounds and flavonoids are essential indicators of honey quality and antioxidant activity. In the present study, total phenolic compounds ranged from 336.9 to 1064.9 µg EAG/g, whereas flavonoids varied between 0 and 151.9 µg EQ/g, with Eucalyptus honey being the richest in flavonoids. However, significant variations were observed when comparing these results with those other studies; different types of Lithuanian honey varieties were evaluated and had total phenolic compound levels of 110–450 µg EAG/g, lower than the highest values observed in the present study [26]. This finding suggests that Eucalyptus honey may have a more complex and elevated phenolic profile than other honey varieties, probably owing to its botanical and climatic characteristics.

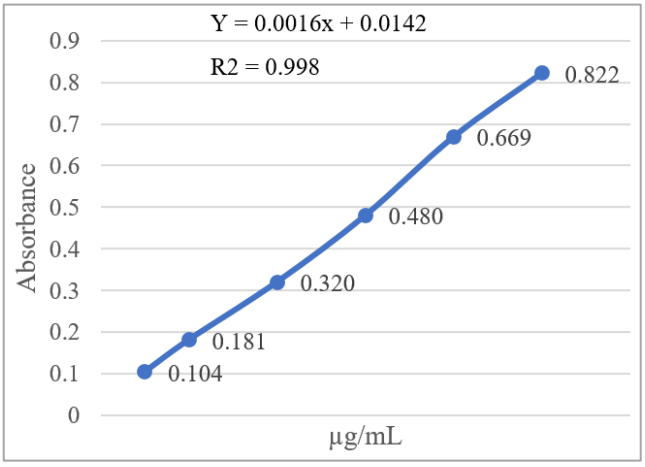

Figure 1. *Total polyphenols equivalent to GAE.*

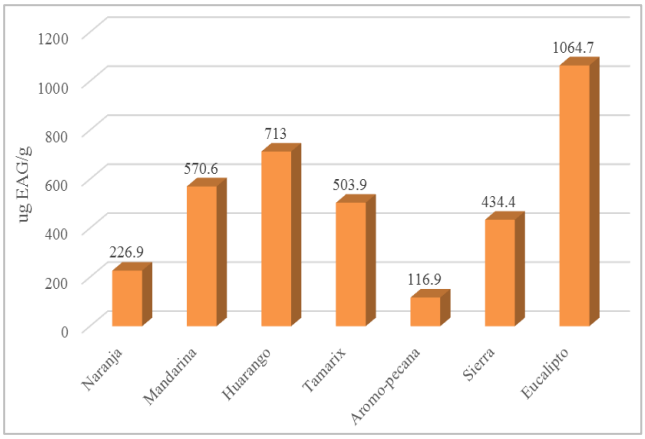

Figure 2. *Determination of polyphenols according to floral origin.*

Brazilian Eucalyptus honey had total phenolic compound concentrations that varied between 400 and 900 µg EAG/g, and that of flavonoids were 30–130 µg EQ/g, which is comparable to the highest values recorded in the present study. These results confirm that Eucalyptus honey varieties consistently show a high antioxidant content [27]. Phenolic compound and flavonoid values of 300–800 µg EAG/g and 20–100 µg EQ/g, respectively, were reported in Spanish honey varieties, which also agrees with the results of the present study, particularly in Eucalyptus honey varieties and other varieties with a specific botanical profile [28].

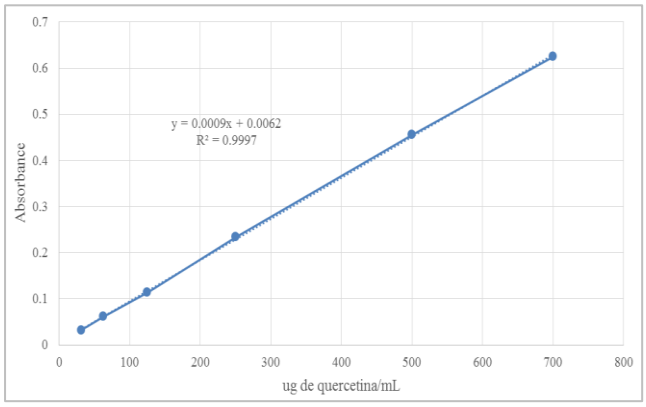

Figure 3. *Quercetin quantification curve for to determine total flavonoid content.*

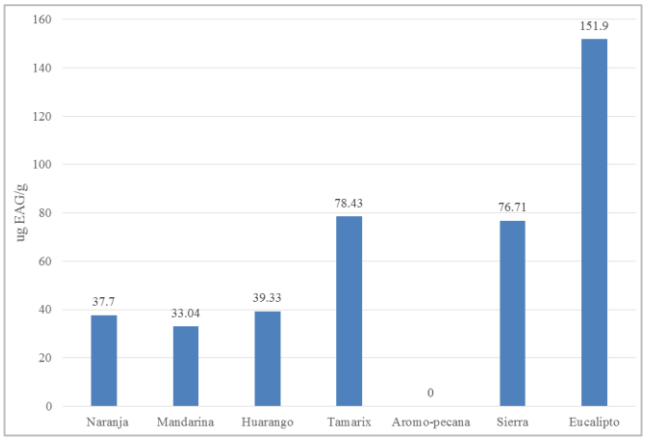

Figure 4. *Flavonoid concentration expressed in µg eq. of AG per gram of sample.*

3.3. Antioxidant Profile

Antioxidant capacity analyses using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) are essential for determining the quality and bioactive potential of honey. In the present study, DPPH values ranged from 0.095 and 0.186 mg TEAC/g, whereas FRAP values ranged from 0.168 and 0.654 mM TEAC/g. Eucalyptus, Tamarix, and Sierra honey samples

had high antioxidant levels, with averages of 0.183, 0.159, and 0.150 mg TEAC/g, respectively, in DPPH and FRAP values of 0.654, 0.527, and 0.475 mM TEAC/g. Similarities and differences were observed in the antioxidant capacity of different honey varieties when comparing these results with those of other studies. A Portuguese study reported DPPH values of 0.12–0.22 mg TEAC/g and FRAP values between 0.200 and 0.580 mM TEAC/g, similar to the findings of the present study, especially in honey varieties of botanical origin such as eucalyptus and chestnut. These results indicate that honey with higher phenol and flavonoid concentrations exhibits greater antioxidant capacity [29]. DPPH values varied between 0.080 and 0.190 mg TEAC/g The first subsection of the results and discussion section did not have a subtitle; therefore, I have suggested one for clarity. Please review whether you agree, and FRAP values ranged between 0.150 and 0.650 mM TEAC/g in Cuban honey. Honey varieties from specific botanical species, such as mangroves and coffee, showed high FRAP values comparable to those of Tamarix and Eucalyptus honey in this study [30]. DPPH values ranged from 0.085 to 0.200 mg TEAC/g, and FRAP values between 0.180 and 0.590 mM TEAC/g in Italian honey. The highest values were observed in chestnut and molasses honey samples, similar to the high antioxidant levels observed in Tamarix and Aroma-Pecan honey samples in this study [31].

These studies indicate that honey samples from different regions and botanical sources can exhibit wide variability in their antioxidant capacity. Eucalyptus honey, both in this study and in other studies, has powerful antioxidant activity and is one of the most consistent varieties in terms of high DPPH and FRAP values.

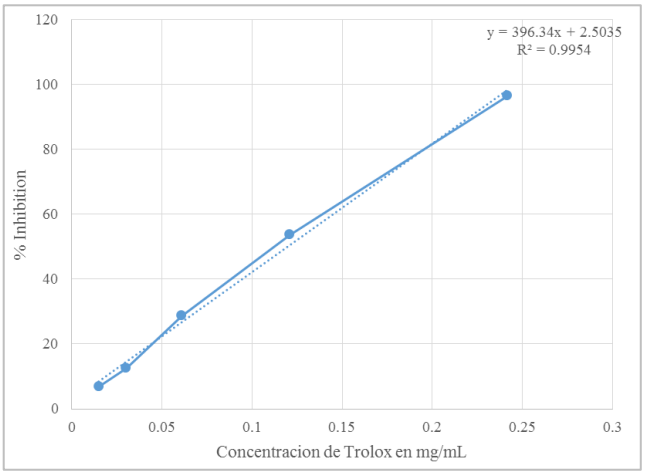

Figure 5. *Trolox calibration curve for 2,2-diphenyl-1-picrylhydrazyl determination.*

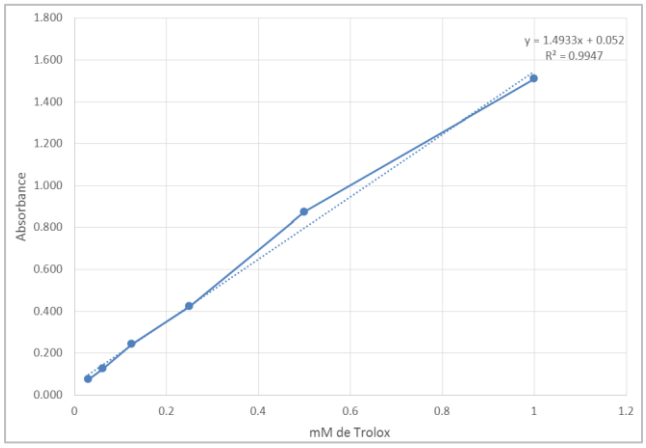

Figure 6. *Trolox curve using the ferric-reducing antioxidant power method.*

3.4. Mineral Composition

Analyzing the minerals in honey is essential to determine its nutritional value and potential health benefits. In this study, notable variability in micronutrient content was observed, especially in the potassium, magnesium, and calcium levels in different honey types (Table 1). The results were especially remarkable for potassium concentrations in Eucalyptus (901.17 mg/kg), Sierra (892.26 mg/kg), and Huarango (549.80 mg/kg) honey; magnesium in Eucalyptus (35.50 mg/kg) and Huarango (32.32 mg/kg) honey; calcium in Eucalyptus (161.55 mg/kg), and Huarango (123.54 mg/kg) honey; and sodium in Aroma-Pecan (172.18 mg/kg), Guarango (129.88 mg/kg), and Eucalyptus (95.08 mg/kg) honey. Similarities and differences in mineral content were observed when comparing these results with those of other studies. Potassium was the predominant mineral, with levels between 400 and 900 mg/kg in Polish honey, which is consistent with the high levels observed in Aroma-Pecan and Sierra honey in this study. These results reaffirm that potassium is the primary mineral in honey, regardless of its geographical origin [32]. In a study on Italian honey varieties, sodium concentrations between 50 and 150 mg/kg were reported. These concentrations were comparable to the values observed in the Tamarix and Eucalyptus honey varieties in this study, indicating their relatively high sodium content. These differences could be associated with soil characteristics and environmental conditions [33]. Honey from various regions of Saudi Arabia was analyzed, and calcium levels between 50 and 200 mg/kg were reported for honey varieties analyzed in Saudi Arabia, similar to the results of the present study, especially in the aromatic and eucalyptus honey varieties, which is consistent with the botanical and geographical characteristics of the producing areas [34].

Furthermore, significant levels of heavy metals, which are positive indicators of food safety, were not detected in any sample in this sample. The absence of heavy metals in the samples is a positive aspect. It coincides with the findings that

no significant traces of toxic elements were observed in high-quality Greek honey, suggesting that these honey varieties are free of industrial or environmental contaminants [35].

Table 2. *Mineral micronutrient content in honey varieties according to floral origin.*

Minerals (mg/kg)	Orange	Tangerine	Huarango	Tamarix	Aroma-Pecan	Saw	Eucalyptus
Boron	3.40	6.08	7.32	2.79	18.50	3.77	4.68
Calcium	72.58	93.00	123.54	70.29	98.09	84.39	161.55
Copper	< 0.0007	< 0.0007	< 0.0007	< 0.0007	< 0.0007	< 0.0007	< 0.0007
Magnesium	9.41	17.74	32,32	21.86	31.05	46.67	35.20
Iron	3.10	2.57	4.39	6.98	2.47	2.83	4.07
Sodium	43.18	87.99	129.88	40.94	172.18	35.79	95.08
Potassium	206.53	424.04	549.80	514.51	569.21	892.26	901.17
Zinc	0.49	0.94	0.98	0.47	2.37	1.88	1.87
Selenium	< 0.0033	< 0.0033	< 0.0033	< 0.0033	< 0.0033	< 0.0033	< 0.0033

4. Discussion

The chemical parameters analyzed (Table 2), such as moisture, acidity, sugars, and hydroxymethylfurfural, met the criteria established by the regulations (Codex Alimentarius), with moisture levels below 20%, acidity under 50 meq/kg, and conductivity below 800 µS/cm. No significant differences or correlations were found between these parameters, aligning with the results reported by Grajales-Conesa et al. (year). Regarding the total polyphenol content, determined through the calibration curve using gallic acid as a standard, eucalyptus honey exhibited the highest value, followed by huarango and tamarix honeys. These values surpass those reported in other studies, while the remaining honeys fall within commonly reported ranges. As for flavonoid content, a significant variability was found. Aromo-pecano honey showed no reading, reporting a value of zero, while eucalyptus honey had the highest concentration at 151.9 µgEQ/g. These findings align with previous studies, such as Nolden et al. (2023), which utilized a modified Folin-Ciocalteu method for determining total phenolic content in honey. [36]

Similarly, antioxidant activity assessed by DPPH and FRAP methods showed comparable results, with the distinction that eucalyptus honey was consistently the most active. A low correlation was established between total flavonoids and antioxidant activity by both methods; however, a high correlation between total polyphenols and antioxidant activity was found, indicating that phenolic compounds, which are not flavonoids, possess antioxidant activity. Regarding minerals of nutritional importance, there was high variability between different floral types. However, concerning potentially harmful heavy metals, such as strontium, the presence was reported in negligible amounts, consistent with Eghbaliferiz et al. (2016), who also found variability in the mineral content of different honeys with minimal presence of potentially harmful substances. [37]

Thus, these results confirm the findings of other studies that identify polyphenols and flavonoids as the main contributors to honey's antioxidant properties and emphasize the low presence of harmful minerals, in line with previous research on the antioxidant activity and mineral profile of various honey types.

5. Conclusions

he honey produced by A. mellifera L. demonstrated a significant antioxidant capacity against the DPPH radical, showing inhibitory activity comparable to the standard Trolox. This antioxidant potential is attributed to its phenolic components, which vary depending on the floral origin and source of the honey. Additionally, high concentrations of potassium were observed in the eucalyptus honey. These findings suggest the potential for utilizing this honey in functional applications within the food and supplement industries. Application to Future Research: Future studies could explore the antioxidant properties of honey from different floral origins, comparing their effectiveness across various assays to better understand their health benefits. Research could also delve into the specific mechanisms by which phenolic compounds and potassium contribute to honey's bioactivity, particularly for potential functional uses in foods or supplements. Additionally, further investigation into the variability of these properties, depending on geographic and environmental

factors, could provide insight into optimizing honey production for specific applications.

Abbreviations

DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
EAG	Gallic Acid Equivalent
EQ	Quercetin Equivalents
TEAC	Trolox Equivalent Antioxidant Capacity
MG	Milligrams
KG	Kilograms
HMF	Hydroxymethylfurfural
pH	Potential of Hydrogen Ions
GAE	Grams of Acid Equivalent
AOAC	Association of Official Analytical Collaboration

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Researching faculty, patent winners.

Author Contributions

Eddie Loyola Gonzales: Supervision, Methodology, Validation, Review

Josefa Bertha Pari Olarte: Methodology, Validation, Review, Writing

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Data Availability Statement

Yes.

Conflicts of Interest

The authors declare no conflicts of interest.

Appendix

Bottles with frosted stoppers: 1000 ml (1 liter)
Petri dishes: 60 mm x 15 mm
Crucibles: 50 ml
Vials: 5 ml
Separatory funnels: 250 ml
Flasks: 50, 100, and 250 ml
Micropipettes: 10, 100, and 1000 µl
Tweezers
Quartz spectrophotometer cuvettes: Standard internal volume: 1 ml (with variants from 0.35 ml to 3 ml).

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