

Determination of the phytochemical quality and botanical origin of bee honey in La Comarca Lagunera

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Abstract

This study aimed to determine the phytochemical quality and botanical origin of bee honey in four municipalities of La Comarca Lagunera, Mexico. The municipalities of Torreón and Francisco I. Madero in the state of Coahuila, and Gómez Palacio and Lerdo in the state of Durango were considered in fixed beekeeping. Variables such as moisture, total soluble solids, color, pH, total phenolic compounds and total flavonoids were analyzed during the spring and summer harvesting seasons. The results indicated significant seasonal differences in moisture, total soluble solids and color, with higher moisture content and darker tonality in the summer season, which are associated with floral diversity and specific climatic conditions. The pH remained stable between seasons. Total phenolic compounds increased significantly in the summer season, whereas total flavonoids remained constant, suggesting that their concentration is less sensitive to seasonal changes. The mellisopalynological analysis revealed that the spring honey was monofloral, from mesquite (*Prosopis juliflora*) flowers, whereas the summer honey was multifloral, reflecting a greater diversity of nectariferous sources. The positive relationship between color and phenolic compounds and flavonoids indicates that darker honeys have greater chemical complexity and putatively, greater antioxidant potential.

Keywords:

antioxidants, nutraceuticals, nutraphenolic compounds, nutraceuticals.



Introduction

Beekeeping is carried out in the 32 states of Mexico; having such varied and favorable climatic conditions in a large part of the national territory, honeybee (*Apis mellifera* L.) colonies proliferate and remain active throughout the year, allowing beekeepers to have two main forms of production, fixed and mobile beekeeping, where the first means that the hives remain in one place all year round, and in the second, the apiaries are moved according to the availability of flows (Baena-Díaz *et al.*, 2022).

This activity has established itself as an important source of income for thousands of families, standing out for its economic value as well as its environmental and social relevance (Ramos-Cuellar *et al.*, 2023). Coahuila has an active community of approximately 178 beekeepers who manage more than 14 500 hives in the state.

Most of the activity is concentrated in the Laguna Region, which accounts for 61% of the total state production, with municipalities such as Torreón and Matamoros standing out (Contreras-García, 2025).

La Comarca Lagunera is a region in north-central Mexico located in the states of Coahuila de Zaragoza and Durango, covering 15 municipalities and an area of almost 48 000 km², representing 2.5% of Mexico (CNANP, 2023).

This region has natural conditions that favor the production of unique honey, from the nectar of characteristic species such as mesquite (*Prosopis juliflora* (Sw.) DC.) and pinabete (*Tamarix aphylla* (L.) H. Karst.) (INIFAP, 2023).

Mexican honey is regulated by the Official Mexican Standard NOM-004-SAG/GAN-2018, which establishes that there is a general characterization of some honeys at the regional level in terms of commercial value (Baena-Díaz *et al.*, 2022). More than 320 types of honey are recognized worldwide, varying according to the plants visited by the bees and the environmental conditions of the place (Vijan *et al.*, 2023).

Bee honey is a natural substance with a sweet taste and viscous consistency that, by enzymatic action, tends to granulate as time passes; it is highly energetic, providing 304 kcal 100 g⁻¹, and is produced and stored by bees as a food source for the colony. It has various uses in gastronomy, but also from a nutraceutical and nutritional point of view, standing out for its content of natural micro- and macronutrients, flavonoids, phenolic acids, polyphenols, vitamins and minerals (FAO, 2020; Faienza *et al.*, 2024; González-Pérez *et al.*, 2024a).

The content of total soluble solids in honey is represented by the value expressed in degrees Brix that is obtained through the refractometer; this variable is related to the moisture content, since water is the second most important component in honey and is expressed as a percentage.

In recent years, interest in honey has expanded beyond its use as a natural sweetener, with a focus on its nutritional and therapeutic value (Faienza *et al.*, 2024; González-Pérez *et al.*, 2024a).

The floral origin of honey is the factor that most determines its phenolic composition; in fact, it can be used as a tool to classify and authenticate its origin, especially concerning unifloral varieties (Cianciosi *et al.*, 2018). Phenolic compounds are beneficial to health due to their ability to eliminate free radicals and regulate enzymes that allow maintaining an oxidation-reduction balance (Jańkiewicz *et al.*, 2025).

It has been observed that darker honeys, by having a higher concentration of these bioactive compounds, have superior antioxidant activity, which reinforces their potential as nutraceuticals in the prevention of oxidative damage (Haque *et al.*, 2023). Nutraceutical is the term for a substance, component or food that has been shown to provide health benefits or clinical effects, such as treatment and prevention of disease.

This study aimed to determine the phytochemical quality and botanical origin of bee honey in four municipalities of La Comarca Lagunera, Mexico.

Materials and methods

Study area

The present work corresponds to a descriptive study aimed at determining the phytochemical quality and botanical origin of bee honey produced in four municipalities of La Comarca Lagunera during two flowering periods in 2024.

The research was conducted in four municipalities of La Comarca Lagunera; on the part of the state of Coahuila, they were Torreón (24° 47' 35.16" N to 25° 41' 29.04" N latitude and 102° 57' 25.20" W to 103° 30' 39.60" W longitude) and Fco. I. Madero (103.2730° W longitude and 25.7753° N latitude); on the part of the state of Durango, they were Gómez Palacio (103° 41' 24.00" W to 103° 19' 08.40" W longitude and 25° 32' 08.16" N to 25° 53' 16.80" N latitude) and Lerdo (103° 58' 44.40" W to 103° 20' 13.20" W longitude and 25° 10' 27.12" N to 25° 46' 05.16" N latitude) (INEGI, 2025).

This region has a warm, dry climate with an average annual temperature of 22.7 °C and two periods of drought: a prolonged one in winter and a shorter one in summer. According to historical data from the Torreón Meteorological Observatory, the season with the highest rainfall runs from June to September, with monthly averages of 30 mm in June, 42.8 mm in July, 40.9 mm in August and 51.6 mm in September (SADER, 2023).

Sampling

Sampling was carried out in spring (April 2024) and summer (July 2024). A total of 16 apiaries distributed across the four municipalities were sampled, yielding 41 randomly selected samples: 24 samples in April and 17 samples in July. The samples were taken directly from the hive super, obtaining a 15×15 cm section weighing approximately 600 g; it was checked that the frame was completely capped; the samples were placed in sterile trays with lids, identified, and stored at a temperature that did not exceed 26 °C so as not to alter their composition, as recommended by Tulandi (2019).

In order to evaluate the quality of the bee honey, physicochemical and phytochemical characterization was performed, including moisture content, TSS (°Brix), color, pH, total phenolic compounds, total flavonoids and botanical origin.

Moisture and total soluble solids

Moisture and TSS were measured using an AN-KA® 106b manual refractometer (China) with a reading range of 58-90 degrees Brix with automatic temperature compensation (ATC).

Color

Color was determined using a Hanna Instruments® c-221 bee honey photocolormeter, with glycerol standard (Hanna Instruments® CAS56-; 81-5; EC200-289-5, Italy) employed to calibrate the equipment. The value provided by the device must be compared with a table of values known as the Pfund scale (Table 1), which classifies honey by color, depending on the measurement provided by the photocolormeter.



Table 1. Color of the bee honey according to the Pfund scale.

Color	Pfund range (scale mm)
Water white	Less than 8
Extra light amber	35 to 50
Light amber	51 to 85
Amber	86 to 114
Dark amber	Greater than 114

Delmoro *et al.* (2010).

pH

The procedure to determine the pH of the honey was carried out according to the methodology of the Institute of Agricultural Research (INIA, 2022), using a Hanna Instruments® HI5521-01 pH meter. Ten grams of honey were dissolved in 75 ml of CO₂ free distilled water. The pre-calibrated pH meter electrode was inserted into the solution, and the reading was recorded, ensuring that the mixture was homogeneous.

Total phenolic compounds and total flavonoids

The content of total phenolic compounds was quantified based on the Folin-Ciocalteu method described by Ciappini *et al.* (2013). A total of 4 ± 0.01 g of honey was weighed, and 25 ml of water was added; 1 ml of this solution was taken and 10 ml of distilled water and 1 ml of Folin-Ciocalteu reagent were added to it; it was then stirred gently and left to stand for two minutes. Then, 2 ml of 10% sodium carbonate solution was added, and the solution was made up to a volume with water (25 ml). It was left to stand for 2 h at room temperature, and the solution's absorbance was read at 725 nm in a Genesys 6 UV-visible spectrophotometer. The results were expressed as gallic acid equivalents (GAE) in 100 g of honey.

Total flavonoids were quantified spectrophotometrically by reacting them with aluminum trichloride according to the techniques described by Ciappini *et al.* (2013).

A total of 2.5 ± 0.01 g of honey was weighed and dissolved with distilled water; then, 0.5 ml of 5% AlCl₃ was added and the solution was made up to a volume of 25 ml with distilled water. It was left to stand for 30 min in the dark, and the solution's absorbance was read at 425 nm in a Genesys 6 UV-visible spectrophotometer. Results were expressed in mg QE 100 g⁻¹ honey.

Botanical origin

The botanical origin was determined by mellisopalynological analysis with the acetolysis technique to clean, concentrate and highlight the external structure (exine) of the pollen grains (Louveaux *et al.*, 1978), to isolate, observe and determine the plant species contained in the honey by identifying the individual pollen, which were compared with the images and measurements reported in the Atlas of the Pollen of La Comarca Lagunera (Reyes-Carrillo *et al.*, 2009).

Experimental design and statistical analysis

A completely randomized experimental design was used, with a single factor (season of the year) and two levels: spring (April 2024) and summer (July 2024). A total of 41 samples of bee honey were analyzed, corresponding to 24 samples from the spring season (April 2024) and 17 samples from the summer season (July 2024).

The data obtained were subjected to analysis of variance (Anova) at a significance level of $p < 0.01$. When significant differences were present, the means were compared using the Minimum Significant Difference (MSD) test. Statistical analyses were performed using SAS® version 9.4 (Statistical Analysis System Institute Inc., Cary, NC, USA).

Results and discussion

Moisture, total soluble solids, color and pH

Moisture

Moisture is a key variable that influences the stability and preservation of honey. In the samples analyzed, a highly significant difference ($p < 0.01$) was observed between seasons of the year, with values of 12.5% in the spring season and 13.36% in the summer season, as observed in Table 2.

Table 2. Physicochemical variables of spring and summer bee honey in La Comarca Lagunera, Mexico.

Season	Moisture (%)	TSS (°Brix)	Pfund color (mm)	pH
Spring	12.52b (11.8-13.3)	85.9a (85-86.5)	Light amber 50b (11-92)	3.78a (3.1-4.8)
Summer	13.36a (12-15.2)	85.4b (84-86.5)	Amber 91a (70-142)	3.74a (3-4.6)

Means accompanied by the same letter are equal to each other ($p \leq 0.01$). Pfund, a numerical scale to classify the color of honey.

The moisture content, considered the second most relevant component of honey, reflects the influence of botanical and climatic factors, as well as the degree of maturity reached at the time of extraction. This parameter is important because a higher proportion of moisture can favor fermentation processes and trigger chemical reactions, directly affecting the stability and quality of the product (González-Pérez *et al.*, 2024b).

The results showed values within the parameters established by the regulations, and even a water content below the recommended limit of 20% moisture was observed (NOM, 2018). This finding suggests that the honey produced in the region presented an adequate maturity, highlighting its quality and stability against deterioration processes.

The increase in moisture in the summer (Table 2), although slight, could be attributed to the relative humidity of the environment (Perez *et al.*, 2016; Fernandez *et al.*, 2018), because according to precipitation records, July usually reports the presence of greater humidity compared to April, when the effects of the Mexican monsoon have not yet been felt (SADER, 2023).

Total soluble solids

Regarding the average TSS values, a highly significant difference ($p < 0.01$) between seasons was determined, with average values of 85.9 °Brix in the spring season and 85.4 °Brix in the summer season. Although both values indicate a high concentration of TSS, characteristic of mature honeys, the slightly higher value in the spring season is statistically significant and could be associated with drier climatic conditions, with nectar of higher concentration in the flowers foraged by bees. The decrease in TSS during the summer season would be related to the increase in humidity on which flowering depends, as well as the higher relative humidity as a result of the rainy season.

Color

The color of the honey showed significant differences between seasons ($p < 0.01$). In spring (April 2024), the average value measured on the Pfund scale was 91 mm, classified as amber, whereas in the summer season (July 2024), it was 50 mm, corresponding to light amber.

The results indicate that the season of the year significantly influenced the color intensity; this is likely due to botanical origin, which is related to the presence of pigments, polyphenols, and minerals, since darker honeys tend to have greater antioxidant activity (García-Chaviano *et al.*, 2022).

pH

When analyzing the pH of the honey samples, the average values were 3.79 in the spring season and 3.75 in the summer season, with no significant differences ($p > 0.01$). The similarity between seasons suggests chemical stability, which is not affected by the characteristic environmental conditions of the two harvest seasons, as well as proper beekeeping practices (García-Chaviano *et al.*, 2022). The low pH value in bee honey is usually an indicator of alteration, mainly caused by fermentation processes characteristic of early extractions with high moisture content (González-Pérez *et al.*, 2024b); however, in this study, the pH values obtained remained within acceptable parameters.

Total phenolic compounds

The contents of total phenolic compounds showed a significant difference ($p < 0.05$) between seasons (Table 3). In the summer season, an average value of 0.6497 was obtained, whereas in the spring season, the value was 0.1605 mg GAE 100 g⁻¹ of honey. This difference indicates significant variation between seasons, suggesting that the environmental or physiological conditions of each period influenced the synthesis and accumulation of phenolic compounds.

Table 3. Total phenolics and total flavonoids of spring and summer bee honey from La Comarca Lagunera, Mexico.

Season	Total phenolics (mg GAE 100 g ⁻¹)	Total flavonoids (mg QAE g ⁻¹)
Spring	0.16b (0.1-0.25)	0.27a (0.1-0.63)
Summer	0.65a (0.19-1.28)	0.27a (0.1-0.64)

Means accompanied by the same letter are equal to each other ($p \leq 0.01$).

This increase suggests either a greater presence of floral sources with high secondary metabolite content or that prevailing climatic conditions favored their accumulation in the nectar (Becerril-Sánchez *et al.*, 2021). The decrease in the content of phenolic compounds during the spring season could be related to a lower availability of plant species rich in secondary metabolites since their flowering is usually reduced by the low amount of rainfall.

Since phenolic content depends on seasonality and local floral diversity, a reduced supply of nectariferous sources could explain this reduction (Cianciosi *et al.*, 2018). In several studies on honey, it has been observed that there is a strong positive correlation between the total content of phenolic compounds and antioxidant capacity, suggesting that these secondary metabolites are primarily responsible for the antioxidant potential of honey.

This relationship indicates that variations in total phenol concentration could be directly reflected in antioxidant activity values, regardless of harvest season or floral origin (Sultana *et al.*, 2024).

Total flavonoids

The total flavonoid content in honey (Table 3) did not show significant differences between the spring season (April) and the summer season (July), indicating that the season of the year did not significantly affect this variable. These results suggest that the flavonoid profile remains relatively stable in honey from La Comarca Lagunera regardless of the season of the year.

Previous studies have also observed that certain individual flavonoids do not vary across seasons and that seasonal harvesting does not always imply differences in the total content of these compounds (Bernklau and Arathi, 2023). The homogeneous distribution between regions suggests a uniform presence of floral sources that provide these compounds in La Comarca Lagunera (Bouacha *et al.*, 2024; Bozkuş, 2025).

Botanical origin

For the spring season, as can be seen in Table 4 of the mellisopalynological analysis, according to Louveaux *et al.* (1978), honey is monofloral, as the pollen content is greater than 45%, with predominance of the flower of the species *Prosopis juliflora*, commonly called mesquite, which represented 45.14% of the total pollen grains, whereas the 'various species' set constituted 40.92%. This suggests that spring honey is derived primarily from a predominant floral resource, with moderate contributions from others.

Table 4. Botanical origin of spring bee honey in La Comarca Lagunera, Mexico.

Species	Presence of pollen in samples	
	Number	(%)
Mesquite (<i>Prosopis juliflora</i>)	256	45.14
Picosilla (<i>Acalypha neomexicana</i> Muell.)	48	8.47
Hierba huevona (<i>Lesquerella fendleri</i>) (Gray) S. Wats.	31	5.47
Various species (78 spp.)	232	40.92
Total	567	100

Of the 78 species found, the outstanding pollens were those whose origins belong to the plants of the following families: Fabaceae (7), Asteraceae (7), Solanaceae (5), Poaceae (5), Euphorbiaceae (4), Rosaceae (4), Ranunculaceae (4) and 22 other families with one or two plants present in the honey samples.

Mellisopalynological analysis

For honey harvested in the summer, the distribution changes: 'various species' account for 50.44% of the pollen grains, whereas the dominant individual species, such as sorghum (*Sorghum vulgare*) with 20.52% and pinabete (*Tamarix aphylla*) with 16.87%, together account for less than 40%. This indicates that summer honey has a distinctly multifloral nature, with several species contributing significantly to the nectar/pollen harvested by bees (Table 5).

Table 5. Botanical origin of summer bee honey in La Comarca Lagunera, Mexico.

Species	Presence of pollen in samples	
	Number	(%)
Sorghum (<i>Sorghum vulgare</i> Pers.)	118	20.52
Pinabete (<i>Tamarix aphylla</i> (L.), Karst.)	97	16.87
Quelite (<i>Amaranthus palmeri</i> S. Wats.)	42	7.3
Abrojo (<i>Cenchrus echinatus</i> L.)	28	4.87
Various species (87 spp.)	290	50.44
Total	575	100

In the information shown in the table, it is noteworthy that there is a greater number of different species present in the honey of the summer harvest (87 spp.) compared to that of the spring harvest (78 spp.). In these species, 35 families are represented, with prominence of Poaceae (9), Asteraceae (9), Brassicaceae (7), Fabaceae (5), Euphorbiaceae (4), Amaranthaceae (3), Solanaceae (3), Rutaceae (3) and 28 families with one or two specimens of each species.

Various studies have reported that honeys of multifloral origin have a greater variability in the content of phenolic compounds compared to monofloral honeys, due to the diversity of plant sources that contribute to their production (Becerril-Sánchez *et al.*, 2021). It was observed that the color of the honey varied between seasons, going from a light amber hue in the spring harvest to amber in the summer harvest.

This change could be attributed to the increase in the content of phenolic compounds and flavonoids, since various studies have reported that the intensity of the color of honey is positively correlated with the concentration of phenolic compounds and flavonoids, with the darkest honeys having the highest values of these compounds (Sultana *et al.*, 2024).

These metabolites participate not only in the coloration but also in the antioxidant capacity of the product, so their increase may reflect greater chemical complexity derived from the floral diversity present during the second season.

Conclusions

When analyzing bee honey, the results indicated significant seasonal differences in moisture, soluble solids and color, with higher moisture content and a darker tone in the summer, which is associated with floral diversity. The pH remained stable between seasons. Total phenolic compounds increased significantly in the summer season, whereas flavonoids remained constant, suggesting that their concentration is less sensitive to seasonal changes.

The mellisopalynological analysis revealed that the spring honey was monofloral, from mesquite (*Prosopis juliflora*) flowers, whereas the summer honey was multifloral, reflecting a greater diversity of nectariferous sources. The positive relationship between color and phenolic compounds and flavonoids indicates that darker honeys have greater chemical complexity and, putatively, greater antioxidant potential.

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