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Changes in physicochemical and antioxidant properties over one year of *Apis mellifera* honey

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ABSTRACT

Objective: Quantify the physicochemical and antioxidant properties of honey harvested in the four seasons of the year, to determine the variation in its quality with respect to official standards.

Design/methodology/approach: Honey samples were collected in the municipality of Tantoyuca, during the four seasons of the year, in the presence or absence of rain. For each sample, color, electrical conductivity, moisture, pH, free acidity, diastase activity, total reducing sugars, Brix degrees, caloric content, phenolic and total flavonoid content and antioxidant capacity were determined by the FRAP and ABTS assays.

Results: The Brix degrees and moisture were found within the limits accepted by NOM-004-SAG/GAN-2018 and CXS 12-1981 throughout the year, however, in the rainy period these variables were higher (82.1 °Brix and 19.6 g 100 g^{-1}). The FRAP and ABTS values showed variation depending on the absence or presence of rain. The highest antioxidant content occurred in the winter season (63.91 and 68.82 μmol TE 100 g^{-1}). The results obtained are attributed to the geographical origin and the floral species present during the bees' foraging.

Limitations on study/implications: Climate change in the region has decreased rainfall, reducing the floristic resource.

Findings/conclusions: The effect of the season of the year affects the characteristics of the honey evaluated, however, it complies with the parameters established in the Mexican standard and the codex alimentarius, which can encourage and support its commercialization in the international market.

Keywords: honeybee, *A. mellifera*, antioxidant compounds, quality, seasonal variation.

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INTRODUCTION

Honey is defined as a sweet and natural food generated from the nectar of plants, collected and transformed by bees. This product consists of various types of carbohydrates, water, minerals, amino acids, proteins, and organic acids. The composition of honey depends on the floral species from which the bees sucks (monofloral, multifloral, and honeydew) (Martínez *et al.*, 2017), as well as the characteristics of the soil, species of the bee, colony physiology, among others (Jean-Prost, 2007; Bogdanov *et al.*, 2008). The nutritional

value and therapeutic and stimulating qualities of honey position it as a highly demanded product in the international market (Escobar and Manresa, 2005). The identity of honey can be determined by analyzing its physicochemical properties, such as moisture content, electrical conductivity, free acidity, pH, and color (Pineda *et al.*, 2019), as well as the total sugar content and other related substances, such as diastase activity. On the other hand, polyphenolic compounds, recognized as responsible for the health benefits provided by honey, are produced in plants and have been studied due to their antioxidant, antimicrobial, and anti-inflammatory activity (Viuda-Martos *et al.*, 2008), as a consequence of their ability to inhibit and/or reduce the production of free radicals that cause oxidative damage to molecules such as carbohydrates, lipids, proteins, and genetic material (Nascimento *et al.*, 2018). Honey production faces various challenges, such as climate change, deforestation, and toxic agrochemicals that threaten the lives of bees. These factors affect production yields and raise doubts about honey quality. La Secretaría de Agricultura y Desarrollo Rural " (SADER) of México, aiming to regulate honey market behavior, issued the Official Mexican Standard NOM-004-SAG/GAN-2018, which outlines the conditions for honey production and marketing (García-Pérez and Fong-Reynoso, 2023). This standard strengthens beekeeping activities in the region and encourages honey commercialization in the international market. Therefore, the objective of this research was to determine the physicochemical properties and evaluate the nutraceutical potential of *A. mellifera* honey produced in a region of the Huasteca Veracruzana to determine the existing variation during the four seasons of the year.

MATERIALS AND METHODS

Study Location

This research was conducted in the Huasteca Veracruzana, located in the northern part of the state. It borders Tamaulipas to the north, Hidalgo, the Gulf of Mexico, and the Totonac region to the south. The Huasteca region lies between the parallels 97° 59' and 98° 24' west longitude and 21° 06' and 21° 40' north latitude. It has a warm sub-humid climate with summer rains and an average annual precipitation of 1100 mm. One of the main productive activities is cattle ranching focused on milk and meat production. In agriculture, the production of maize, citrus fruits, and sugar cane is prominent. The coexisting ecosystems include subtropical evergreen forest types, with species such as guarumbo, jonotes, guanacaxtle, and sangre de grado, along with important crops like sesame, peanuts, zucchini, sweet potatoes, beans, watermelon, sorghum, wheat, tobacco, tomatoes, coconuts, mangoes, and papayas (Alan and Martínez, 2010; INEGI, 2021).

Sample Collection

A total of 32 honey samples were collected from the localities of San Jerónimo, San Sebastián, Pensador Mexicano, Zapote Largo, Ixcanelco, Las Martas, and Mincuiní, all belonging to the municipality of Tantoyuca, Veracruz. Samples were obtained during the winter of 2022 and the spring, summer, and autumn of 2023, with eight samples taken from each apiary. The supers were selected randomly, and a manual extractor was used to

avoid honey contamination. The collected samples (approximately 500 mL) were stored at room temperature and protected from light until analysis.

Reagents and Instrumentation

The Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium bisulfite, quercetin (95% purity), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), ferric chloride (III) hexahydrate, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Absorbances for the quantification of phenols, flavonoids, and antioxidant capacity were measured using a Synergy 2 Microplate Reader, with Gen5 software (BioTek Instruments Inc., Winooski, VT, USA). Physicochemical parameter color was measured using a Hunter colorimeter (MiniScan XE Plus 45/0-L, HunterLab, Reston, Virginia, USA) on the CieLab scale (L*, a*, b*). The chromatic coordinates a* and b* are expressed on a scale of −100 to 100. For a*, the negative end indicates green, while the positive end indicates red. In the chromatic coordinate b*, the negative end indicates blue, and the positive end indicates yellow. The values for hue (Hue; h*) and chroma (Chroma; C*) were calculated using equations 1 and 2, while the value L* was taken as luminosity (Karabagias *et al.*, 2017).

$$h^* = \mathrm{Tan}^{-1}\left(b^*/a^*\right) \quad (1)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

Electrical conductivity, moisture content, pH, free acidity, and diastase activity were measured according to the procedures described by Bogdanov *et al.* (2002). All determinations were performed in triplicate.

The concentration of reducing sugars was determined following the method of Miller (1959) with some modifications: 0.5 mL of the aqueous honey solution (0.8 mg mL^{-1}) was mixed with 0.5 mL of 3,5-dinitrosalicylic acid (10 mg mL^{-1}). The mixture was heated to 90 °C for 10 minutes and then cooled in ice water for 10 minutes. The absorbance of the reaction mixture was read at 540 nm on a microplate reader. A glucose calibration curve was prepared in the concentration range of 0.2 to 1.0 mg mL^{-1}. The results were reported as grams of glucose equivalents per 100 grams of honey (g GluE 100 g^{-1}).

The percentage of soluble solids (°Brix) was determined using a pocket digital refractometer PAL-3 from Atago, with a range of 0.0 to 93.0%, calibrated with distilled water. An approximate volume of 1 g of sample was introduced, each in triplicate.

The caloric content of the samples was determined in a Parr 6400 calorimeter, and the results were reported in kilocalories per 100 grams of honey (kcal 100 g^{-1}).

Antioxidant properties

The antioxidant capacity was evaluated in aqueous solutions of honey (1:5 w/v). Total phenolic content was determined using the Folin-Ciocalteu method, adapted to microplates

(Hernández-Rodríguez *et al.*, 2016). Absorbance was measured at a wavelength of 760 nm. The results were expressed in milligrams of gallic acid equivalents per 100 grams of honey (mg GAE 100 g^{-1}). The calibration curve was prepared from a stock solution of 0.5 mg mL^{-1} of gallic acid in the linear concentration range of 0.02 to 0.21 μg mL^{-1}. The samples were analyzed in quadruplicate, taking four absorbance readings.

Total flavonoid content was quantified according to Chang *et al.* (2002). Absorbance was measured at a wavelength of 415 nm. The results were expressed in milligrams of quercetin equivalents per 100 grams of honey (mg QE 100 g^{-1}). The calibration curve for quercetin was prepared in a concentration range of 0.5 to 10 μg mL^{-1}. The samples were analyzed in quadruplicate, taking four absorbance readings.

Antioxidant capacity was determined using the FRAP (Benzie & Strain, 1996) and ABTS (Re *et al.*, 1999) assays, adapted to microplates. The results were reported in micromoles of Trolox equivalents per 100 grams of honey (μmol TE 100 g^{-1}). The calibration curve for Trolox was prepared in concentration ranges of 4 to 46 μM for the FRAP assay and 5 to 60 μM for the ABTS assay. The samples were analyzed in quadruplicate, taking four absorbance readings.

Experimental design and statistical analysis

A completely randomized experimental design was used, considering the season and the presence or absence of rainfall as sources of variation. A one-way ANOVA was performed for statistical analysis. Homogeneity was evaluated using Bartlett's test, and normality was assessed using the Shapiro-Wilk test. Mean comparisons were conducted using Tukey's test ($p<0.05$). The statistical analysis was carried out using Statistica v.10 software.

The proposed general linear model for this research is as follows:

$$y_{ij} = \mu + \alpha_i + \varepsilon_i$$

where: y_{ij}=response variable; μ=overall population mean; α_i=effect of the i-th season and presence or absence of rainfall during the year; εj=Associated experimental error.

RESULTS AND DISCUSSION

Physicochemical properties

Color is an important characteristic in the quality of honey. It can vary from light yellow, light amber, amber, reddish amber to almost black (Tuberoso *et al.*, 2013). The color parameters L*, a*, and b* of the samples evaluated in the four seasons and two periods of the year are presented in Figure 1. It was observed that autumn honey showed the highest luminosity value (L*=13.8), indicating that honey collected in this season was lighter. Honey from spring and winter was slightly darker (L*=9.9 and 9.3), and the lowest luminosity value was recorded in summer honey (L*=7.5), resulting in the darkest samples compared to the other seasons. The chromatic coordinates (a* and b*) of the honey in winter, spring, and autumn were similar. The honey samples evaluated over the periods showed that honeys with elevated L*, a*, and b* values were present during the rainy season, indicating that the samples collected during this period were lighter than those collected during the dry season.

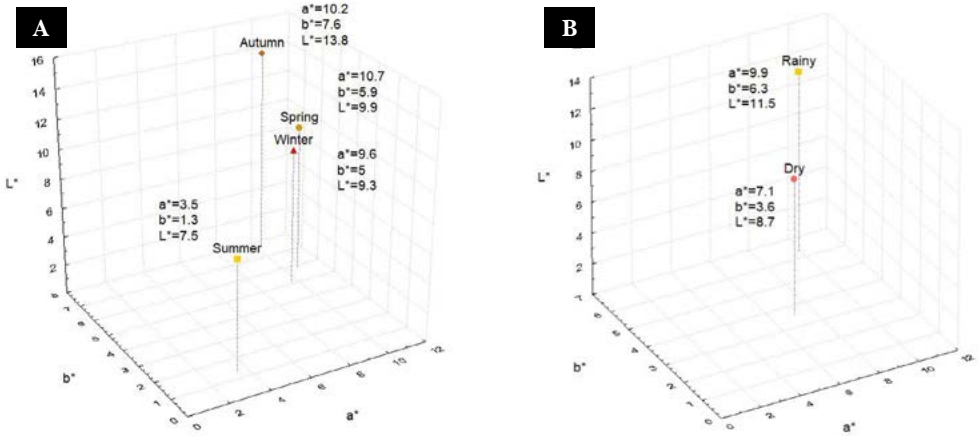

Figure 1. Chromatic coordinates and luminosity (a, b, and L*) of *A. mellifera* honeys in the CIE Lab space. A. Honey color by season. B. Honey color by period of the year.

The color of honey is a physical property influenced by the floral composition of the nectar, the extraction process, and the storage temperature. It is described by three chromatic attributes: luminosity (the color closest to white or black), hue (the perceived color: yellow, red, blue, green), and chroma (saturation and purity of the color). The luminosity of the honey samples evaluated was higher in autumn than in the other seasons ($p<0.05$) (see Figure 1). In this regard, Starowicz *et al.* (2021) studied the relationship between browning index and phenolic content, color, and antioxidant capacity in honey from Poland. Using the CIE L* a* b* method, they reported different luminosity values (8.7, 9.17, and 47.52) in monofloral honeys (*Acacia*). The similarity of the values found in the Huasteca region with those of these authors is noteworthy, given that they evaluated monofloral honeys, unlike this research. The edaphoclimatic conditions in Poland determine the local floral resources that could be responsible for the coloration of the honey.

The hue attribute had a higher value in autumn honey (0.761) compared to other seasons ($p<0.05$). In contrast, the hue of honey collected in summer showed the lowest

Table 1. Color attributes h* and C* in honey samples from the Huasteca Veracruzana.

Season / Period	Color	
	h*	C*
Spring	0.540^{b}	12.35^{a}
Summer	0.343^{c}	3.75^{b}
Autumn	0.761^{a}	12.99^{a}
Winter	0.453^{bc}	10.99^{a}
Standard error	0.04	1.23
Rain	0.442^{b}	8.05^{a}
Dry	0.607^{a}	11.99^{a}
Standard error	0.03	0.97

Note: Means within a column with different letters indicate statistical differences (Tukey $p<0.05$).

value (0.343), while the honeys harvested in spring and winter seasons (0.540, 0.453) did not differ from each other ($p>0.05$). The hue values were higher during the rainy season (0.607) than in the dry season (0.442). The results obtained are lower than those reported by Piotraszewska-Pająk and Gliszczyńska-Świgło (2015) in multifloral honeys from Poland. On the other hand, the chroma values for autumn, spring, and winter honey were 12.99, 12.35, and 10.99 ($p>0.05$), respectively. The saturation value in summer honey was lower (3.75) compared to the other seasons, indicating a statistical difference. The chroma values during the dry and rainy periods showed no statistical differences, with values of 8.05 and 11.99, respectively ($p>0.05$). These results are slightly lower (18.64, 9.71, 19.45, 23.11, 25.33, and 27.44) than those reported for multifloral honeys in temperate climates (Piotraszewska-Pająk and Gliszczyńska-Świgło, 2015).

The fluctuations found in this study regarding the color attributes could be attributed to the beekeeper's management practices, the conditions under which the honey is extracted, the storage duration, and the temperature at which the honey is kept. Various authors state that the color intensity in honey is related to the content of phenols, flavonoids, and antioxidants, which are provided by the plants (Becerril-Sánchez *et al.*, 2021).

The electrical conductivity of honey samples collected in the spring and summer was the highest (0.63 and 0.58 mS cm^{-1}) and did not show significant differences ($p<0.05$). The samples with the lowest conductivity were obtained in winter (0.18 mS cm^{-1}). The data indicate that there were no differences between the spring and summer seasons ($p<0.05$). However, this group exhibited higher conductivity compared to autumn and winter ($p<0.05$). The electrical conductivity of honey is related to its mineral content. Estimating this property is important for classifying honey and determining its origin. The variation in the obtained results may be a consequence of the presence of mineral substances from the botanical source, the degree of maturity at the time of extraction, and the storage conditions (Campo and Hincapié, 2023). On the other hand, the electrical conductivity of honey was higher ($p<0.05$) during the dry period (0.61 mS cm^{-1}) than during the rainy season (0.302 mS cm^{-1}). According to the Mexican Official Standard (NOM-004-SAG/GAN-2018) and the Codex Alimentarius (CXS 12-1981), the electrical conductivity of honey should not exceed 0.80 mS cm^{-1}. Thus, the honeys studied were within the established range.

Ezin *et al.* (2018) conducted a physicochemical characterization of honey produced in Benin during two seasons (dry and rainy) and found that during the dry season, the electrical conductivity was measured at 0.63 mS cm^{-1}, which is similar to the findings of this study. The similarity in results may be attributed to the climatic conditions and the sampling periods in both research studies.

The moisture content of the summer and winter samples was higher than that of the spring and autumn samples ($p>0.05$). Humidity is an important parameter that determines the maturation of honey and its physicochemical properties (García-Chaviano *et al.*, 2022). Humidity can vary according to the relative humidity at the collection site and the storage conditions. Percentages above 20%, as established in NOM-004-SAG/GAN-2018, facilitate the fermentation of honey, leading to changes in its physicochemical characteristics (Moyano *et al.*, 2023). The humidity values during the dry and rainy periods

show a statistical difference. These results are similar with those reported by Albú *et al.* (2022), who found humidity values ranging from 15.41% to 19.49% in multifloral honey from honeybees. The humidity range obtained is within the parameters established by NOM-004-SAG/GAN-2018. Environmental conditions and the collection of samples from capped frames could explain the similarity between these studies.

As shown in Table 2, the pH values of the samples collected in spring and summer did not show significant differences ($p>0.05$). No differences were observed between the autumn and winter samples either. The results obtained in autumn and winter honey are agree with those reported by Al-Ghamdi *et al.* (2019), who conducted a comparison of the chemical composition of honey samples from *A. mellifera* and *A. florea* subjected to different thermal processes. In their research, they found pH values of 3.62 in honey from *A. mellifera*. The appropriate pH range is between 3.2-4.5. This range is capable of inhibiting the growth of microorganisms (Da Silva *et al.*, 2016). The pH values of the samples evaluated during the dry and rainy periods showed no statistical difference and were similar to those reported by Ezin *et al.* (2018), who assessed the variation of the physicochemical properties of honey during two seasons (dry and rainy) and reported pH values ranging from 3.7-4.1. On the other hand, Albú *et al.* (2022) found pH values ranging from 3.25 to 5.03 in monofloral honeys from the east and southeast of Romania. The values reported in the studied samples could be associated with the floral resources available to the bees during foraging. The variation in pH may be due to the salivary secretion of the bees, which is responsible for the enzymatic and fermentative processes during the nectar processing (Ezin *et al.*, 2018).

Table 2. Electrical conductivity, moisture, pH, free acidity, diastatic index, reducing sugars, °Brix, and calorific value of *A. mellifera* honey samples from the Huasteca Veracruzana and the standard quality value.

Season/ Period /Quality standards	Electrical conductivity (mS cm^{-1})	Moisture (g 100 g^{-1})	pH	Free acidity (mEq kg^{-1})	Diastatic index (Schade units)	Reducing sugars (g GluE 100 g^{-1})	°Brix	Calorific value (kcal 100 g^{-1})
Spring	0.63±0.17^{a}	19.1±1.2^{ab}	4±0.2^{a}	50.3±12.4^{b}	19.5±13.3^{a}	42.3±15.6^{c}	81.8±1.1^{b}	311.6±3.2^{a}
Summer	0.58±0.22^{a}	18.5±1^{b}	4±0.2^{a}	49.5±7.6^{b}	13.8±6.8^{a}	61±13.7^{a}	81.1±0.9^{b}	312.1±3.4^{a}
Autumn	0.42±0.18^{b}	19.1±1.3^{ab}	3.6±0.1^{b}	60.9±7.9^{a}	16.2±6.4^{a}	52.9±9.7^{b}	81.6±1.7^{b}	312.7±5.3^{a}
Winter	0.18±0.07^{c}	20.1±2^{a}	3.6±0.06^{b}	62.5±8.4^{a}	16.2±4.9^{a}	64.9±8.1^{a}	82.6±1.5^{a}	315.1±3.1^{a}
Rainy	0.61±0.20^{a}	18.8±1.2^{b}	4.02±0.2^{a}	49.9±10.2^{b}	16.7±10.9^{a}	49.9±10.2^{b}	81.4±1.0^{b}	311.8±3.2^{b}
Dry	0.30±0.18^{b}	19.6±1.7^{a}	3.6±0.1^{b}	61.75±8.13^{a}	16.2±5.7^{a}	58.9±10.7^{a}	82.1±1.7^{a}	313.9±4.4^{a}
NOM-004-SAG/GAN-2018	Max. 0.8	Max. 20	SVR	Max. 50	Min. 8	Mín. 60	NRV	NRV
CXS 12-1981	Not more than 0.8	Not more than 20	SVR	Not more than 50	Not less than 8	Not less than 60	NRV	NRV

Note: Means in the same column with different letters indicate statistical differences (Tukey $p<0.05$). NRV=no reference value.

The free acidity values in autumn and winter honey were higher and statistically different from those in spring and summer ($p<0.05$). Free acidity is a parameter that indicates the freshness and deterioration of honey. The results reported in the four seasons are similar to those found by Al-Ghamdi *et al.* (2019) in multifloral honey from *A. mellifera* with values ranging from 51.80 to 84.6 mEq kg^{-1}. The honeys evaluated during the dry period showed lower acidity compared to those collected in the rainy season (see Table 2). Da Silva *et al.* (2016) mention that this parameter can be affected by the location and time of harvest.

The diastase index values showed no significant differences; however, they were higher than the maximum allowable range according to the Official Mexican Standard for Honey Production and Specifications and CXS 12-1981. The diastase index is an indicator of honey freshness. This indicator is determined by the floral resources accessible to the bees. Its content can vary based on the age of the hive, the nectar collection period, and the high concentration of sugars. A diastase level below 8 Schade units (DN) could indicate the premature collection of honey (Da Silva *et al.*, 2016).

The diastase index of honey during the dry and rainy periods showed no statistical difference (16.70 and 16.08 DN, respectively). These results are similar to those reported by Velásquez and Goetschel (2019), who determined the physicochemical quality of honey sold in markets south of Quito, Ecuador. The diastase index reported in their research ranged from 11.04 to 16.44 DN. Both studies prioritize understanding the physicochemical qualities of the evaluated honeys.

The reducing sugars contained in the samples collected in winter and spring (61 and 64 g glucose 100 g^{-1}) showed no significant difference between them ($p>0.05$). The determination of sugars in honey is used to assess its quality and possible adulteration. According to quality standards, the content must be at least 60 g glucose+fructose 100 g^{-1}. The concentration of sugars in the dry and rainy periods (51.66 and 58.91 g glucose 100 g^{-1}) was statistically different ($p<0.05$). Castillo *et al.* (2022) compared the sugar composition and °Brix in multifloral honeys from *A. mellifera* and *Melipona beecheii* from different states in Mexico. In that comparison, they found values of 28.9 g glucose 100 g^{-1} for *A. mellifera* and 28.2 g glucose 100 g^{-1} for *Melipona beecheii*. Their results were lower than those reported in this research. The variation between results can be attributed to the different floral sources present in each state. The soluble solids content (°Brix) in winter was higher (82.69) than in the other seasons. The values estimated in spring, summer, and autumn samples did not show statistical differences ($p>0.05$). Soluble solids represent the percentage of sugars present in honey. The results from the dry and rainy periods showed a statistical difference, with the rainy period (82.15) being higher than the dry period (81.49). Castillo *et al.* (2022) reported values between 76.7 and 81.5 °Brix in multifloral honeys from *A. mellifera*, coinciding with the values found in this study. This could be attributed to the diverse floral origin of honey in both investigations.

The caloric content does not show statistical differences ($p>0.05$). The monosaccharides glucose and fructose are rapidly absorbed and allow honey to provide energy. The energy content of honey fluctuates between 294-320 kcal 100 g^{-1} (García-Chaviano *et al.*, 2022). The heat of combustion showed a statistical difference between the dry and rainy periods; however, it is similar to the values reported for honeys from La Patagonia Verde, Chile

(Lobos *et al.*, 2021). The similarity of the obtained results can be attributed to environmental conditions, floral resources, and the timing of collection in both investigations.

Phenolic content and Antioxidant Capacity

The content of phenols and flavonoids, as well as the antioxidant capacity in the honey samples, is shown in Table 3. According to Adaškeviciute *et al.* (2019), phenolic compounds in honey are responsible for its antioxidant, antimicrobial, and anti-inflammatory properties. The phenolic content of summer honey (28.87 mg GAE 100 g^{-1}) was higher than that of the other seasons ($p<0.05$).

The flavonoid content of the evaluated samples indicated that the highest value was obtained in summer honey (0.757 mg QE 100 g^{-1}). Flavonoids are part of phenolic compounds. Their origin varies according to the floral source, which could explain the variation of its concentration between seasons.

The FRAP method is used to measure the antioxidant capacity of foods, beverages, and dietary supplements containing polyphenols. The results obtained for this parameter indicate that the calculated value in summer honey (93.39 μmol TE 100 g^{-1}) was higher than in the other seasons ($p<0.05$). In the samples of autumn and winter, the values obtained showed no statistical differences (57.75 and 63.91 μmol TE 100 g^{-1}, respectively).

The ABTS method measures the capacity of antioxidants to eliminate the $ABTS^{\bullet+}$ cation radical (oxidizing agent) (Mercado-Mercado *et al.*, 2013). The autumn and spring samples showed higher values of antioxidant capacity (100.12 and 98.46 μmol TE 100 g^{-1}) ($p>0.05$).

The results for phenolic content, flavonoids, and antioxidant capacity during the dry and rainy periods are presented in Table 3. In the dry period, a value of 27.31 mg GAE 100 g^{-1} was found, which was higher than that found in the rainy period (25.32 mg GAE 100 g^{-1}) ($p<0.05$). The content of polyphenolic compounds in honey can vary according to geographical origin, floral source, and climatic conditions (Becerril-Sánchez *et al.*, 2021). The results obtained during the studied periods were higher than those presented by Perna *et al.* (2013). They evaluated the antioxidant properties, polyphenol content, and colorimetric characteristics in mono- and multifloral honeys from different regions of southern Italy. In their research, they found values of 11.79 mg GAE 100 g^{-1} for multifloral honeys and 12.15 mg GAE 100 g^{-1} in citrus honeys. Becerril-Sánchez *et al.*

Table 3. Content of Phenolic Compounds and Antioxidant Capacity of Honey Samples Produced in a Region of Huasteca Veracruzana During the Four Seasons of the Year.

Season	Phenols mg GAE 100 g^{-1}	Flavonoids mg QE 100 g^{-1}	FRAP μmol TE 100 g^{-1}	ABTS μmol TE 100 g^{-1}
Spring	25.74 ± 3.94^{b}	0.562 ± 0.18^{b}	72.18 ± 25.35^{b}	98.46 ± 32.70^{a}
Summer	28.87 ± 4.83^{a}	0.757 ± 0.17^{a}	93.39 ± 23.90^{a}	79.85 ± 23.83^{b}
Autumn	25.92 ± 7.95^{b}	0.518 ± 0.22^{b}	57.57 ± 22.24^{c}	99.84 ± 59.96^{a}
Winter	24.86 ± 4.65^{b}	0.503 ± 0.18^{b}	63.91 ± 23.77^{c}	68.82 ± 20.59^{b}
Rainy	27.31 ± 4.67^{a}	0.660 ± 0.20^{a}	82.78 ± 26.78^{a}	89.15 ± 30.04^{a}
Dry	25.39 ± 6.51^{b}	0.510 ± 0.20^{b}	60.76 ± 23.20^{b}	84.21 ± 47.23^{a}

Note: Means in each column with different letters indicate statistical differences (Tukey $p<0.05$).

(2021) concluded that the content of phenols and flavonoids is related to the botanical origin. They assume that variations in phenolic content are related to conditions at the sampling site (country and/or region). In their study, they found that monofloral honeys can have higher phenolic content, ranging from 203 to 217.0 mg GAE 100 g^{-1}. However, they mention that multifloral honeys have been reported with values of 20.32 and 28.26 mg GAE 100 g^{-1}, respectively. These results align with those found in the honey samples evaluated in this study during the dry and rainy periods (27.31 mg GAE 100 g^{-1} and 25.32 mg GAE 100 g^{-1}). The similarity in results is attributed to the floral resources available to the bees during foraging.

The flavonoid content was higher during the dry period (0.660 mg QE 100 g^{-1}) than during the rainy period (0.507 mg QE 100 g^{-1}) ($p<0.05$). The results obtained were lower than those reported by Perna *et al.* (2013), who found values of 8.94 mg QE 100 g^{-1} in multifloral honeys and 5.49 mg QE 100 g^{-1} in monofloral (citrus) honeys. On the other hand, Cabrera *et al.* (2017) evaluated phenolic compounds, flavonoids, and antioxidant capacity in relation to color. In their study, they reported values ranging from 6.94 to 37.47 mg QE 100 g^{-1}. The variation in results can be attributed to the different floral sources and geographical origin in each study.

The calculated values for FRAP and ABTS in the evaluated periods indicate that the dry period (82.78 and 89.15 μmol TE 100 g^{-1}) was higher than the rainy period (60.84 and 84.41 μmol TE 100 g^{-1}) ($p<0.05$). These results are similar to those of Rodríguez *et al.* (2011), who evaluated the antioxidant and antimicrobial properties in multifloral and monofloral honeys from Mexico. The FRAP values for multifloral and monofloral honeys were 182.6 and 749.4 μmol TE 100 g^{-1}, respectively. Likewise, the ABTS results were 76.8 and 910.2 μmol TE 100 g^{-1}. The floral resources available to the bees are likely the reason for these differences.

Correlation Between Color and Antioxidants

The chromatic coordinates L*, a*, and b* of the color indicate a negative correlation with the flavonoid content (Table 4).

It was observed that as the values of L*, a*, and b* increased, the flavonoid content decreased. The presence of phytochemicals is greater in dark honey samples. This finding is consistent with the studies by Anklam *et al.* (1988), Frankel *et al.* (1998), and Vanhanen *et al.* (2011), who have studied and found that dark honeys have a higher concentration of minerals and pigments (phenols, flavonoids, carotenoids) that provide antioxidant properties to the honey.

Table 4. Correlation Between Color Attributes and Flavonoid Content.

Color attributes	Phenols compounds y antioxidant capacity	Goodness-of-fit-attributes		
		r	r^2	Valor p
L*	Flavonoids QE mg 100 g^{-1}	−0.753	0.568	0.001
a*	Flavonoids QE mg 100 g^{-1}	−0.867	0.752	0.001
b*	Flavonoids QE mg 100 g^{-1}	−0.797	0.636	0.001

Note: r=Correlation; r^2=Determination coefficient.

CONCLUSIONS

The physicochemical properties studied in *A. mellifera* honey showed statistical differences due to the season and time of year. However, the reported values were within the parameters established by NOM-004-SAG/GAN-2018 and CXS 12-1981. The presence of phenols and flavonoids was evidenced. The FRAP and ABTS assays confirm that throughout the year, the honey produced in the region has antioxidant properties; however, that produced in summer stands out in this regard. It is important to investigate the quality of honey in other regions of the state in order to promote its properties, generate a designation of origin, and encourage its commercialization in international markets.

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