Determination of bioactive compounds and physicochemical parameters of honey produced in the state of Veracruz, Mexico

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ABSTRACT

Veracruz occupies one of the first places as a producer of honey. However, there are few studies that address the typing of bioactive compounds, and the physicochemical characteristics present in Veracruz honey.

Objective: Determine the physicochemical and antioxidant parameters, and total phenols (TP) of Veracruz honey.

Design/methodology/approach: Honey samples were collected at 17 sites in the state of Veracruz, and physicochemical, TP and antioxidant parameters were analyzed.

Results: The determined physicochemical parameters presented values within the ranges set by NOM-004-SAG/GAN-2018 and the *Codex Alimentarius*. The color distribution showed the following values: dark (47.6%), amber (19.04%), white (19.04%), and the colors aqua white, light amber and extra light showed values of 4.77% each. Dark honeys presented ~370 μg GAE/mL, compared to the contents shown by light-colored honeys of ~200 μg GAE/mL. Sayula de Aleman honey had the highest antioxidant content with 143 μg TE/g honey. On the other hand, honey from San Pedro Soteapan showed the lowest contents (53 μg TE/g honey).

Limitations on study/implications: The selection of Veracruz honey apiaries and lack of flora information.

Findings/conclusions: The honeys presented physicochemical parameters within ranges of national standards. These Veracruz honeys exhibited a range of colors from dark to extra light. A positive correlation was shown between color and TP content. The antioxidant content was dependent on the botanical origin and color of these honeys.

Keywords: honey color, phenolic compounds, antioxidants.

Citation: Sánchez-Mundo, M. L., Nila-Méndez, G. A., Espinosa-Solis, V., Gabino-Roman, J.F., Hernández-Nava, R.G., Chipol-Pava, D.N., & Hernández-Domínguez, E. (2024). Determination of bioactive compounds and physicochemical parameters of honey produced in the state of Veracruz, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i2.2701

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: October 06, 2023.
Accepted: January 15, 2024.
Published on-line: April 01, 2024.

Agro Productividad, 17(2). February. 2024. pp: 109-117.

INTRODUCTION

Bee honey by definition is a sweet natural compound, which is produced thanks to the participation of an insect commonly known as "bee", which includes several species such as: *Apis mellifera*, *Melipona beecheii*, among others. The bee collects nectar from flowers, secretions or living parts of the plant, which can be combined with different substances from its saliva, so these are transformed and then stored in the honeycombs of the hive, where the chemical transformation culminates to become honey (*Codex Alimentarius*, 2019). This process has been identified as usually occurring in two parts: the first involves evaporation, where this nectar loses an average of a third of its moisture during storage. In the next step, the enzyme invertase hydrolyzes sucrose to produce the monosaccharides fructose and glucose (Lichtenberg-Kraag, 2014; Nicolson *et al.*, 2022; Alaerjani *et al.*, 2022).

Honey contains approximately ∼180 different molecules ranging from carbohydrates, water, organic acids, enzymes, amino acids, pigments, pollen, among others. Therefore, this gives honey a high nutritional value, in addition to attributing various bioactivity properties to it. Some studies have revealed the antioxidant effects of honey, coupled with its antimicrobial, anti-inflammatory and anti-proliferative properties (Samarghandian *et al.*, 2017; Silva *et al.*, 2021). Particularly, antioxidants are compounds that can prevent and/or inhibit free radicals, which are responsible for oxidative stress in humans and cause various diseases. It has been reported that these antioxidants could correspond to phenolic compounds, flavonoids, ascorbic acid, catalase, peroxidase, alkaloids, amino acids and chlorophyll derivatives, among others. Among the antioxidants detected in honey are α-tocopherol, carotenoids, proteins and melanoidins (Brudzynski & Miotto, 2011; Al-Farsi *et al.*, 2018). It has been proposed that the chemical composition of honey is a function of several factors, such as the flowers that the bee visits to collect nectar, beekeeping management, storage, soil chemistry, geographical conditions, and even the climatic conditions.

It is worth mentioning that for the world market, Mexican honey has great acceptance and demand due to its quality, nutritional value, pleasant sensory characteristics, and color. According to statistics, Mexico occupies ninth place as a honey producer and thirteenth as the largest exporter, with a value of ∼118.6 million dollars, with the United States, the European market (Germany, Netherlands, France, the United Kingdom, Switzerland, Belgium and Spain), Saudi Arabia and Japan as its main clients (SIAP, 2022). The main honey producing states are Yucatán, Campeche, Jalisco, Chiapas, Veracruz, Oaxaca, Quintana Roo, Puebla, Michoacán, Guerrero, Zacatecas, Morelos, Hidalgo and San Luis Potosí. The state of Veracruz occupies 5th place as one of the main honey producers in the country (SIAP, 2022).

This beekeeping activity, both in the country and in the state, is facing several problems, among which we can mention the attack of pests, particularly the Varroa destructor mite, climate change, which in recent years has caused long periods of drought, lack of infrastructure in the honey production chain, and/or an increase in the market for honey adulterated with fructose; all these factors have strongly impacted the productivity and profitability of beekeeping activity (Magaña-Magaña *et al.*, 2016). All of the above has caused the majority of apiaries to fail to standardize the quality of honey, which is why a differential number of batches arrive on the market that do not always meet the specifications required and established by the Mexican Standard (NOM-004-SAG/GAN-2018). Beekeeping in the state of Veracruz requires, in the medium term, to strengthen the honey production chain. For this, among its first actions, it is necessary to carry out work aimed at complying with the quality parameters that allow it to obtain certifications and compete in national and international markets.

Therefore, it is necessary to carry out basic research focused on establishing the bases to classify the physicochemical quality, the content of phenolic compounds, as well as the antioxidant compounds present in honey produced in the state of Veracruz.

MATERIALS AND METHODS

The honey samples were provided by producers from 17 different sites in the state of Veracruz (north, center and south); Soconusco (17° 57' 47"N - 94° 52' 50" W), Texistepec (17° 53' 43" N - 94° 49' 01" W), San Pedro Soteapan (18° 13' 48" N - 94° 52' 19" W), Cuatotolapan, Hueyapan de Ocampo (18° 08' 46" N - 95° 17' 59" W), Playa Vicente (17° 49' 48" N - 95° 48' 48" W), Villa Juanita (17° 48' 32" N - 95° 13' 11" W), San Andrés, Tuxtla (18° 26' 18" N - 95° 12' 45" W), Almagres (17° 48' 25" N - 94° 55' 02" W), Valle del Uxpanapa (17° 14' 18" N - 94° 26' 21" W), Sayula de Alemán (17° 52' 47" N - 94° 57' 30" W), Comején (18° 03' 39" N - 94° 53' 03" W), Álamo Temapache (20° 54' 22" N - 97° 41' 24" W), Hueyapan de Ocampo (18° 08' 58" N - 95° 08' 38" W), Colmena, Soconusco (17° 59' 36" N - 94° 50' 31" W), Aguilera, Sayula de Alemán (17° 48' 40" N - 95° 00' 58" W), Coatepec (19° 26' 59" N - 96° 57' 31" W), Chinameca (18° 01' 11" N - 94° 40' 44" W). Consequently, the northern zone has a semiwarm subhumid climate with sub-deciduous forest and palm grove vegetation, while the center of the state has a temperate climate with mesophilic mountain forest vegetation, and in the southeast of the state, the climate is semiwarm and humid with tropical forest vegetation, grasslands, and secondary vegetation (INEGI, 2020). These honey samples with characteristic aroma, flavor and without signs of fermentation. These were compared with two honey samples from two commercial brands: Carlota® and Vita Real®.

To determine the pH and total acidity, a HANNA 211® potentiometer was used, according to the Mexican Standard (NOM-004-SAG/GAN-2018). A portable dual ATC® refractometer was used to determine, according to the Mexican Standard (NOM-004-SAG/GAN-2018), the content of soluble solids and humidity of the collected honey samples (g/100 g honey).

The color of the honey was determined by spectrophotometry (BioRad SmartSpec Plus®; Codex Alimentarius, 2019). The readings were made at an absorbance of 560 nm and according to the Pfund scale. The extraction of total phenols (TP) was carried out using the protocol published by Elvia *et al.*, 2015, to which some modifications were made. The analysis of TPs was performed spectrophotometrically at 760 nm using the Folin-Ciocalteu method, reported by Singleton *et al.*, 1999 with some modifications. The antioxidant capacity was estimated using ABTS•+(2,2'-zino-bis(3-ethylbenzothiazolin)-6-sulfonic acid, A-1888), potassium persulfate ($K_2S_2O_8$) and as standard Trolox (6-hydroxy acid -2,5,7,8-tetramethylchromium-2-carboxylic acid 97%) as reference oxidizing agent. The antioxidant activity was expressed as grams of Trolox Equivalent (TE) per gram of honey (g TE/g honey) according to what was reported by Re *et al.*, 1999, with modifications. Assays were performed in triplicate and the results were expressed as the mean values with standard deviations (±). The significant differences represented by letters were obtained by a one-way analysis of variance (ANOVA) followed by Tukey's ($P<0.05$) and Pearson's correlation analysis to obtain the r-value with the Minitab® 17 software.

RESULTS AND DISCUSSION

The results show that honey is a food of acidic nature, having a pH range of 3.28±0.14 to 4.42±0.23, and total acidity in a range of 20±1.45 to 35±2.75. These data coincide with what was reported by (*Codex Alimentarius*, 1981; NOM-004-SAG/GAN-2018; Pauliuc & Oroian, 2020; García-Chaviano *et al.*, 2022; Table 1). Furthermore, due to the pH ranges shown in this study, it is suggested that they could be honeys with floral origins because they are within the reported pH range of Cervera & Cervera, 1994, that was between 3.4 to 4.6. The total acidity was also evaluated, which is formed by the hydroxylated acids in honey, that is, they can be both acids and alcohols (Cervera & Cervera, 1994). The honey samples analyzed present values between 20±1.45 to 35±2.75 meq/Kg, which correspond to the limits marked by the standards (*Codex Alimentarius*, 1981; NOM-004-SAG/GAN-2018). It has been proposed that these acidity ranges protect honey from pathogenic microbial growth and, in turn, may contribute to aroma. Regarding soluble solids, honey samples collected in the state presented ranges from 76±0.0 to 89.6±0.0 °Brix. These values are similar and are in the same range as those already reported (Castillo-Martínez *et al.*, 2022), with values between 77 and 86 °Brix for honey from *Apis mellifera* and *Melipona beecheii*. On the other hand, the water content present in honey should not be greater than 23%, because it is very likely that honey will ferment, change its smell, flavor, and/or tend to crystallize. The humidity range presented by the honeys analyzed in this study was from 14.0 to 20.6%, values that are within the permissible limits (Table 1), being, therefore, another good indication of the maturity of these honey samples from Veracruz (Makhloufi *et al.*, 2010).

In the case of the color of the honey samples, the results were grouped by colors, where dark tones predominated, with an average of 47.6%, followed by the amber color with 19.04%, the white color with 19.04%, for the light amber only 4.77%, similar to aqua white with 4.77%, and finally extra light amber with the remaining 4.77%. This diversity in the types of honey, where they differ in both color and flavor, is perhaps due to the floral origin of each of them (Muñoz-Jáuregui *et al.*, 2014; Ávila *et al.*, 2019; Becerril-Sánchez *et al.*, 2021; Figure 1), due to the diversity that each of the honey collection areas present and its vegetation. In this sense, for example, Alamo Temapache honey, whose color is extra light amber, also presents notes of citrus flavors, this is since it is found among *Citrus* plantations in the region.

It has been proposed that the color of honey is due to pigments in the nectar of flowers and other parts of plants (Becerril-Sánchez *et al.*, 2021). Phenolic compounds are the main factor that can give the color and flavor properties of honey.

It is worth mentioning that it has been reported that dark honeys tend to be the richest in the content of minerals (Na, K, Ca, Mg, Fe, Cu, Zn, Al, Ni, Cd and Mn) and vitamins (B and C), while light-colored honeys have a higher content of vitamin A (Solayman *et al.*, 2016, Hungerford *et al.*, 2020).

Table 1. Physicochemical properties determined from honey samples collected in the different regions of the state of Veracruz.

Collection sites (and code)	pH	Total acidity (meq/kg)	°Brix	% Moisture
Soconusco (S)	4.42 ± 0.23^{a}	31 ± 1.80^{cde}	88 ± 0.11^{bc}	16 ± 2.0^{abcde}
Texistepec (T)	4.18 ± 0.15^{abcd}	33 ± 5.03^{a}	89.6 ± 0.00^{d}	15 ± 1.0^{de}
San Pedro Soteapan (SPS)	4.07 ± 0.06^{abcd}	$30\pm.50^{fgh}$	82.6 ± 0.30^{bcd}	15.5 ± 1.4^{bcd}
Cuatotolapan, Hueyapan de Ocampo (CHO)	3.28 ± 0.14^{h}	$26\pm.36^{cdef}$	78 ± 0.11^{d}	20.3 ± 0.9^{ab}
Playa Vicente (PV)	3.82 ± 0.40^{bcdef}	$22\pm.55^{fghij}$	85.3 ± 0.21^{cd}	14.6 ± 1.8^{de}
Villa Juanita (VJ)	4.01 ± 0.17^{abcd}	30 ± 2.225^{ghij}	80 ± 0.11^{d}	18.5 ± 0.5^{abcde}
San Andrés Tuxtla (SAT)	3.90 ± 0.08^{bcdef}	31 ± 1.04^{bc}	81 ± 0.20^{d}	17.4 ± 1.5^{abcde}
Almagres (ASA)	3.60 ± 0.11^{fg}	$28\pm.43^{cde}$	78.6 ± 0.1^{bcd}	20 ± 3.0^{abc}
Valle de Uxpanapa (VU)	3.81 ± 0.05^{bcdef}	23 ± 1.2^{cdefg}	80.7 ± 0.11^{bcd}	17.8 ± 0.2^{abcde}
Sayula de Aleman (SA)	3.91 ± 0.06^{abcde}	34 ± 4.07^{c}	82 ± 0.35^{bcd}	16.4 ± 1.4^{abcde}
Comején (C)	3.57 ± 0.07^{ef}	20 ± 1.451^{cde}	79.3 ± 0.28^{bcd}	19.5 ± 1.5^{abcd}
Álamo, Temapache (AT)	4.32 ± 0.18^{ab}	28 ± 0.32^{b}	86 ± 0.00^{bc}	14 ± 0.8^{e}
Hueyapan de Ocampo (HO)	3.88 ± 0.07^{bcdef}	$30\pm.52^{cdef}$	76 ± 0.00^{a}	20 ± 3.0^{abc}
Colmena, Soconusco (CS)	4.17 ± 0.12^{abcd}	27 ± 2.6^{cd}	81.3 ± 0.00^{b}	17.5 ± 2.0^{abcde}
Aguilera (AGSA)	3.85 ± 0.09^{cdef}	30 ± 0.52^{ef}	80 ± 0.34^{cd}	18.4 ± 1.4^{abcde}
Coatepec (CO)	3.80 ± 0.11^{def}	35 ± 2.75^{efgh}	83.3 ± 0.41^{bcd}	15.2 ± 2.0^{cde}
Chinameca (CHI)	4.32 ± 0.16^{abc}	$29\pm.1.2^{defg}$	82 ± 0.23^{ab}	16.4 ± 1.4^{abcde}
Carlota (CA)®	3.85 ± 0.02^{bcdef}	26 ± 0.75^{cdefg}	78 ± 0.57^{b}	20.6 ± 1.3^{a}
Vita Real (VI)®	3.90 ± 0.74^{bcdef}	25 ± 1.29^{fghij}	81.3 ± 0.00^{bcd}	17.1 ± 0.3^{abcde}

* Note: Tukey's analysis: Means that do not share a letter are significantly different (one-way ANOVA, $p<0.05$).

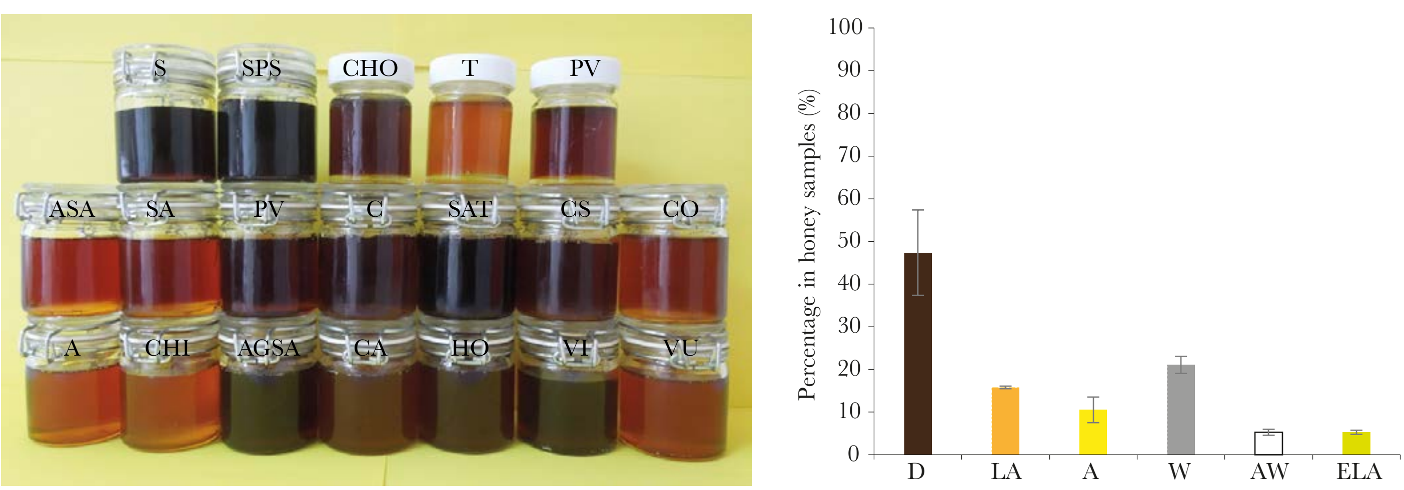

Figure 1. Classification of the color variation of honey samples collected from the different regions of the state of Veracruz. a) Color of honey samples, b) Color classification by the spectrophotometry method according to the Pfund scale. (D; Dark, LA; Light Amber, A; Amber, W; White, AW; Aqua White and ELA; Extra Light Amber).

The determination of the total phenol contents in the Veracruz honeys was carried out, and the results show approximate ranges from 200 to 370 GAE (μg gallic acid equivalent / mL) (Figure 2). The honey with the highest phenol content was the collected in Soconusco (S), with approximately 370 GAE (μg gallic acid equivalent/mL), while the lowest phenol content was found in the honey collected in the communities of Texistepec and Almagres, municipality of Sayula de Aleman, with 200~204 GAE (μg gallic acid equivalent/mL).

When comparing these results, a positive correlation (r=0.54) is shown with the color of honey, that is, the higher the phenol content, the darker the honey, and the lower the phenol content of the honey, the lighter the color.

In this sense, having honeys with high phenolic compounds can be a strong indication of the presence of a high antioxidant capacity, which results in health benefits and can even reduce cellular stress and, therefore, delay aging in these organisms (Aljadi & Kamaruddin, 2004; Rodríguez *et al.*, 2012).

These phenolic compounds contained in honey are directly related and attributed to the bioactive properties of honey (Aljadi & Kamaruddin, 2004, Becerril-Sánchez *et al.*, 2021). Consequently, it is usually one of the characteristics that is important to market and provide added value to honey. In this sense, the price of honey for the international market depends largely on its color, flavor, and moisture content (Ciappini *et al.*, 2013).

Another of the important compounds in honey are antioxidants, considered as heterogeneous substances that are made up of vitamins (A, E and C), minerals, natural

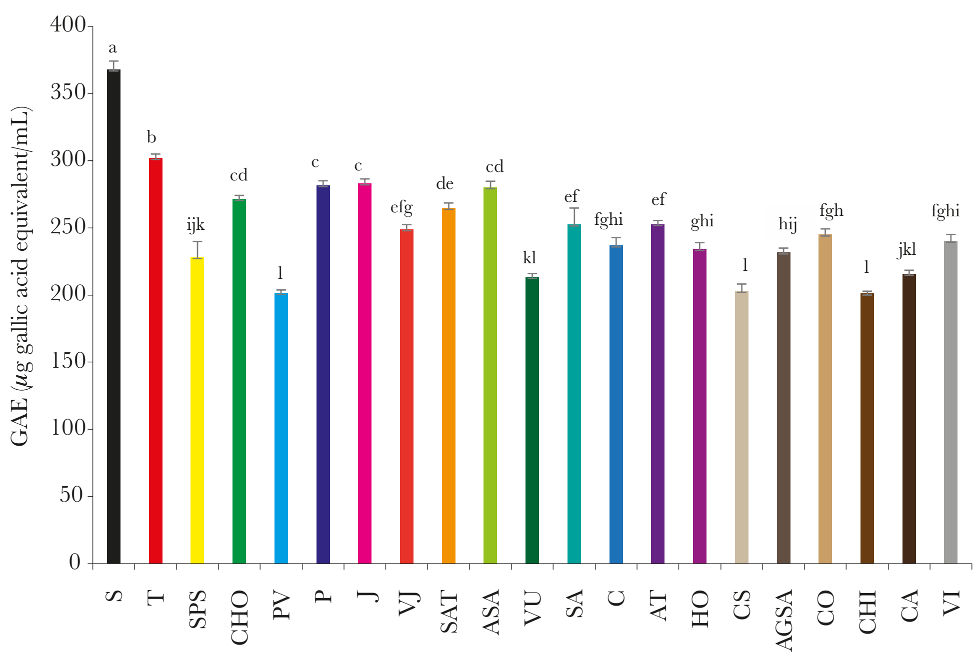

Figure 2. Determination of the total phenolic content in honey samples collected in the different regions of the state of Veracruz. Values are presented as mean ± standard deviation; furthermore, different letters in the columns indicate statistically significant differences (one-way ANOVA, $p<0.05$). (S) Soconusco, (T) Texistepec, (SPS), San Pedro Soteapan, (CHO), Cuatotolapan, Hueyapan de Ocampo, (PV), Playa Vicente, (VJ) Villa Juanita, (SAT) San Andrés Tuxtla, (ASA) Almagres, (VU) Valle de Uxpanapa, (SA) Sayula de Alemán, (C), Comejen, (AT), Álamo Temapache, (HO) Hueyapan de Ocampo, (CS) Colmena, Soconusco, (AGSA) Aguilera, (CO) Coatepec, (CHI) Chinameca, (CA) Carlota®, (VI) Vital Real®.

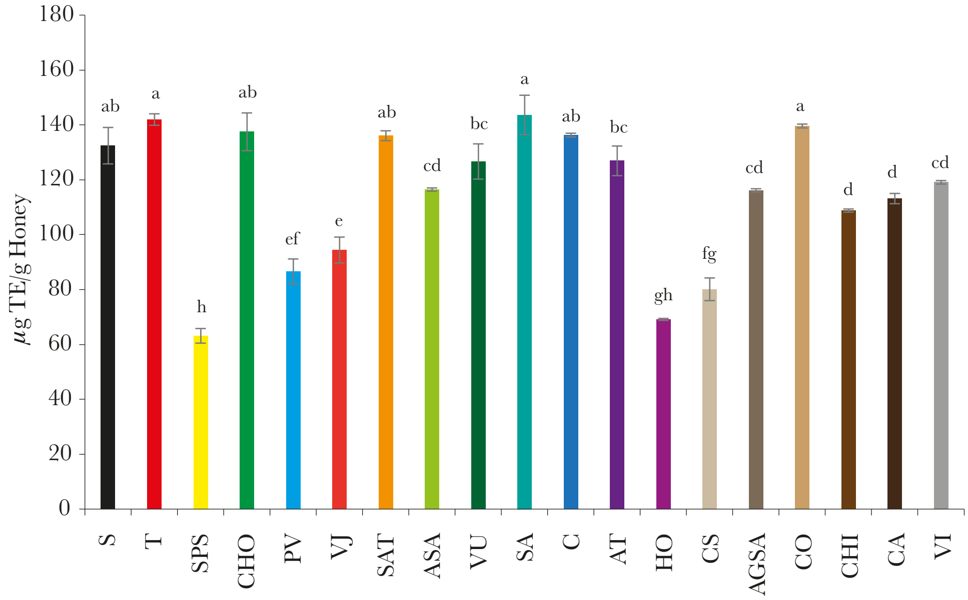

Figure 3. Determination of antioxidant content in honey samples collected in the different regions of the state of Veracruz. Values are presented as mean ± standard deviation; furthermore, different letters in the columns indicate statistically significant differences. Values are presented as mean ± standard deviation; furthermore, different letters in the columns indicate statistically significant differences (one-way ANOVA, $p<0.05$). (S) Soconusco, (T) Texistepec, (SPS), San Pedro Soteapan, (CHO), Cuatotolapan, Hueyapan de Ocampo, (PV), Playa Vicente, (VJ) Villa Juanita, (SAT) San Andrés Tuxtla, (ASA) Almagres, (VU) Valle de Uxpanapa, (SA) Sayula de Alemán, (C), Comejen, (AT), Álamo Temapache, (HO) Hueyapan de Ocampo, (CS) Colmena, Soconusco, (AGSA) Aguilera, (CO) Coatepec, (CHI) Chinameca, (CA) Carlota®, (VI) Vital Real®.

pigments (flavonoids, carotenoids, and polyphenols), enzymes (coenzymes Q, catalases, and oxidases), in addition to nitrogenous compounds, among others (Cianciosi *et al.*, 2018).

The antioxidant content of honey samples from Veracruz presented values between 52-145 μg TE/g honey. The samples that presented the highest contents were from Sayula de Aleman, Texistepec and Coatepec with values between 140,142 and 143 μg TE/g honey, respectively. While the honey samples with the lowest values were from San Pedro Soteapan and Hueyapan de Ocampo, with only 53 and 69 μg TE/g honey, respectively.

It has been reported that clover, eucalyptus and alfalfa honeys present values similar to those shown in the Veracruz honeys analyzed (Cianciosi *et al.*, 2018). Furthermore, as has been reported by various researchers, these antioxidant properties have been linked to both color and moisture content, possibly due to the presence of carotenes and flavonoids (pigments) found in the pollen of the flora where bees percolate (Contreras-Martínez *et al.*, 2020; Chuah *et al.*, 2023).

CONCLUSIONS

In general, the Veracruz honeys had good quality according to the results obtained from the physicochemical analyses, and they are also within the limits of both the national and international regulations that regulate honey. This group of honeys presents a wide spectrum of colors ranging from dark, amber, light amber, extra light to white and aqua white. The color of honey is very important at a commercial level since it defines its price and consequently strongly influences the consumer's purchasing choice. In this sense, the honeys that displayed dark colors were those with the highest contents of total phenols and antioxidants. This suggests that these compounds are directly responsible for providing greater antioxidant capacity and being involved in the color of the honey samples analyzed.

ACKNOWLEDGEMENTS

This work was carried out at the Plant Biotechnology Research Unit (UNIBVE) of the Higher Technological Institute of Acayucan (ITSA).

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