Color influence on phenolic compounds and bioactive properties of honey from Guerrero, Mexico

Influencia del color en compuestos fenólicos y propiedades bioactivas de la miel de Guerrero, México

Mendoza-Bacilio CI[1], Epifanio-Gómez R[1], Yam-Puc A[3], Avila-Caballero LP[1], Palemón-Alberto F[2], Torres-Guzmán F[4] and Bello-Martínez J[1]*

[1] Universidad Autónoma de Guerrero, Facultad de Ciencias Químico Biológicas, Av. Lázaro Cárdenas S/N Col. La Haciendita CU Sur C.P. 39070. Chilpancingo, Guerrero, México.
[2] Departamento de Agronomía, Facultad de Ciencias Agropecuarias y Ambientales de la Universidad Autónoma de Guerrero, C. P. 40020 Iguala de la Independencia, Guerrero, México.
[3] Instituto Tecnológico de Mérida. Avenida Tecnológico km. 4.5 s/n. CP. 97118. Mérida, Yucatán, México.
[4] Universidad Autónoma de Guerrero, Unidad de Ciencias Naturales. Rancho Xalaco, Petaquillas Guerrero, México.

ABSTRACT

Honey is mainly composed of glucose and fructose between 80 and 85 %, which come from the nectar collected by bees; therefore, it is considered a great caloric source. The present study aimed to evaluate the influence of color, on chemical composition and bioactive properties of polyfloral honey collected in different geographical regions of Guerrero state, Mexico. Honey samples from the 2018 harvest were analyzed to determine their total phenolic and flavonoids content, as well as their antioxidant and antimicrobial activity. The phenolic and flavonoids content varied considerably, and the highest values were obtained for dark amber honeys. Similarly, dark amber honeys showed higher antioxidant activity. The antibacterial activity was more effective against Gram-positive bacteria than Gram-negative bacteria; also, results indicated that fungi were less susceptible than bacteria. A positive correlation between color and antioxidant capacity was found. Correlation existed also between color vs phenolics content, vs flavonoid content or between phenolic vs flavonoid. Principal component analysis (PCA) on bioactive results was a useful tool to characterize different types of honey based on the variables of each group in relation to the similarity between the samples.

Key words: Antioxidant activity, Antimicrobial activity, Honey bee, PCA.

RESUMEN

La miel está compuesta principalmente por glucosa y fructosa entre un 80 y 85 %, que provienen del néctar recolectado por las abejas; por tanto, se considera una gran fuente calórica. El presente estudio tuvo como objetivo evaluar la influencia del color en la composición química y las propiedades bioactivas de la miel polifloral de varias regiones geográficas del estado de Guerrero, México. Las muestras de miel fueron cosechadas en 2018 y se analizaron para determinar su contenido total de fenoles y flavonoides, así como su actividad antioxidante y antimicrobiana. El contenido total de fenoles y flavonoides varió considerablemente y los valores más altos se obtuvieron para las mieles de color ámbar oscuro. De manera similar, las mieles de color ámbar oscuro mostraron una mayor actividad antioxidante. La actividad antibacteriana fue más eficaz contra bacterias Gram-positivas que contra bacterias Gram-negativas; Además, los resultados indicaron que los hongos eran menos susceptibles que las bacterias. Se encontró una correlación positiva entre el color y la actividad antioxidante. También existió correlación entre el color y el contenido fenólico, el contenido de flavonoides o entre fenoles y flavonoides. El análisis de componentes principales (PCA) sobre los resultados bioactivos fue una herramienta útil para caracterizar diferentes tipos de miel en función con la similitud entre las muestras.

Palabras clave: Actividad antioxidante, Actividad antimicrobiana, Miel de abeja, ACP.

INTRODUCTION

Mexico is the third largest exporter of honey. The state of Guerrero (GRO) is the sixth largest producer in Mexico, with an annual production of ~ 2,029 tons in 2019. According to the Census of Secretaría de Agricultura y Desarrollo Rural, there are >4,000 beekeeper`s in this state. The Small Coast (SC) and Big Coast (BC) mesoregions are the most productive (~53 % of the state`s total) (SIAP, 2019). The chemical composition and organoleptic properties (color, aroma, and flavor) of honeybee depend firstly on flowers, climate, and geographical regions (Kadri *et al*., 2017). Regarding composition, it has been demonstrated that some phytochemicals like phenolic compounds (phenolic acids and flavonoids) present in honey have antioxidant properties (da Silva *et al*., 2016; Deng *et al*., 2018). Also, certain enzymes (glucose oxidase and catalase), ascorbic acid, proteins and carotenoids have been associated with these properties (Alvarez-Suarez *et al*., 2010).

Other authors have also studied the correlations between color and antioxidant activity, the phenolic and flavonoid contents of honey to determine if there is a correlation with floral origin (Panseri *et al*., 2013; García-Tenesaca

*Autor para correspondencia: Jorge Bello Martínez
Correo electrónico: belloj@uagro.mx
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et al., 2018). Studies have demonstrated the antioxidant, antibacterial, anti-inflammatory and antitumoral properties of honey (Cruz *et al*., 2014; Giménez-Bastida *et al*., 2015; Bueno-Costa *et al*., 2016; Cheng *et al*., 2017; Stagos *et al*., 2018; Pereira *et al*., 2020). Honey production in Mexico has a very long tradition, dating back to ancient times (Rodríguez *et al*., 2012). However, data on these properties in Mexican honeys are limited on the composition and bioactive properties of honeys from different geographical regions in Guerrero, Mexico. It is currently very important to determine parameters in honey samples, especially due to the productive and economic relevance on the Mexican honey market. Therefore, the present study aimed to evaluate the color influence on bioactive properties of polyfloral honey collected in different geographical regions of Guerrero state, Mexico.

MATERIALS Y METHODS

Honey samples

A total of 20 polyfloral honey samples (*Apis mellifera*) were collected in autumn 2018 by beekeepers, at different geographical regions in the state of Guerrero (Mexico) (Figure 1). All samples were stored at −4 °C in amber glass vials until use. A sugar analog (SA) was used as control, and it was composed by sucrose (1.5 g), 7.5 g maltose (7.5 g), 40.5 g fructose (40.5 g), glucose (33.5 g) and 17 mL of distilled water.

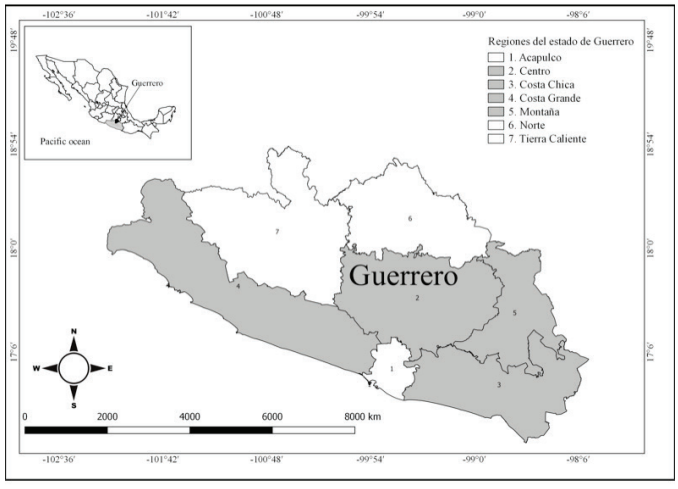

Figure 1. Mesoregions of the state of Guerrero, Mexico.
Figura 1. Regiones del Estado de Guerrero, México.

Color intensity

The color of honey samples was determined using a digital honey colorimeter (C221, Hanna® Instrument, CA, USA). The results were expressed in mm Pfund scale (0-150 mm) and named in accordance with the standard nomenclature (Ferreira *et al*., 2009).

Total phenolic content

The total phenolic content (TPC) of honey samples was determined according to the Folin-Ciocalteu's phenol (FCP) method (Singleton *et al*., 1999), with slight modifications. Briefly, 5 g of each sample was mixed with 50 mL of distilled water to obtain a stock solution (10 % w/v). The resultant solution (0.5 mL) was mixed with 2.5 mL of FCP reagent (0.2 N) for 5 min, then 2 mL of sodium carbonate (Na_2CO_3) (0.7 M) were added. The solution was incubated at 25 °C for 2 h, in the dark. Absorbance was measured at 760 nm (VIS Genesys® 20, Thermo Scientific, NY, USA). TPC was determined using gallic acid as standard and expressed as mg of gallic acid equivalents/100 g of honey (mg GAE/100 g honey).

Total flavonoids content

The total flavonoids content (TFC) of honey samples was determined by the aluminum chloride ($AlCl_3$) complex formation method (Marghitas, *et al*., 2009), with slight modifications. Briefly, 1 mL of honey solution (1 mg/mL) was mixed with 0.3 mL of sodium nitrite ($NaNO_2$) (5 %), and 0.3 mL of $AlCl_3$ (10 %) was added, after five minutes. Subsequently, after six min, the reaction mixture was neutralized with 2 mL of sodium hydroxide (NaOH) (1 M). The absorbance was measured at 510 nm (VIS Genesys® 20, Thermo Scientific, NY, USA). TFC was determined using quercetin as standard and expressed as mg of quercetin equivalents/100 g of honey (mg QE/100 g honey).

Antioxidant activity assays

Free-radical scavenging activity. Antiradical activity was determined using the stable radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) method (Rodriguez *et al*., 2012). Twenty µL of honey dissolved in methanol (10 %) was mixed with 200 µL of $DPPH^{\cdot}$ solution (150 µM, in 80 % of methanol). The absorbance was measured at 517 nm after 30 min. Results were obtained using Trolox as standard and expressed as µmol of Trolox equivalents/100 g of honey (µmol TE/100 g honey).

Trolox equivalent antioxidant capacity (TEAC) assay. The TEAC assay was conducted following the modified methodology described by Vidal-Gutierrez *et al*. (2020). The radical solution was obtained by mixing 19.3 mg of ABTS in 5 mL of H_2O, with 88 mL of $K_2S_2O_8$ solution (140 mM) and incubated for 16 h in darkness. Subsequently, the radical absorbance was adjusted to an OD of 0.7 at 730 nm. Five µL of an aqueous honey solution (50 %) mixed with 245 µL of adjusted radical, were incubated for 5 min in the dark. The absorbance was measured at 730 nm. Results were reported as µM Trolox Equivalent (µmol TE)/100 g honey.

Ferric reducing antioxidant power (FRAP) assay. The FRAP assay was performed by the previous reported method (Tuberoso *et al*., 2011). Briefly, the ferric complex was prepared by mixing 300 mM acetate buffer (pH 3.6), 2,4,6-tri(2-pyridyl)-striazine (TPTZ) (40 mM, dissolved in 40 mM HCl) and 20 mM aqueous ferric chloride ($FeCl_3$) in a 10:1:1 proportion. Then, 20 ml of an aqueous honey solution (20 %) were mixed with 280 µL of ferric complex The absorbance was measured at 630 nm in a microplate reader after 30 min of incubation in the dark. Results were expressed as µM Fe(II)/ 100 g honey.

Antimicrobial assay

Standard strains analyzed were *Escherichia coli* ATCC® 25922™ (Gram-negative bacteria) and *Staphylococcus aureus* ATCC® 25923™ (Gram-positive bacteria), maintained in tryptone soy broth (TSB) at 4 °C, as well as *Candida albicans* ATCC® 90028™ (fungal strain), maintained in Brain Heart Infusion broth (BHI) at 4 °C. The inoculums were prepared in TSB and BHI for bacteria and yeast respectively at 37 °C for 24 h. Cell suspensions were diluted in peptone water (0.1 %), to a 0.5 of McFarland scale concentration ($1.5x10^6$ CFU/mL). Minimum inhibitory concentration (MIC) was determined with adapted method by Bueno-Costa *et al*. (2016). Briefly, 10 µL of each $1.5x10^6$ CFU/mL suspension, 90 µL of Trytone TSB or BHI and 100 µL of each honey solution (12.5-400 mg/mL) were added to each pool. Negative control was distilled water, while the antibiotic ciprofloxacin (15 µg/mL) and nystatin (100 IU/mL) were used as positive control. Plates with microdilution were incubated at 37 ºC for 24 h and measured at 620 nm in a microplate reader (Thermo Scientific™ Multiskan™ FC, NY, USA).

Statistical analysis

Statistical analysis was performed through the IBM® SPSS® Statistics, 2020 software. A Principal Component Analysis (PCA), Pearson's correlation and linear regression analysis were carried out to evaluate the color influence on phenolic composition (TPC and TFC), antioxidant and antibacterial activity. The significance was set a $p < 0.05$.

RESULTS AND DISCUSSION

Color intensity

Color of honey samples were high, ranging from 17.0 to 146.0 mm Pfund, with five colors found in the studied honeys (Table 1), White (20 %), Extra Light Amber (10 %), Light amber (25 %), Amber (25 %) and Dark amber (20 %) (Table 1). This is in agreement with Bueno-Costa *et al*. (2016), who reported Light Amber color for Brazilian honeys collected at different zones. Probably the dark tone found in Mexican honey is due to the wild origin of most honey produced in the country. In Guerrero state, Mexico, there is a wide variety of vegetation, which favors the dominance of wild honey, such as Tropical Deciduous Forest, Rain Forest and Coniferous Forest. Light honeys, such as the honey from the Harenna Forest in Ethiopia, showed Pfund scales between 34 and 85 mm (Belay *et al*., 2015).

Total polyphenol content

Table 2 show the results of TPC in honey samples. The total phenolic compounds were higher in Dark Amber honey (101.5 mg GAE/100 g honey), followed by Amber (66.4 GAE/100 g honey), Light Amber (68 mg GAE/100 g honey), Extra Light (42.5 mg GAE/100 g honey) and White (17.3 mg GAE/100 g honey). These results show a high correlation observed between color and TPC ($r = 0.895$, $p < 0.01$) (Table 4) this implies that the amount and type of polyphenolic substances in honey are variable and essentially depend on the floral origin (Küçük *et al*., 2007). Similarly, the presence of higher phenolic contents in darker color honeys than lighter honeys and their strong correlations are well documented for Cuban (Alvarez-Suarez *et al*., 2010), Argentina (Isla *et al*., 2011), and Brazilian (Bueno-Costa *et al*., 2016) honeys.

Table 1. Geographical origin and Color of the honey samples from the Guerrero State, Mexico.
Tabla 1. Origen geográfico y color de las muestras de miel del estado de Guerrero, México.

Samples	Region	Pfund scale[a]	Color
C1	Central	86	Amber
C2	Central	78	Light Amber
C3	Central	40	Extra Light Amber
C4	Central	146	Dark Amber
C5	Central	100	Amber
MT6	Mountain	17	White
MT7	Mountain	21	White
MT8	Mountain	18	White
MT9	Mountain	29	White
MT10	Mountain	35	Extra Light Amber
SC11	Small Coast	88	Amber
SC12	Small Coast	94	Amber
SC13	Small Coast	120	Dark Amber
SC14	Small Coast	129	Dark Amber
SC15	Small Coast	123	Dark Amber
BC16	Big Coast	89	Amber
BC17	Big Coast	72	Light Amber
BC18	Big Coast	82	Light Amber
BC19	Big Coast	64	Light Amber
BC20	Big Coast	68	Light Amber

a: In milimeters.

Total flavonoid content

The TFC of honeys samples are displayed in Table 2. The results showed that Dark Amber honey possess a high content of total flavonoids (22.45 mg CE/100 g honey), followed by Amber (16.24 mg QE/100 g honey), Light Amber (14.77 mg QE/100 g honey), Extra Light Amber (14.77 mg QE/100 g honey) and White (9.58 mg QE/100 g honey). These results also showed a high correlation between TPC and TFC ($r = 0.814$, $p < 0.01$) (Table 4). In addition, these results showed a high correlation between color values of the honey and TFC ($r = 0.864$, $p < 0.01$) (Table 4). TFC are also related to the floral sources as discussed previously Bueno-Costa *et al*. (2016).

Antioxidant activity

In this study, three *in vitro* assays were used to determine antioxidant activity. The DPPH· radical scavenging activity varied significantly among most honey samples (Table 2). The highest antioxidant activity (24.0 **µ**M TE/100 g honey) was observed in the Dark Amber honey sample (SC13), whereas the lowest activity (2.9 µM TE/100 g honey)

Table 2. Chemical composition and antioxidant activity of the honey samples from the Guerrero State, Mexico.
Tabla 2. Composición química y actividad antioxidante de las muestras de miel del Estado de Guerrero, México.

Samples	TPC (mg GAE/100 g honey)	TFC (mg QE/100 g honey)	DPPH• (µM TE/100 g honey)	TEAC (µM TE/100 g honey)	FRAP (µM Fe(II)/100 g honey)
SA	2.0 ± 0.1	0.0 ± 0	0.0 ± 0	17.0 ± 0.5	27.0 ± 0.1
C1	60.0 ± 1	10.0 ± 0.6	8.2 ± 1	125.0 ± 15	361.0 ± 32
C2	58.0 ± 2	9.5 ± 0.8	8.4 ± 2	123.0 ± 29	345.0 ± 35
C3	50.0 ± 2	8.0 ± 0.2	7.8 ± 2	117.0 ± 11	332.0 ± 29
C4	101.0 ± 9	17.0 ± 0.1	19 ± 3	210.0 ± 34	405.4 ± 48
C5	59.0 ± 7	7.5 ± 0.3	7.9 ± 1	122.0 ± 18	367.0 ± 42
MT6	16.0 ± 8	4.0 ± 0.1	3.0 ± 2	40.0 ± 8	285.5 ± 31
MT7	19.0 ± 6	3.9 ± 0.1	2.9 ± 5	45.0 ± 5	275.6 ± 27
MT8	23.0 ± 9	3.5 ± 0.2	5.3 ± 1	86.0 ± 19	268.3 ± 17
MT9	80.0 ± 9	19.0 ± 0.9	9.1 ± 2	167.0 ± 32	295.4 ± 49
MT10	35.0 ± 4	7.1 ± 0.3	7.5 ± 1	127.0 ± 21	318.0 ± 35
SC11	65.0 ± 9	9.0 ± 0.9	10.1 ± 2	160.0 ± 29	390.0 ± 33
SC12	75.0 ± 9	9.5 ± 0.8	11.2 ± 1	189.0 ± 30	400.0 ± 17
SC13	119.0 ± 10	3.0 ± 0.1	24.0 ± 4	290.0 ± 28	465.7 ± 21
SC14	80.0 ± 9	18.0 ± 0.8	10.0 ± 1	174.0 ± 33	451.8 ± 11
SC15	106.0 ± 9	17.5 ± 0.7	21.0 ± 3	245.0 ± 25	432.1 ± 31
BC16	73.0 ± 6	7.5 ± 0.6	13.0 ± 1	185.0 ± 29	396.0 ± 26
BC17	54.0 ± 5	6.3 ± 0.5	8.7 ± 1	120.0 ± 17	370.0 ± 19
BC18	95.0 ± 4	5.8 ± 0.5	11.9 ± 3	200.0 ± 15	408.0 ± 11
BC19	63.0 ± 3	6.9 ± 0.1	8.3 ± 2	139.0 ± 29	299.0 ± 47
BC20	70.0 ± 2	7.0 ± 0.1	6.9 ± 1	148.0 ± 34	326.0 ± 39

* Values represent a mean ± SD (n = 3) of three independents experiments. SA: sugar analog, C: Central, SC: Small Coast, BC: Big Coast and MT: Mountain.

Table 3. Antimicrobial activity of honey samples from the Guerrero State, Mexico.
Tabla 3. Actividad Antimicrobiana de mieles del estado de Guerrero, México.

Samples	Antimicrobial activity (mg/mL)[a]		
	S. aureus	*E. coli*	*C. albicas*
SA	> 400	> 400	> 400
C1	75.0 ± 8.0	200.0 ± 22.0	75.0 ± 8.0
C2	75.0 ± 9.0	200.0 ± 14.0	50.0 ± 3.0
C3	50.0 ± 1.0	150.0 ± 5.0	75.0 ± 10.0
C4	12.5 ± 2.0	75.0 ± 4.0	25.0 ± 9.0
C5	75.0 ± 3.0	150.0 ± 11.0	300.0 ± 11.0
MT6	75.0 ± 5.0	150.0 ± 9.0	75.0 ± 9.0
MT7	75.0 ± 4.0	150.0 ±1 0.0	75.0 ± 9.0
MT8	50.0 ± 2.0	100.0 ± 12.0	50.0 ± 8.0
MT9	12.5 ± 1.0	75.0 ± 10.0	50.0 ± 9.0
MT10	50.0 ± 3.0	150.0 ± 22.0	75.0 ± 11.0
SC11	75.0 ± 2.0	150.0 ± 20.0	75.0 ± 10.0
SC12	75.0 ± 1.0	150.0 ± 11.0	50.0 ± 9.0
SC13	12.5 ± 2.0	50.0 ± 7.0	50.0 ± 7.0
SC14	50.0 ± 3.0	75.0 ± 10.0	75.0 ± 8.0
SC15	12.5 ± 1.0	75.0 ± 8.0	75.0 ± 5.0
BC16	50.0 ± 2.0	150.0 ± 9.0	75.0 ± 9.0
BC17	75.0 ± 5.0	150.0 ± 10.0	250.0 ± 4.0
BC18	25.0 ± 5.0	75.0 ± 12.0	50.0 ± 3.0
BC19	25.0 ± 4.0	100.0 ± 11.0	75.0 ± 9.0
BC20	75.0 ± 5.0	200.0 ± 12.0	75.0 ± 8.0

*Values represent a mean ± SD (n = 3) of three independents experiments. a: Antimicrobial activity by Minimum Inhibitory Concentration (MIC_{50}) necessary to inhibit 50 % of the microbial growth *in vitro*. SA: sugar analog.

were observed in White honey sample (MT7). Higher correlations were observed between the DPPH• activity and TPC ($r = 0.945$, $p < 0.01$), DPPH• activity and TFC ($r = 0.792$, $p < 0.01$), and between TPC and TFC ($r = 0.814$, $p < 0.01$). These results are similar with the reports of Ferreira *et al*. (2009), Alvarez-Suarez *et al*. (2010), Saxena *et al*. (2010) and Azonwade *et al*. (2018), who found that there is a positive correlation between DPPH•, TPC, and TFC.

Free radical scavenged activity of honey samples was also determined through the TEAC assay (Table 2). Results evidenced that evaluated honey exhibited scavenging activity against $ABTS^{\bullet+}$ radical, between 40.0 and 290 µM TE/100 g honey, Dark Amber showing the highest TEAC values, with a correlation found between color and TEAC ($r = 0.824$) (Table 4). The results present here are similar with those of other authors who demonstrated a correlation between honey color and TEAC (Alvarez-Suarez *et al*., 2012; Chen *et al*., 2017). Moreover, data obtained by the FRAP evaluation are presented (Table 2). The results obtained in Dark Amber exhibited a high ferric reducing activity (465.7 µM Fe(II)/100 g honey). The antioxidant properties of the Dark Amber and Amber honeys were within the ranges of the antioxidant values reported for Polish honey (Kuś *et al*., 2016). The differences in antioxidant properties of the honey samples could be due to the variations in phytochemicals of the respective plants and their geographical origins (Amarowicz *et al*., 2004; Jasicka-Misiak, 2012). Higher correlations were observed between the FRAP and color ($r = 0.903$, $p < 0.01$), TPC ($r = 0.888$, $p < 0.01$) and TFC ($r = 0.830$, $p < 0.01$). The Dark Amber honeys exhibited higher antioxidant activity ($p < 0.01$) in all the antioxidant assays.

Antimicrobial activity

All honey samples showed antimicrobial activity against the two bacteria and yeast tested (Table 3). The antibacterial activity was more effective against Gram-positive than Gram-negative bacteria. Thus, more efficient results occurred against *S. aureus* with averagely range from 12.5 to 75.0 mg/mL. With regard to gram-negative bacteria, the MIC of the studied honeys samples varied from 50.0 to 200 mg/mL. Other authors reported that Gram-positive bacteria were more sensitive to the honeys antibacterial activities than Gram-negative ones (Alvarez-Suarez *et al*., 2010; Isla *et al*., 2011). Regarding the antifungal activity against *C. albicas*, the study indicated that fungi were less susceptible than bacteria ranging from 50.3 to 300 mg/mL. The lower susceptibility of fungi to different honey samples, in comparison of bacteria, is documented (Kačániová *et al*., 2011; Al-Waili *et al*., 2013).

Table 4. Pearson's co-relation coefficient between evaluated parameters of honey samples from the Guerrero State, Mexico.
Tabla 4. Coeficiente de correlación de Pearson entre parámetros evaluados de muestras de miel del Estado de Guerrero, México.

	Color	TPC	TFC	DPPH·	TEAC	FRAP	AASA	AAEC	AACA
Color	1.000								
TPC	0.895**	1.000							
TFC	0.864**	0.814**	1.000						
DPPH·	0.809**	0.913**	0.792**	1.000					
TEAC	0.824**	0.840**	0.800**	0.786**	1.000				
FRAP	0.903**	0.888**	0.830**	0.815**	0.834**	1.000			
AASA	-0.695	-0.608	-0.535	-0.522	-0.489	-0.653	1.000		
AAEC	-0.144	-0.053	-0.160	-0.023	-0.034	-0.226	0.265	1.000	
AACA	0.204	0.212	0.087	0.140	0.046	0.202	0.007	0.511*	1.000

Pearson's co-relation between color (Color), total phenol content (TPC), total flavonoids content (TFC), antioxidant activity with (DPPH·), antioxidant activity with ($ABTS^{\cdot+}$), antioxidant activity with (FRAP), and antimicrobial activity in *Staphylococcus aureus* (AASA), *Escherichia coli* (AAEC) and *Candida albicans* (AACA).
*Significant at $p < 0.05$, ** Significant at $p < 0.01$.

However, other factors, in addition to the phenolic composition, such as the presence of hydrogen peroxide, catalase and glucose oxidase, which are known to be present in honeys of diverse origins (Stagos *et al*., 2018), may have contributed to the antimicrobial activity of the studied honeys. Moreover, the presence of a high content of flavonoids could contribute to its bioactivity. The same samples also showed the best results with regard to antioxidant activities, both of light amber color and showed intermediate compounds of phytochemicals. Moreover, the more antimicrobial properties of some of the honeys could be due to their higher phenolic content and antioxidant properties. The strong relations of antimicrobial properties of honeys with their antioxidant properties and phenolic contents is well discussed (Isla *et al*., 2011).

PCA analysis

Two major factors were extracted using PCA and the results are shown in Figure 2. These main components (PC1 and PC2) explained 60.9 and 17.5 % of the variability, respectively. The screen graph suggested that PC1 contained most

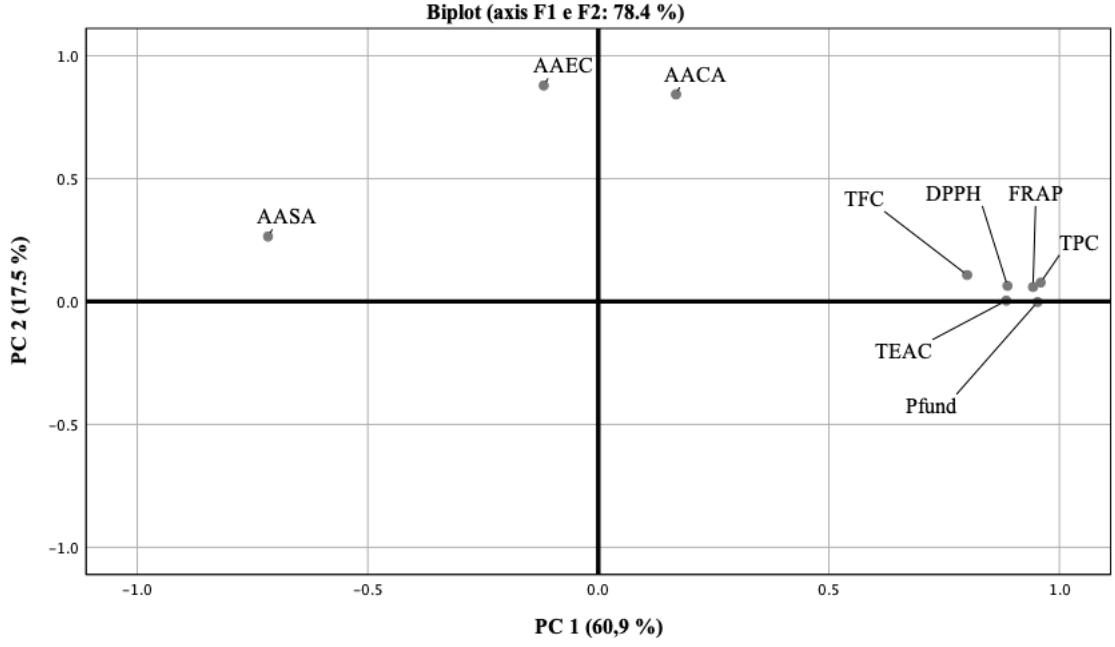

Figure 2. Principal component analysis plot of the evaluated parameters.
Figura 2. Gráfico de componentes principales de los parámetros evaluados.

of the information, followed by PC2. The important variables in PC1 were Color, Total Phenol Content (TPC), Total Flavonoid Content (TFC) and Antioxidant Activity with, $DPPH^{\cdot}$, TEAC and FRAP and finally PC2 was influenced by the antimicrobial activity against *S. aureus* (AASA), antimicrobial activity against *E. coli* (AAEC) and antimicrobial activity against *C. albicas* (AACA). A regression analysis was performed and Pearson's correlation coefficients were calculated to determine in detail the correlations between the variations in the biological properties of the samples. The graphs of the first two components clearly indicated that the darker honeys had higher content of phenol, flavonoids and antioxidant capacity than the lighter honeys, and that the antioxidant capacity was strictly related to the total phenolic content (Fig. 2). The multivariate linear analysis showed a high association between variables ($p \leq 0.01$). Color was correlated with total phenols ($r = 0.895$), total flavonoids ($r = 0.864$) and antioxidant capacity ($DPPH^{\cdot}$ $r = 0.809$), (TEAC $r = 0.824$) 1 and (FRAP $r = 0.903$). No statistically significant correlations were found between color and antimicrobial activity.

CONCLUSION

Dark amber honey shows the highest antioxidant activity values. Strong correlations were shown between phenolic content, antioxidant activity and color, showing that TPC, TFC and antioxidant activity are higher in dark honeys. On the other hand, the antimicrobial activity, especially with gram-positive microorganisms such as *S. aureus*, suggests that the analyzed honeys may play a relevant role as natural antibacterial products to reduce the effects of bacterial infections and contribute to better treatment.

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