



ANTIOXIDANT CAPACITY AND PHENOLIC CONTENT OF BEE HONEY PRODUCED IN ZACATECAS, MEXICO

CAPACIDAD ANTIOXIDANTE Y CONTENIDO FENÓLICO DE MIEL DE ABEJA PRODUCIDA EN ZACATECAS, MÉXICO

Cristina S. Contreras-Martínez, Juan P. Macías-Nieves,
J. Manuel García-González, Verónica I. Trejo-Guardado y José Carranza-Concha*

Universidad Autónoma de Zacatecas, Programa Académico de Nutrición. Ejido La Escondida, Zacatecas, Zacatecas, México.

*Corresponding author (joseconcha10@hotmail.com)

SUMMARY

Bee honey has been used since ancient times due to its good nutritional and therapeutic properties. It is produced worldwide and plays an important role as an antioxidant, anti-inflammatory and antibacterial agent; it also increases the adherence of skin grafts and the process of wound healing. The relationship between the consumption of certain foods and health derives from the presence of a series of nutrients in these foods, as well as other non-nutritive elements, yet with a key role in the prevention of certain diseases. Polyphenols are bioactive compounds found in food that contribute to improving various physiological activities, playing an antimicrobial role, with anti-inflammatory, antitumor, and anticancer effects. The objective of this research was to determine the antioxidant activity and total phenolic content of bee honey produced in Zacatecas, Mexico. Four varieties of bee honey [multiflora, mezquite (*Prosopis laevigata*), gatuño flower (*Mimosa* spp.), and creamy gatuño flower (*Mimosa* spp.)] were analyzed in terms of antioxidant capacity (methods ABTS^{•+}, DPPH and FRAP) and the total phenolic content (Folin-Ciocalteu method) by two extraction procedures (methanolic and water extraction). Data were submitted to one-way analysis of variance. Statistically significant differences ($P \leq 0.05$) were found among the samples in terms of the total phenolic content, pH, ° Brix and water content, as well as in antioxidant capacity through the ABTS^{•+} and FRAP methods. Methanolic extraction showed the highest total phenolic content (144 mg of GAE 100 g⁻¹), while the water extraction showed the highest antioxidant capacity when analyzed through the DPPH method (1118.3 µmol equivalents of Trolox 100 g⁻¹).

Index words: *Apis mellifera*, antioxidants, polyphenols, Zacatecas.

RESUMEN

La miel de abeja se ha utilizado desde la antigüedad por sus buenas propiedades nutricionales y terapéuticas. Se produce en todo el mundo y juega un papel importante como agente antioxidante, antiinflamatorio y antibacteriano; también aumenta la adherencia de los injertos de piel y el proceso de curación de heridas. La relación entre el consumo de ciertos alimentos y la salud deriva de la presencia en éstos de una serie de sustancias nutritivas y otras no nutritivas, pero con un papel clave en la prevención de determinadas enfermedades. Los polifenoles son compuestos bioactivos que se encuentran en los alimentos, contribuyen a mejorar diversas actividades fisiológicas, desempeñando un papel antimicrobiano, con propiedades antiinflamatorias, antitumorales y anticancerígenas. El objetivo

de esta investigación fue determinar la actividad antioxidante y el contenido fenólico total de miel de abeja producida en Zacatecas. Se analizaron cuatro variedades de miel de abeja [multiflora, mezquite (*Prosopis laevigata*), flor de gatuño (*Mimosa* spp.) y flor de gatuño cremosa (*Mimosa* spp.)] en términos de capacidad antioxidante (métodos ABTS^{•+}, DPPH y FRAP) y el contenido fenólico total (método Folin-Ciocalteu) por dos procedimientos de extracción (extracción metanólica y acuosa). Los datos se sometieron a análisis de varianza de una vía. Se encontraron diferencias estadísticamente significativas ($P \leq 0.05$) entre las muestras en términos de contenido fenólico total, pH, °Brix y humedad, así como en la capacidad antioxidante por los métodos ABTS^{•+} y FRAP. La extracción metanólica mostró el contenido fenólico más alto (144 mg of GAE 100 g⁻¹), mientras que la extracción acuosa presentó la mayor capacidad antioxidante cuando fue analizada con el método DPPH (1118.3 µmol equivalentes de Trolox 100 g⁻¹).

Palabras clave: *Apis Mellifera*, antioxidantes, polifenoles, Zacatecas.

INTRODUCTION

Honey bees are bees from the single genus *Apis* from the *Apini* tribe, where the most popular subspecies is *Apis mellifera* (Penk Kek *et al.*, 2014). Nowadays, bees are at home in almost all habitats in the world, from the equatorial rainforests and tropical deserts to the Sub-arctic regions of Eurasia and North America (Bankova *et al.*, 2018). According to the Codex Alimentarius (2001), honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature (Nordin *et al.*, 2018).

Honey has been known for its nutritional and therapeutic values thanks to the benefits of its consumption as well as the use and enrichment of other products (Alqarni *et al.*, 2016). China, Turkey, Argentina, Iran, Ukraine, the United

States of America, India, the Russian Federation, Mexico, and Ethiopia were the main producers of honey in 2018. Mexico is the ninth-largest producer in the world with an annual production of 64,253.04 t of honey (FAOSTAT, 2018). The state of Zacatecas, one of the main producers of honey in Mexico, produced in 2016 1929 t, increasing this amount the following year (2078 t) (SIAP, 2019). In the state of Zacatecas, different types of honey are produced, including Mezquite honey (white), Multiflora honey (variable color), and Gatuño honey (dark color), however, there is little information about them.

There are about 320 different varieties of honey from various floral sources, so that the taste, color and smell of a specific type of honey depend on the various sources of liquid flowers and plants visited by the bee. The different types of honey are comparable in terms of temperature, rainfall, seasonal, and climatic changes, while the color of honey varies from light brown to dark brown depending on where the bees pollinated, differing in organoleptic properties as well as chemically from one country to another, even in different regions within the same country due to its floral origin, soil composition and other factors (Meo *et al.*, 2017). Honey contains approximately 20 % moisture, monosaccharides (75 % glucose and fructose), disaccharides (3 - 10 % sucrose) and proteins, vitamins and minerals, as well as antioxidants (Alqarni *et al.*, 2016).

Antioxidants are an element that can inhibit the oxidation of other molecules. Oxidation is a biochemical reaction that generates free radicals that may harm the cells, tissues, and ultimately the physiological functions. Antioxidants such as vitamin C terminate the chain reactions to protect the body from free radicals. To balance the oxidative status, the human body maintains complex systems of overlapping antioxidants. The food containing antioxidants has been shown to improve health (Meo *et al.*, 2017).

The term 'phenolic' or 'polyphenol' can be defined chemically as a substance that possesses an aromatic ring bearing one or more hydroxyl substituents including functional derivatives (esters, methyl esters, and glycosides). These bioactive substances are widely distributed in the plant kingdom and are closely associated with the sensory and nutritional quality of fresh and processed plant food (Román-Cortés *et al.*, 2018). Some phenolic compounds are extremely widespread, while others are specific to certain plant families or are found only in certain plant parts or at specific developmental stages (Hossen *et al.*, 2017).

Different solvents and techniques of extraction could lead to different compositions of phenolic compounds in extracts because the solubility of each compound

in a given solvent would be quite different, this implies that the phenolic compounds with more hydrophobic characteristics might occur in lower amounts than those with hydrophilic characteristics, consequently, the bioactivity of an extract might also be affected (Lou *et al.*, 2014).

Due to the above and in the absence of information about the functional value of the different varieties of honey produced in Zacatecas, this research paper aims to determine the antioxidant capacity and the total phenolic content in the honey varieties multiflora, mesquite, gatuño flower and creamy gatuño flower using water (Kavanagh *et al.*, 2019) and methanolic (Tomás-Barberán *et al.*, 2001) extraction for bioactive compounds.

MATERIAL AND METHODS

Raw material

Multiflora, mesquite, gatuño, and creamy gatuño kinds of honey were obtained on the limits of the locality of Las Norias, in the municipality of Guadalupe, Zacatecas (22° 52' 56.0" N, 102° 23' 20.0" W). The creamy gatuño honey was mechanically mixed with a blender at room temperature until the desired consistency was obtained. Samples were analyzed for pH, °Brix, moisture content, as well as the total phenolic content and antioxidant activity. A pH meter (Ohaus, Parsippany-Troy Hills, NJ, EUA) was used to measure the pH of a 10 % (w/v) solution of honey prepared in deionized water (Kavanagh *et al.*, 2019). °Brix and moisture content (from refraction index, using the Wedmore, 1955 Table) of all honey samples was measured at 20 °C in triplicate using an ATAGO® refractometer (NAR 3T, Tokyo, Japan).

The extraction for the quantification of the total phenols (CFT) and determination of the antioxidant capacity were carried out by an adaptation of the method described by Tomás-Barberán *et al.* (2001). For this, 1 g of honey was mixed with 25 mL of a solution of MeOH and 6 N HCl 80:20 (v/v), dissolved with continuous agitation for 30 min at room temperature, and then filtered on Wathman 1 paper (methanolic extraction). A second extraction was performed according to Kavanagh *et al.* (2019) diluting 1g of honey in 20 mL of deionized H₂O (water extraction) with continuous agitation for 30 min at room temperature, it was then filtered on Wathman paper 1.

Total phenolics (TPC)

Total phenolic content (TPC) was quantified using the Folin-Ciocalteu test (Li *et al.*, 2006); 250 µL of the extract was mixed with 15 mL deionized water and 1.25 mL of

Folin- Ciocalteu phenol reagent (Sigma-Aldrich, MO, USA). After 5 min 3.75 mL of Na_2CO_3 (7.5 %) was added and leveled to 25 mL with deionized water. Absorbance was measured at 765 nm in a spectrophotometer UV-Vis (Thermo Scientific 10S, Thermo Fisher Scientific Inc, Waltham, MA, USA) after a 2.5 min reaction at 20 °C. The results were reported as mg of gallic acid (mg GAE 100 g⁻¹).

Antioxidant capacity (AC)

Regarding the antioxidant capacity (CA), this was quantified by the spectrophotometric technique of ABTS•+ (Re *et al.*, 1999), DPPH (Brand-Williams *et al.*, 1995) and FRAP. In all cases, the results were expressed in micromoles of Trolox (TEAC) in 100 g of sample.

ABTS•+ radical scavenging ability

The same extract obtained for TPC quantification was used to evaluate AC. The AC was determined through a modification of the spectrophotometric technique developed by Re *et al.* (1999), using 7 mM ABTS•+ radical (Sigma) generated by 2.45 mM potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$). The mixture of the honey remained in the dark at room temperature (~20 °C) for 16 h before use, and then the ABTS•+ solution was diluted to give an absorbance of 0.7 ± 0.1 at 734 nm. Afterwards, 100 µL of honey extract were mixed with 900 µL of the ABTS•+ diluted solution, incubated 2.5 min at 20 °C the absorbance was measured at 734 nm. The results were expressed as antioxidant activity equivalent to µmol units of Trolox (TEAC) 100 g⁻¹. All the experiments were replicated thrice.

DPPH radical scavenging activity

Also, a slightly modified version of the method described by Brand-Williams *et al.* (1995) to analyze the AC of the samples was used: 100 µL of honey extract was added to 1 mL of 2,2-diphenyl- 1-picrylhydrazyl (DPPH) mM (3 mg 100 mL⁻¹ in methanolic solution). The scavenging activity of the free radicals, using the free radical reaction DPPH, was evaluated by measuring the absorbance at 515 nm, after a 2.5 min reaction at 20 °C, in a spectrophotometer. Results were expressed in µmol equivalents of Trolox (TEAC) 100 g⁻¹.

FRAP (ferric reducing antioxidant power)

The FRAP assay was carried out based on the procedure described in the literature (Benzie and Strain, 1996). Briefly, the FRAP reagent was prepared with 2.5 mL of sodium acetate buffer (300 mmol L⁻¹, pH 3.6), 2.5 mL of 10 mmol L⁻¹ TPTZ solution (40 mmol L⁻¹ HCl as solvent) and 25 mL of 20 mmol L⁻¹ iron (III). The FRAP reagent was prepared

freshly every day and warmed to 37 °C in a water bath prior to use. One hundred µL of the diluted sample were added to 1 mL of the FRAP reagent. The absorbance of the mixture was measured at 593 nm after 30 min. The results were expressed as antioxidant activity equivalent to µmol units of Trolox (TEAC) 100 g⁻¹. All the experiments were replicated thrice.

The experimental design was completely randomized with three replications. All analyses were carried out in triplicate and the results expressed as mean and standard deviation ($\bar{X} \pm \text{SD}$). To determine statistically significant differences between the data of the variables in the honey varieties, a one-way ANOVA was carried out and, if significant, a Tukey test was applied ($P \leq 0.05$). The values of antioxidant activity and total phenol content were analyzed using the Pearson correlation. All statistical analyses were performed using Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Table 1 shows the results of the physicochemical analysis of the four honey varieties analyzed, where it can be observed that there were significant differences between them, in °Brix, pH, and moisture content.

The data obtained concurs with that reported by Alqarni *et al.* (2016) who found values of 70 to 85 °Brix in 23 samples of honey, from countries such as Germany, Malaysia, Egypt, Australia, New Zealand, Yemen, and Saudi Arabia. With regard to water content, Alqarni *et al.* (2016) reported from 12.2 to 27.4 % as well as values of 3.03 to 4.76 in pH, while Leyva-Daniel *et al.* (2017) found 18.4 % moisture and a pH of 3.8. Kavanagh *et al.* (2019) reported a pH range of 3.24 to 4.84, as well as 65.42 to 85.42 in °Brix and a moisture content of 12.9 to 24.2 %.

The soluble solids serve as an indicator parameter of the rate in solution solids such as sugars, organic acids, and minerals, nonetheless directly related to sugars and the water levels in the samples, being related to higher water content with lower sugar content (Biluca *et al.*, 2016). According to the standards of the Codex Alimentarius Committee on Sugars (2001), the minimum amount of reducing sugars is 60 g 100 g⁻¹ for floral honey. The sugar composition of honey is influenced by the types of flowers used by the bees, as well as regions and climate conditions (Da Silva *et al.*, 2016).

The pH value in honey is not directly related to free acidity because of the buffering action of various acids and minerals present. The pH of honey varied from 3.42 to 6.10 (Alqarni *et al.* 2016). The pH is a useful criterium of

Table 1. Mean values and standard deviation (n = 3) in °Brix, pH and moisture content (% Xw) of honey samples.

Honey variety	°Brix	pH	% Xw
Multiflora	79.46 (0.06) a	3.426 (0.005) a	18.96 (0.02) d
Mezquite	80.00 (0.05) b	3.443 (0.005) a	18.23 (0) c
Gatuño	82.66 (0.28) c	4.486 (0.011) b	15.60 (0.30) b
Creamy gatuño	83.53 (0.06) d	4.493 (0.005) b	14.65 (0.02) a

Means with equal letters in the same column are not statistically different according to the ANOVA (Tukey test, $P \leq 0.05$).

possible microbial growth. Most bacteria grow in a neutral and mildly alkaline environment, while yeasts and molds grow in acidic ones (Alqarni *et al.*, 2016). Also, pH is used for discrimination between honeydew (high pH values) and blossom honeys (low ones). There is no standard in terms of pH for honey (Vit *et al.*, 2004).

Water content in honey is responsible for its stability against fermentation and granulation. Normally ripe honey has a moisture content below 18.6 % (Alqarni *et al.*, 2016). In 2001, the Codex Alimentarius was revised and the parameters for honey quality were set (International Honey Commission, 2009). Good quality honey should have a moisture content that is no more than 20 g 100 g⁻¹ (Nordin *et al.*, 2018). Honey with a high-water content translates to a greater fermentation potential, resulting in difficult preservation and storage (Nordin *et al.*, 2018). Water content is the second largest constituent of honey and is closely related to several factors, such as the floral and geographical origin of the nectar, soil characteristics, climatic conditions, conditions during harvesting, degree of maturation, manipulation by beekeepers during harvest, extraction, processing methods and storage conditions (Bobis *et al.*, 2020).

Mexico has a wide variety of honey due to its diverse climates and flora, which depend directly on the geographic origin, resulting in honey with particular physicochemical parameters for each blooming process. These parameters are dependent on pollen grains, allowing for honey classification in unifloral and multifloral (Frausto-Reyes *et al.*, 2017). Zacatecas is located in the northern region of Mexico, this region is characterized by xerophytic vegetation and large areas of grassland, thorny and coniferous forests. It also includes irrigated agricultural areas for export products, which has facilitated the development of pollination with bees. Honey production in this region is considered to be of excellent quality and takes place from March to May and from August to October (Martínez-Pérez *et al.*, 2017). Three-fourths of the territory of the state of Zacatecas (56,463 km²) correspond to arid zones and shrubs (Mata-Páez *et al.*, 2016).

Figure 1 shows the values in total phenols, where a very

marked difference from the honey of gatuño and gatuño creamy can be seen. In addition, a better extraction of the phenolic compounds was observed using the methanolic extraction, especially in Gatuño honey. The values were 56.5 mg of GAE 100 g⁻¹ in Mezquite honey, 67.9 mg of GAE 100 g⁻¹ in multiflora honey, as well as 141 and 144 mg of GAE 100 g⁻¹ in honey of gatuño and creamy gatuño, respectively, when the methanolic extraction was applied, while with water extraction values of 50.47 were obtained in the multiflora honey, 55.8 in Mezquite honey, as well as 108 and 102 mg of GAE 100 g⁻¹ in gatuño flower and creamy gatuño flower.

Phytochemical extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used for extraction. The presence of different antioxidant compounds with diverse chemical characteristics and polarities may or may not be soluble in a particular solvent. Polar solvents are frequently used for recovering polyphenols from plant matrices. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, or ethyl acetate. Methanol has been generally found to be more efficient in the extraction of lower molecular weight polyphenols. The combined use of water and organic solvent may facilitates the extraction of chemicals that are soluble in water and/or organic solvent (Do *et al.*, 2014).

On the other hand, the result of the Folin-Ciocalteu reaction in honey samples should be interpreted as a quantitative estimation of total phenols because the reducing sugars of honey can also react with the Folin-Ciocalteu reagent (Alves *et al.*, 2013). Reductive sugars (such as fructose, glucose, and sucrose), organic acids (such as ascorbic, citric, and tartaric acids), ferrous sulfate, and sodium sulfite are potentially capable of interfering with the assessment of phenolic compounds in food matrices by the FC assay (Bridi *et al.*, 2017). However, the cleaning process during Amberlite XAD-2 or PVPP (Polyvinylpolypyrrolidone) extraction can remove sugars and other polar compounds, including glycosylated phenolic compounds, which can also contribute to the antioxidant capacity of honey, sugars and other compounds in the entire honey may have a good reducing capacity (Ferreira *et al.*, 2009).

Leyva-Daniel *et al.* (2017) found 29.89 mg of GAE 100 g⁻¹, while Alqarni *et al.* (2016) from 42 to 84 mg of GAE 100 g⁻¹ in honeys from different countries, Kavanagh *et al.* (2019) reported a range from 2.59 to 81.10 mg of GAE 100 g⁻¹ in Irish multiflora honey, while Al-Mamary *et al.* (2002) found a range between 56.3 and 246.2 mg of GAE 100 g⁻¹ in nine varieties of honey produced in Yemen. Peng-Kek *et al.* (2014) found a range from 51.04 to 105.88 mg of GAE 100 g⁻¹ in Malaysian honey. A higher phenolic content was observed in gatuño flower honey, which has a darker color than the mezquite and multiflora varieties which are pale yellow, agreeing with those found by Alqarni *et al.* (2016) who observed that darker honeys, e.g. Acacia, Manuka and Tualang seemed to have more phenolic compounds than light ones.

Many phenolic compounds are found in honey with different quality and quantity according to the floral source. Total phenolic content is a good criterion to determine the quality and curative properties of honey (Al-Mamary *et al.*, 2002). Among the phytochemical compounds present in honey, the phenolic compounds play a major role in the antioxidant activity. The phenolic compounds found in honey are free phenols, phenolic acids, polyphenols (usually in the form of flavonoids), anthocyanins, procyanidins, and pigments (Oroian and Ropciuc, 2017). Polyphenols mainly exert their antioxidant activity by neutralizing free radicals, which is accomplished by donating an electron or hydrogen atom (Hossen *et al.*, 2017).

Table 2 shows the values of the antioxidant capacity of the honey samples analyzed, where significant differences can be observed between the samples. There were also differences between the extraction methods. The methanolic extraction showed a higher yield in the gatuño flower samples when the antioxidant capacity was analyzed with the ABTS•+ method; on the contrary, the extraction did not suppose differences between the multiflora honey samples, neither between the mezquite honey samples analyzed by the ABTS•+. In contrast, when the antioxidant capacity was measured with the DPPH and FRAP methods, greater antioxidant power was observed when water extraction was used.

The ferric reducing antioxidant power (FRAP) assay is a measure of overall antioxidant capacity, while 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and high Trolox (a vitamin E analog) equivalent antioxidant capacity (TEAC) assays measure capacity to scavenge radicals. All are recognized as valid methods used to determine the antioxidant activity of food and beverages.

The composition of a particular honey sample greatly depends on the composition of nectar, whence it originates. Polyphenols have been recognized as the main responsible for the antioxidant activity of honey that is mainly associated with the ability of free radical scavengers, through the formation of more stable and less toxic molecules. Phenolic compounds stabilize free radicals when they give off hydrogen from one of their hydroxyl group; the degree of activity is related to the

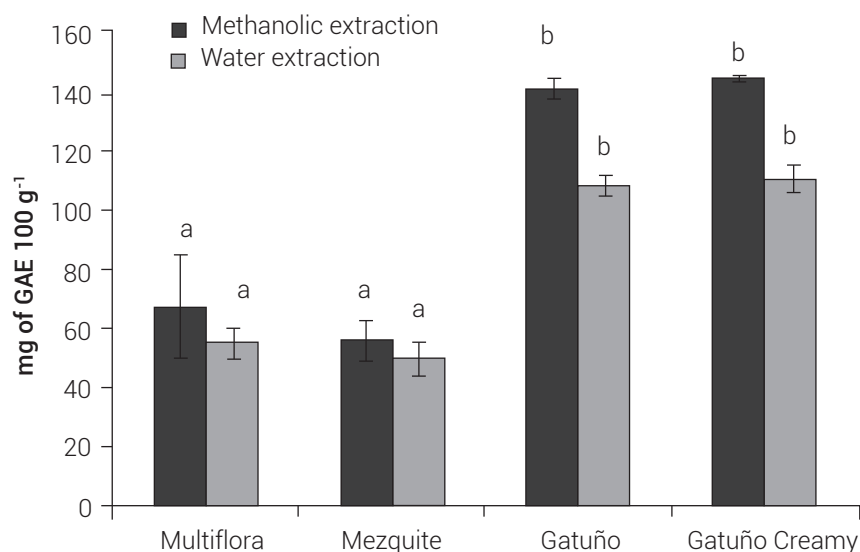


Figure 1. Total phenolic content of the different honey samples analyzed according to the extraction method. Means with equal letters between the bars for the same extraction are not statistically different according to the ANOVA (Tukey test, $P \leq 0.05$).

Table 2. Mean values and standard deviation (n = 3) in the antioxidant capacity of honey [μmol equivalents of Trolox (TEAC) 100 g⁻¹].

Extraction	Honey variety	ABTS•+	DPPH	FRAP
Methanolic extraction	Multiflora	16.67 (2.61) a	387.07 (53.03) b	70.4 (22) a
	Mezquite	16.03 (3.41) a	282.11 (26.62) a	71.5 (14.2) a
	Gatuño	308.75 (9.32) b	306.3 (18.8) ab	624.1 (44.7) b
	Gatuño Creamy	325.03 (11.73) b	333.3 (11.2) ab	647.80 (8.03) b
Water extraction	Multiflora	16.56 (3.68) a	1097.3 (83.8) a	215.7 (28.8) a
	Mezquite	31.71 (14.04) a	1063.1 (61.3) a	205.9 (12.9) a
	Gatuño	183.09 (22.91) b	1118.3 (55.6) a	680.4 (49.9) b
	Gatuño Creamy	179.96 (9.85) b	1113.1 (41.3) a	774.8 (18.7) c

Means with equal letters in the same column under the same extraction are not statistically different according to the ANOVA (Tukey test, $P \leq 0.05$).

number of their hydroxyl groups (Cianciosi *et al.*, 2018). The natural antioxidants, especially flavonoids, exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, anti-allergic, anti-thrombotic, and vasodilatory actions.

Phenols are very efficient scavengers of peroxy radicals because of their molecular structures which include an aromatic ring with hydroxyl groups containing mobile hydrogens. Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ions which catalyzes lipid peroxidation. The differences in activities of antioxidants depend on structural dissimilarities, primarily the degree of hydroxylation and methylation of the compounds (Al-Mamary *et al.*, 2002). The AC of honey is given primarily by phenolic compounds, but enzymes, amino acids and carotenoids also contribute to this ability. Radical scavenging and protection against the lipid peroxidation of honey can reduce and prevent diseases and physiological situations where oxidative stress plays an important role (Cianciosi *et al.*, 2018).

Analysis of the correlation between the antioxidant capacities and total phenolic contents of the honey samples showed a positive correlation ($R = 0.9692$ ABTS•+) ($R = 0.8488$ FRAP). High TPC honeys have been shown to exhibit significant antioxidant capacity using the FRAP, DPPH, and TEAC assays (Alvarez-Suarez *et al.*, 2013). Literature suggests that honey contains potent antioxidative agents. The role of honey also depends on its concentration and its geographical origin. As an antioxidant, honey has numerous preemptive properties against many clinical conditions such as inflammatory disorders, coronary artery diseases, neurological worsening, aging and cancer. The increase in phenolic compounds in honey provides antioxidant properties (Meo *et al.*, 2017). The composition of honey is difficult to define since it is a mixture of different active compounds and it depends on

several factors. They contain almost the same phenolic profile, including p-coumaric acid, eugenol, ferulic acid, caffeic acid and flavonoids like pinobanksin, pinocembrin, chrysin, quercetin, apigenin and naringin in different concentration (Badolato *et al.*, 2017). As honey samples are different and may not contain all of the polyphenols described and because not all polyphenols exert the same protective effects, consumption of a wide variety of honey samples at any one time is recommended (Hossen *et al.*, 2017).

CONCLUSIONS

Gatuño flower and creamy gatuño flower honey have a greater total phenolic content compared to Mezquite and multiflora varieties. The total phenolic content of honey correlated positively with its antioxidant capacity. Methanolic extraction showed better performance in total phenols and antioxidant capacity (ABTS•+) while water extraction did when DPPH and FRAP methods were applied. Climatological, geographical factors, as well as the type of flora, influence and favor the sensory, physical-chemical and functional diversity of honey. In this sense, honey produced in the state of Zacatecas can compete commercially with other national or even international honeys and at the same time contribute to the ecological balance through the practice of beekeeping.

ACKNOWLEDGEMENTS

Authors acknowledge the Research and Food Safety Laboratory of the Nutrition Department of the Autonomous University of Zacatecas for their technical support and allowing us the use of their facilities. Authors also acknowledge Miguel Angel Bernal Rayas.

BIBLIOGRAPHY

- Alqarni A. S., A. A. Owayss and A. A. Mahmoud (2016) Physicochemical characteristics, total phenols and pigments of national and international honeys in Saudi Arabia. *Arabian Journal of Chemistry* 9:114-120, <https://doi.org/10.1016/j.arabjc.2012.11.013>
- Al-Mamary M., Al-Meer A. and Al-Habori M. (2002) Antioxidant activities and total phenolics of different types of honey. *Nutrition Research* 22:1041-1047, [https://doi.org/10.1016/S0271-5317\(02\)00406-2](https://doi.org/10.1016/S0271-5317(02)00406-2)
- Alvarez-Suarez J. M., F. Giampieri and M. Battino (2013) Honey as a source of dietary antioxidants: Structures, bioavailability and evidence of protective effects against human chronic diseases. *Current Medicinal Chemistry* 20:621-638, <https://doi.org/10.2174/0929867311320050005>
- Alves A., A. Ramos, M. M. Gonçalves, M. Bernardo and B. Mendes (2013) Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. *Journal of Food Composition and Analysis* 30:130-138, <https://doi.org/10.2174/0929867311320050005>
- Badolato M., G. Carullo, E. Cione, F. Aiello and M. C. Caroleo (2017) From the hive: Honey, a novel weapon against cancer. *European Journal of Medicinal Chemistry* 142:290-299, <https://doi.org/10.1016/j.ejmech.2017.07.064>
- Bankova V., M. Popova and B. Trusheva (2018) The phytochemistry of the honeybee. *Phytochemistry* 155:1-11, <https://doi.org/10.1016/j.phytochem.2018.07.007>
- Benzie I. F. F. and J. J. Strain (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry* 239:70-76, <https://doi.org/10.1006/abio.1996.0292>
- Biluca F. C., F. Braghini, L. V. Gonzaga, A. C. Oliveira C. and R. Fett (2016) Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (*Meliponinae*). *Journal of Food Composition and Analysis* 50:61-69, <https://doi.org/10.1016/j.jfca.2016.05.007>
- Bobis O., A. R. Moise, I. Ballesteros, E. Sánchez R., S. Sánchez D., J. Sánchez-Sánchez, ... and J. M. Alvarez-Suarez (2020) Eucalyptus honey: Quality parameters, chemical composition and health-promoting properties. *Food Chemistry* 325:126870, <https://doi.org/10.1016/j.foodchem.2020.126870>
- Brand-Williams W., M. E. Cuvelier and C. Berset (1995) Use of free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology* 28:25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Bridi R., G. Nuñez-Quijada, P. Aguilar, P. Martínez, E. Lissi, A. Giordano and G. Montenegro (2017) Differences between phenolic content and antioxidant capacity of quillay Chilean honeys and their separated phenolic extracts. *Ciencia e Investigación Agraria* 44:252-261, <https://doi.org/10.7764/rcia.v44i3.1756>
- Cianciosi D., T. Y. Forbes-Hernández, S. Afrin, M. Gasparini, P. Reboredo-Rodríguez, P. P. Manna, ... and Maurizio Battino (2018) Phenolic compounds in honey and their associated health benefits: A review. *Molecules* 23:2322, <https://doi.org/10.3390/molecules23092322>
- Da Silva P. M., C. Gauche, L. V. Gonzaga, A. C. O. Costa and R. Fett (2016) Honey: Chemical composition, stability and authenticity. *Food Chemistry* 196:309-323, <https://doi.org/10.1016/j.foodchem.2015.09.051>
- Do Q. D., A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Huynh, F. E. Soetaredjo, S. Ismadji and Y. H. Ju (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. *Journal of Food and Drug Analysis* 22:296-302, <https://doi.org/10.1016/j.jfda.2013.11.001>
- FAO, Food and Agriculture Organization of the United Nations (2018) Codex Alimentarius. Codex Standard for Honey. Codex Stan 12-1981. Adopted in 1981. Revisions 1987 and 2001. Secretariat of the Joint FAO/WHO Food Standards Program. Rome. 8 p.
- FAOSTAT, Food and Agriculture Organization of the United Nations (2018) Livestock primary. Production of Honey, natural: Top 10 Producers. <http://www.fao.org/faostat/en/?#data/QL/visualize> (April 2020).
- Ferreira I. C. F. R., E. Aires, J. C. M. Barreira and L. M. Estevinho (2009) Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry* 114:1438-1443, <https://doi.org/10.1016/j.foodchem.2008.11.028>
- Frausto-Reyes C., R. Casillas-Peñuelas, J. L. Quintanar-Stephano, E. Macías-López, J. M. Bujdud-Pérez and I. Medina-Ramírez (2017) Spectroscopic study of honey from *Apis mellifera* from different regions in Mexico. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 178:212-217, <https://doi.org/10.1016/j.saa.2017.02.009>
- Hossen M. S., M. Y. Ali, M. H. A. Jahurul, M. M. Abdel-Daim, S. H. Gan and M. I. Khalil (2017) Beneficial roles of honey polyphenols against some human degenerative diseases: A review. *Pharmacological Reports* 69:1194-1205, <https://doi.org/10.1016/j.pharep.2017.07.002>
- International Honey Commission (2009) Harmonised Methods of the International Honey Commission. Swiss Bee Research Centre. Liebefeld, Switzerland. 63 p.
- Kavanagh S., J. Gunnoo, T. Marques P., J. C. Stout and B. White (2019) Physicochemical properties and phenolic content of honey from different floral origins and from rural versus urban landscapes. *Food Chemistry* 272:66-75, <https://doi.org/10.1016/j.foodchem.2018.08.035>
- Leyva-Daniel D. E., Z. Escobedo-Avellaneda, F. Villalobos-Castillejos, L. Alamilla-Beltrán and J. Welti-Chanes (2017) Effect of high hydrostatic pressure applied to a Mexican honey to increase its microbiological and functional quality. *Food and Bioprocess Technology* 102:299-306, <https://doi.org/10.1016/j.fbp.2017.01.001>
- Li B. B., B. Smith and M. M. Hossain (2006) Extraction of phenolics from citrus peel: II. Enzyme-assisted extraction method. *Separation and Purification Technology* 48:189-196, <https://doi.org/10.1016/j.seppur.2005.07.019>
- Lou S. N., Y. S. Hsu and C. T. Ho (2014) Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. *Journal of Food and Drug Analysis* 22:290-295, <https://doi.org/10.1016/j.jfda.2014.01.020>
- Martínez-Pérez de Ayala L. R., J. F. Martínez-Puc y W. R. Cetzal-Ix (2017) Apicultura: Manejo, Nutrición, Sanidad y Flora Apícola. Universidad Autónoma de Campeche. Campeche, México. 112 p.
- Mata-Páez R. I., P. A. Domínguez-Calleros, J. R. Goche-Télles y M. A. Díaz-Vásquez (2016) Importancia ecológica de los gatuños (*Mimosa* spp.) en el Parque Nacional Sierra de Órganos. *Revista Mexicana de Ciencias Forestales* 7:53-64, <https://doi.org/10.29298/rmcf.v7i35.74>
- Meo S. A., S. A. Al-Asiri, A. L. Mahesar and M. J. Ansari (2017) Role of honey in modern medicine. *Saudi Journal of Biological Sciences* 24:975-978, <https://doi.org/10.1016/j.sjbs.2016.12.010>
- Nordin A., N. Q. A. V. Sainik, S. R. Chowdhury, A. B. Saim and R. B. Idrus (2018) Physicochemical properties of stingless bee honey from around the globe: A comprehensive review. *Journal of Food Composition and Analysis* 73:91-102, <https://doi.org/10.1016/j.jfca.2018.06.002>
- Oroian M. and S. Ropciuc (2017) Honey authentication based on physicochemical parameters and phenolic compounds. *Computers and Electronics in Agriculture* 138:148-156, <https://doi.org/10.1016/j.compag.2017.04.020>
- Peng-Kek S., N. L. Chin, Y. A. Yusof, S. W. Tan and L. S. Chua (2014) Total phenolic contents and colour intensity of Malaysian honeys from the *Apis* spp. and *Trigona* spp. Bees. *Agriculture and Agricultural Science Procedia* 2:150-155, <https://doi.org/10.1016/j.aaspro.2014.11.022>
- Re R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26:1231-1237, [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Román-Cortés N. R., M. R. García-Mateos, A. M. Castillo-González, J. Sahagún-Castellanos y Ma. A. Jiménez-Arellanes (2018) Características nutricionales y nutraceuticas de hortalizas de uso ancestral en México. *Revista Fitotecnica Mexicana* 41:245-253, <https://doi.org/10.35196/rfm.2018.3.245-253>
- SIAP, Servicio de Información Agroalimentaria y Pesquera (2019) Avance de la producción pecuaria por estado. http://infosiap.siap.gob.mx/repoAvance_siap_gb/pecAvanceEdo.jsp (May 2019).
- Tomás-Barberán F. A., M. I. Gil, P. Cremin, A. L. Waterhouse, B. Hess-Pierce and A. A. Kader (2001) HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *Journal of Agricultural and Food Chemistry* 49:4748-4760, <https://doi.org/10.1021/jf0104681>
- Vit P., M. Medina and M. E. Enríquez (2004) Quality standards for medicinal uses of *Meliponinae* honey in Guatemala, Mexico and Venezuela. *Bee World* 85:2-5, <https://doi.org/10.1080/0005772X.2004.11099603>
- Wedmore E. B. (1955) The accurate determination of the water content

of honeys: Part I. Introduction and results. *Bee World* 36:197-

206 <https://doi.org/10.1080/0005772X.1955.11094904>