Article **ECORFAN Journal-Ecuador**
December 2023, Vol.10 No.19 18-22

Seasonal variation in the concentration of phenolic compounds in bee honey

Variación estacional en la concentración de compuestos fenólicos en miel de abeja

DE LA CRUZ-MARTÍNEZ, Wendy Madahí†, ARRIETA-GONZÁLEZ, Armando*, SILVA-MARTÍNEZ, Karla Lissette and GUERRA-RAMÍREZ, Diana

Tecnológico Nacional de México- Instituto Tecnológico Superior de Tantoyuca, Desviación Lindero Tametate S/N, La Morita, Tantoyuca, Veracruz, CP 92100, México.

ID 1st Author: *Wendy Madahí, De la Cruz-Martínez* / **ORC ID:** 0009-0001-5382-5159, **CVU CONAHCYT ID:** 1230131

ID 1st Co-author: *Armando, Arrieta-González* / **ORC ID:** 0000-0001-7810-6743, **CVU CONAHCYT ID:** 360256

ID 2nd Co-author: *Karla Lissette, Silva-Martínez* / **ORC ID:** 0000-0002-2010-6123, **SNI CONAHCYT ID:** 303867

ID 3rd Co-author: *Diana, Guerra-Ramirez* / **ORC ID:** 0000-0001-9840-975X, **SNI CONAHCYT ID:** 74680

DOI: 10.35429/EJE.2023.19.10.18.22 Received July 20, 2023; Accepted December 30, 2023

Abstract

The phenolic compounds in honey provide healthy properties to this food. These products have been studied due to their ability to inhibit and reduce the production of free radicals, which cause oxidative damage to molecules. This research aimed to determine the concentration of antioxidants in *Apis mellifera* honey harvested during winter and spring in the Huasteca Veracruzana region. To assess the honey, 16 samples were collected and diluted (1:5 w/v) to quantify the total phenol content by the Folin-Ciocalteau method; flavonoids according to Kubola and Syriamonrpun; antioxidant capacity through FRAP and ABTS assays adapted to microplates. One-way ANOVA was performed considering the season of the year as a variation factor. Homogeneity was evaluated with Bartlett, normality with Shapiro-Wilk and the comparison of means with Tukey ($p < 0.05$). The concentration of phenols, flavonoids, FRAP and ABTS was higher in the spring season ($p < 0.05$). The content of flavonoid in samples harvested in spring was 0.562 mg QE/100 g^{-1}; while those collected in winter the was 0.502 mg QE/100 g^{-1}. These differences could be attributed to the availability of flora between these seasons.

Antioxidants, Phenols, Flavonoids

Resumen

Los compuestos fenólicos en la miel de abeja, proporcionan propiedades saludables a este producto alimenticio; éstos han sido estudiados debido a su capacidad para inhibir y reducir la producción de radicales libres, los cuales ocasionan daños oxidativos en las moléculas. El objetivo de esta investigación fue determinar la concentración de antioxidantes presentes en la miel de *Apis mellifera* cosechada durante invierno y primavera en la Huasteca Veracruzana. 16 muestras fueron colectadas y diluidas (1:5 p/v), para cuantificar el contenido total de fenoles por el método Folin-Ciocalteau; flavonoides de acuerdo con Kubola y Siriamonrpun (2011); capacidad antioxidante a través de ensayos FRAP y ABTS adaptados a microplacas. Se realizó ANOVA de una vía considerando como factor de variación la estación del año. La homogeneidad se evaluó con Bartlett, normalidad con Shapiro-Wilk y la comparación de medias con Tukey ($p < 0.05$). La concentración de fenoles, flavonoides, FRAP y ABTS fue mayor en la estación de primavera ($p < 0.05$), en el caso particular del contenido de flavonoides en miel cosechada en primavera se estimó un contenido de 0.562 mg EQ/100 g^{-1} mientras que la de invierno 0.502 mg EQ/100 g^{-1}. Estas diferencias podrían atribuirse a la disponibilidad de flora entre estaciones.

Antioxidantes, Fenoles, Flavonoides

Citation: DE LA CRUZ-MARTÍNEZ, Wendy Madahí, ARRIETA-GONZÁLEZ, Armando, SILVA-MARTÍNEZ, Karla Lissette and GUERRA-RAMÍREZ, Diana. Seasonal variation in the concentration of phenolic compounds in bee honey. ECORFAN Journal-Ecuador. 2023. 10-19:18-22.

* Author's Correspondence (E-mail: armando.arrieta@itsta.edu.mx)
† Researcher contributing as first author.

Introduction

Honey is defined as a sweet, natural food that is generated from the nectar of plants, which is collected and transformed by bees. According to the floristic resource it can be classified as monofloral (where a single floral species predominates), multifloral or polyfloral (representing different floral species), and honeydew (coming from secretions of living parts of plants) (Martínez et al., 2017).

Honey is in great demand on the international market thanks to its nutritional value and its therapeutic and stimulating qualities (Escobar and Manresa, 2005). It is made up of carbohydrates, with monosaccharides such as fructose and glucose, disaccharides such as sucrose, trisaccharides such as melecithiose, and oligosaccharides, water, minerals, amino acids, proteins and organic acids. Their composition may depend on factors such as floral species, soil, species, colony physiology, among others (Bogdanov et al., 2008; Jean-Prost, 2007). Polyphenolic compounds, such as phenolic acids and flavonoids, are known to be the constituents responsible for the health-promoting properties provided by honey. These compounds are able to inhibit and/or reduce the production of oxidative compounds, also known as free radicals that can cause oxidative damage to molecules such as carbohydrates, lipids, proteins and their genetic material (Nascimento et al., 2018). These chemicals produced by plants have been studied due to their antioxidant, antimicrobial, anti-inflammatory and other health effects (Viuda-Martos et al., 2008). The objective of this research was to determine the variation in the concentration of antioxidants present in Apis mellifera honey harvested during winter and spring in the Huasteca Veracruzana.

Methodology

This research was carried out in the Huasteca region of the state of Veracruz, which lies between parallels 97º 59' and 98º 24' west longitude and 21º 06' and 21º 40' north latitude. This region has a warm sub-humid climate with summer rainfall and an annual precipitation of around 1100 mm. Relative humidity can reach values of 64% and the average annual temperature ranges between 22 - 26 ºC. (INEGI, 2021).

The main productive activities in the region are cattle raising and agriculture, focused on the production of corn, citrus fruits and sugar cane. Other crops of equal importance for the regional economy are sesame, peanuts, courgette, sweet potato, beans, watermelon, sorghum, wheat (in Huayacocotla), soybean (Pánuco), tobacco, tomato, coconut, mango and papaya. In some municipalities there are important fragments of mesophyll forest, pine forest and medium forest in the lower elevations of the mountain range. There are also important fragments of mangrove and hydrophilic vegetation distributed in some municipalities (INEGI, 2021).

Sixteen samples were collected from different apiaries during two seasons in the municipality of Tantoyuca, Veracruz. From each apiary, one hive was selected from which a hive frame was taken to an extraction room, where it was castrated and introduced into a manual extractor to avoid contamination of the honey. An approximate volume of 500 mL was obtained from each apiary, which was packed in jars wrapped with aluminium to avoid the entry of light, labelled and stored at room temperature until analysis. The concentration of phenols, flavonoids and antioxidant capacity by FRAP and ABTS assays were evaluated in vitro using honey solutions (1:5 w/v) in the Natural Products Laboratory of the Agricultural Preparatory Department at the Autonomous University of Chapingo. Phenolic content was determined according to Singleton and Rossi (1965), by the Folin-Ciocalteau method. The calibration curve was prepared from a solution of gallic acid. The absorbance was measured at 760 nm, the results were expressed milligrams of gallic acid equivalents per 100 g of honey. (mg GAE/100 g^{-1}). Total flavonoid content was quantified using the technique supported by Kubola and Siriamonrpun (2011) with some modifications in sample preparation. An aliquot of the 1:5 solution (0.5 mL) was mixed with 0.1 mL of 10% $AlCl_3$ $6H_2O$ and 0.1 mL of 1M CH_3CO_2K. The calibration curve was prepared for quercetin, and the results were expressed as milligrams quercetin equivalents per 100 g honey. (mg QE/100 g^{-1}). Antioxidant capacity was calculated by means of FRAP and ABTS assays performed under the protocol described by Benzie and Strain (1996) and Re et al. (1999), adapted to microplates.

ISSN-On line: 1390-9959

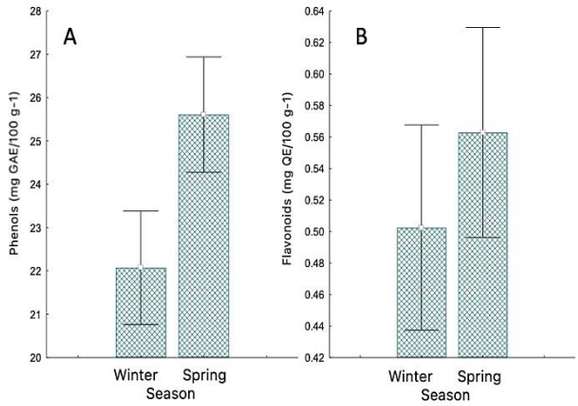
Calibration curves for Trolox (6-hydroxy-2,5,7,8-tetramethylchroman2-carboxylic acid) were prepared at a concentration of 0.25 mg/mL^{-1}, and data were reported in µmol Trolox equivalents per 100 g of honey (µmol TE/100 g^{-1}). One-way ANOVA was performed considering season as a factor of variation. Homogeneity was evaluated with Bartlett, normality with Shapiro-Wilk and comparison of means with Tukey ($p < 0.05$).

Results

The total phenol and flavonoid contents of the samples analysed during two seasons are shown in Table 1. Table 1 shows that the phenolic and flavonoid content was higher in spring, showing a statistical difference ($p < 0.05$) on the phenolic values obtained from the winter samples compared to the spring samples (Figure 1A and 1B). The difference among the concentrations of these compounds could be due to the greater availability of floristic resources during spring. These results are similar to those reported by Avila-Uribe et al. (2020), who evaluated honey samples from different municipalities in the state of Quintana Roo, where the bee floristic composition was characterised by a marked difference, which is attributed to the sampling site. Their results show that floristic characteristics influenced the concentration of phenolics and flavonoids. The samples they analysed showed values of 27.85 ± 0.045 mg GAE/100 g^{-1} and 5.75 ± 0.31 mg QE/100 g^{-1} respectively.

Station	Phenols mg GAE/100 g^{-1}	Flavonoids mg QE/100 g^{-1}
Winter	22.074^{b}	0.502^{b}
Spring	25.605^{a}	0.562^{a}
Standard error	0.565	0.018
Means per column with different literals show statistical difference (Tukey $p < 0.05$).		

Table 1 Concentration of total phenols and flavonoids in winter 2022 and spring 2023 bee honey.



Graphic 1 Concentration of phenols and flavonoids present in bee honey during the winter 2022 and spring 2023 seasons

On the other hand, Quintero-Lira et al. (2019) in their research on the concentrations of phenols and flavonoids in different regions of the state of Hidalgo, reported that the sampling site associated with the floristic composition was the main factor responsible for the statistical differences between the honey samples evaluated. In their analysis, they report that monofloral honey (*Citrus sinensis*) obtained the lowest concentrations of total phenols 16.61 mg GAE/100 g^{-1} and 97.92 mg QE/100 g^{-1} of phlovonoids.

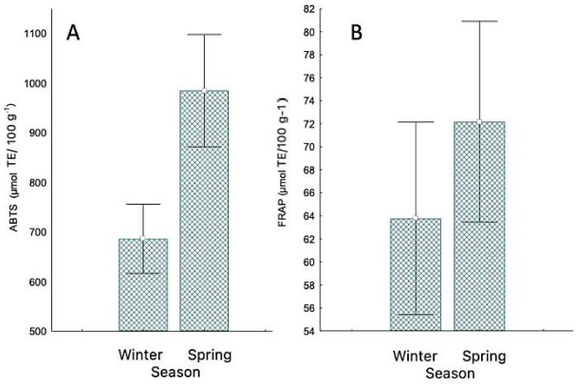
Station	FRAP µmol TE/100 g^{-1}	ABTS µmol TE/100 g^{-1}
Winter	63.771^{b}	686.41^{b}
Spring	72.182^{a}	984.91^{a}
Standard error	2.746	31.558
Means per column with different literals indicate significant difference (Tukey $p < 0.05$).		

Table 2 Concentration of total phenols and flavonoids in bee honey from winter 2022 and spring 2023

For both the FRAP and ABTS assays, samples from the spring season showed a higher antioxidant capacity (Table 2). These results were similar to those reported by Ávila-Uribe et al. (2022) in honeys from the state of Quintana Roo, which were 30.55 ± 0.09 - 137.54 ± 0.10 for FRAP and for ABTS 83.97 ± 0.04 - 109.30 ± 0.05 µmol TE/100 g^{-1}. Concentration of total phenols and flavonoids in bee honey from winter 2022 and spring 2023.

ISSN-On line: 1390-9959

For both the FRAP and ABTS assays, samples from the spring season showed a higher antioxidant capacity (Table 2). These results were similar to those reported by Ávila-Uribe et al. (2022) in honeys from the state of Quintana Roo, which were 30.55 ± 0.09 - 137.54 ± 0.10 for FRAP and for ABTS 83.97 ± 0.04 - 109.30 ± 0.05 μmol TE/100 g^{-1}.



Graphic 2 Antioxidant capacity by FRAP and ABTS assays in honeys produced in winter 2022 and spring 2023

As shown in Figure 2A and 2B, FRAP and ABTS values were higher in spring. The results obtained indicate that the honey produced in the Huasteca Veracruzana does have a certain antioxidant capacity.

Acknowledgement

Instituto Tecnológico Superior de Tantoyuca. Universidad Autónoma de Chapingo. Universidad Veracruzana campus Tuxpan, Veracruz.

Conclusions

The antioxidant properties of bee honey are a function of the time of the year in which it is produced. The presence of phenolic compounds is higher in spring than in winter.

The concentrations of antioxidant compounds found in the honey produced in the Huasteca Veracruzana, in general, were lower than those found in other places, this being attributed to the environmental conditions and floristic diversity available in each sampling site.

References

Ávila-Uribe, G., Salgado-Escobar, I., Guerra-Ramírez, D., Reyes-Trejo, B., y Pérez-López, A. 2022. *Compuestos marcadores y propiedades antioxidantes de mieles de Quintana Roo, México*. [Tesis doctoral, Universidad Autónoma Chapingo] Repositorio Chapingo. https://repositorio.chapingo.edu.mx/items/011c76b8-4302-4170-85eb-f95090ce63a6

Benzie, I. F. F. & Strain, J. J. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry, 239 (1): 70-76. https://doi.org/10.1006/abio.1996.0292

Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. 2008. Honey for nutrition and health: A review. Journal of the American College of Nutrition, 27 (6): 677–689. https://doi.org/10.1080/07315724.2008.10719745

Escobar, C.M., y Manresa, G.A. (2005). Clasificación de mieles cubanas a partir de sus propiedades fisico-químicas. *Revista CENIC Ciencias Biológicas., (36).* http://www.redalyc.org/articulo.oa?id=181220525088

González, S. R., Catalán, H. C., Domínguez, M. V. M., Luna, L. C., Hernández, C. E., Damián, N. A., Cruz, L. B. y Palemón, F. A. 2016. Análisis Palinológico de los recursos florales utilizados por *Apis mellifera L.* (Hymenoptera apidae) en cuatro municipios del estado de Guerrero, México. Tropical and Subtropical Agroecosystems, 19 (1): 19-28. https://www.redalyc.org/pdf/939/93945700008.pdf

INEGI (Instituto Nacional de Estadística y Geografía). 2021. Biblioteca Digital. México. Disponible en https://www.inegi.org.mx/app/mapas/?ag=30

Jean-Prost, P. 2007. *Apicultura: conocimiento de la abeja. Manejo de la colmena (4a. ed.)*. México. Mundi-Prensa. p.440. https://elibro.net/es/lc/itsta/titulos/55228

ISSN-On line: 1390-9959

Kubola, J. & Siriamonrpun, S. 2011. Phytochemicals and antioxidant activity of different fruit factiosn (peel, Pulp, aril and seed) of Thal gac (Momordica cochinchinensis Spreng). Food Chemistry, 127 (3): 1138-1145. https://doi.org/10.1016/j.foodchem.2011.01.115

Martínez, P. L. R., Martínez, P. J. F. y Cetzal-Ix, W. R. 2017. *Apicultura: manejo, nutrición, sanidad y flora apícola*. México. Universidad Autónoma de Campeche. p.30. https://www.academia.edu/35836633/Apicultura_Manejo_Nutrici%C3%B3n_Sanidad_y_Flora_ap%C3%ADcola

Nascimento, K. S. D., Gasparotto, S. J. A., Lauer, M. L. F., Serna, G. C. V., Pereira de Melo, I. L., Da Silva Araújo, E., Granato, D., Sattler, A., & de Almeida-Muradian, L. B. 2018. Phenolic compounds, antioxidant capacity and physicochemical properties of Brazilian Apis mellifera honeys. LWT - Food Science and Technology, 9: 85–94. https://doi.org/10.1016/j.lwt.2018.01.016

Quintero-Lira, A., López-Oliveira, A. C. F., Martínez-Juárez, V. M., Del Razo-Rodríguez, O. E., Jiménez-Alvarado, R. y Campos, M. R. G. 2019. Determinación de compuestos biactivos y contenido de selenio en diversas mieles del estado de Hidalgo. Journal of Energy, Engineering Optimization and Sustainability, 3 (2): https://doi.org/10.19136/jeeos.a3n2.3405

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., y Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cationdecolorzation assay. Free Radicals in Biology and Medicine, 26 (9/10): 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Singleton, V. L. & Rossi, J. A. 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, 16 (3): 144-158. https://www.ajevonline.org/content/16/3/144.short

Viuda-Martos, M., Ruiz, N. Y., Fernández-López, J. y Pérez-Álvarez, J. A. 2008. Propiedades funcionales de la miel, propóleo y jalea real. Revista de Ciencia de los Alimentos, 73 (9): R117-R124. https://doi.org/10.1111/j.1750-3841.2008.00966.x.

ISSN-On line: 1390-9959