Emirates Journal of Food and Agriculture. 2021. 33(7): 583-588
doi: 10.9755/ejfa.2021.v33.i7.2719
http://www.ejfa.me/

RESEARCH ARTICLE

Capsaicin, dihydrocapsaicin content and antioxidants properties of habanero pepper (*Capsicum chinense* Jacq.) Oleoresin during storage

Mariel Guadalupe Valencia-Cordova[1], Ángela Suárez-Jacobo[2], Nelly del Socorro Cruz-Cansino[1], Esther Ramírez-Moreno[1], Quinatzin Yadira Zafra-Rojas[1], José Alberto-Ariza-Ortega[1], Ernesto Alanís-García[1]*

[1]Centro de Investigación Interdisciplinario, Área Académica de Nutrición, Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Circuito Ex Hacienda La Concepción S/N, Carretera Pachuca-Actopan, C.P. 42160 San Agustín Tlaxiaca, Hidalgo, Mexico
[2]Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C., Subsede Noreste-Tecnología Alimentaria, Parque de Investigación e Innovación Tecnológica PIIT-Vía de la Innovación 404, C.P. 66628 Apodaca, Nuevo León, México

ABSTRACT

The objective of the present study was to evaluate the capsaicin (CAP) and dihydrocapsaicin (DHC), total phenolic content (TPC) as well as antioxidants properties of Habanero pepper (*Capsicum chinense* Jacq.) oleoresin (HPO) during storage. Identification and quantification of CAP and DHC were performed by HPLC, while TPC and antioxidant activity by ABTS and DPPH were by spectrophotometry. Retention time of CAP and DHC was 19.23 and 20.47 min, respectively. During storage, CAP and TPC remained stable until day 35 (14.35 to 15.72 mg CAP/g HPO and 29.13 to 28.31 mg GAE/mL HOP, respectively), while DHC had similar values (11.45 - 9.61 mg CAP/g HPO) ($p > 0.05$) through storage time. On the other hand, antioxidant activity by ABTS and DPPH decreased from day 15 (6.08 µmol TE/mL) and 35 (10.18 µmol TE/mL), respectively. The results showed that CAP, DCH and TP were stable for more than a month of storage.

Keywords: Habanero pepper; Oleoresin; Capsaicinoids; Antioxidant activity; Storage

INTRODUCTION

The Mexican population is characterized by consuming foods with a high content of pepper in the dishes or in sauces of different styles, so most of the Mexican population is accustomed to spicy flavor (Estrada-García et al., 2002). The tradition of pepper consumption has been established in Mexico since pre-Hispanic times and is part of the daily mexican diet (Castellón-Martínez et al., 2012). There are about 5 species of chilies described and domesticated in Mexico, which *Capsicum annuum, Capsicum chinense, Capsicum frutescens, Capsicum baccatum* and *Capsicum pubescens* are native (Castellón-Najeta et al., 2010), among them the *Capsicum chinense* Jacq. known as Habanero pepper which is positioned as one of the hottest in the world (Tapia-Vargas et al., 2016). The spicy flavor of peppers is due to a group of alkaloids called capsaicinoids, which are synthesized in the placenta of the fruit and are secondary metabolites (Vázquez-Flora et al., 2007) such as vanillylamine (phenolic compound) and 8-methyl-6-nonenoyl-coA (fatty acid) (Sanchez and Gutiérrez, 2016). The most important capsaicinoids are capsaicin (CAP) and dihydrocapsaicin (DHC) since constitute 90% of the content of these compounds in peppers (Orellana-Escobedo et al., 2013). Remarkable antioxidant a8-methyl-6-nonenoyl-coActivity of these foods is attributed to capsaicinoids (Mendoza-López et al., 2015) with biological activity as anti-obesity (2.53 mg CAP/day) (Janssesns et al., 2013), analgesic (patches at 8% capsaicin) (Giordano et al., 2011), apoptosis in cells of renal carcinoma (5 mg/kg weight) (Liu et al., 2016) and diabetes (0.015% capsaicin) (Kang et al., 2011). Therefore, these molecules could be used in order to benefit the consumer´s health, and one of the ways which can be consumed is in the addition of food from oleoresin. An oleoresin is an extract obtained from oily plants or species,

***Corresponding author:**
Ernesto Alanís García, Centro de Investigación Multidisciplinario, Área Académica de Nutrición, Instituto de Ciencias de la Salud (ICSa), Universidad Autónoma del Estado de Hidalgo, Circuito Actopan-Tilcuautla s/n. Ex hacienda La Concepción. San Agustin Tlaxiaca, C.P. 42160 Pachuca, Hidalgo, México. **Tel:** 7717172000 ext. 4316. **E-mail:** ernesto_alanis@uaeh.edu.mx

Received: 14 May 2021; **Accepted:** 28 June 2021

to which the extraction solvent evaporates (Restrepo, 2006). Extraction of oleoresin with different solvents (acetonitrile, acetone and ethanol), to identification and quantification of capsaicinoids in habanero pepper have been previously reported, obtaining less oleoresin by ethanol, however is recommend to use ethanol for food consumption (Nagoth et al., 2014). The storage of oleoresin has relevance in the application of the food industry due high compounds concentration in oleoresin, practically free of water, low degradation by oxidation and flavor loss (Restrepo et al., 2007). There are no studies reporting on *Capsicum chinense* ethanolic oleoresins storage behavior, only in *Capsicum annum* sp. powder at room temperature (Giuffrida et al., 2014), in Habanero pepper oleoresin purchased from a trademark which was microencapsulated and stored at 25, 35, and 45ºC (Domínguez-Cañedo and Beristain-Guevara, 2011). While in another study demonstrated that pharmaceutical grade capsaicin in pure solutions with different molarities reported stability at low temperatures (3ºC) for at least 12 weeks (Constanzo et al., 2014; Kopec et al., 2002). For the above mentioned, the objective of the present study was to evaluate the capsaicin and dihydrocapsaicin, phenolic content and antioxidant capacity from Habanero pepper oleoresin during storage.

MATERIALS AND METHODS

Images of the overall experiment can be seen in Fig. 1.

Chemicals and reagents

Acetonitrile (Baker, USA), Folin–Ciocalteu 2N reagent, 2,2,1-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) ≥ 98%, 1,1-di-phenyl-2-picrylhydrazyl (DPPH), Trolox 97%, standard for HPLC Capsaicin natural, 8-Methyl-N-vanillyl-trans-6-nonenamide (Sigma-Aldrich, Germany) Anhydrous sodium carbonate, gallic acid, crystals of potassium persulfate, absolute ethanol (Meyer, Mexico) Formic acid grade HPLC (Formic acid 98% - 100%) (Sigma-Aldrich, Germany).

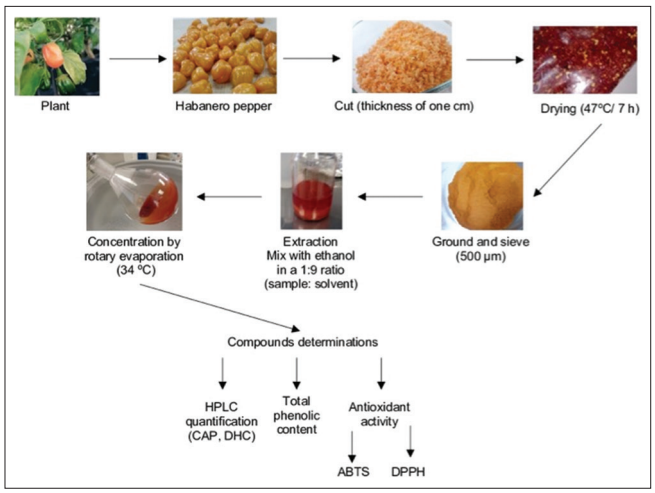

Fig 1. General diagram of the experiment.

Plant materials and sample preparation

Habanero peppers (*Capsicum chinense* Jacq.) were purchased at the local central Pachuca, Hidalgo, México of supplies during the months of May to December 2018. Were selected in a single stage of maturation defined by the reddish-orange coloration. For identification, the plant was deposited at the herbarium of the Center for Biological Research-Universidad Autónoma del Estado de Hidalgo; the sample was identified and the number assigned was *Capsicum chinense* Jacq., 05 (HGOM) which is registered in the Index Herbariorum: http://sweetgum.nybg.org/science/ih/herbarium-details/?irn=152964. The edible parts of pepper were separated, washed and weighed. Then, were cut into pieces with a thickness of one cm, later were dried using a commercial dehydrator (Weston,74-1001-W, USA) at 47ºC until the weight was kept constant (7 hours), subsequently, the dry sample was ground (A11, IKA, USA) and sieve at a particle size of 500 µm.

Habanero pepper oleoresin (HPO) extraction

Capsaicinoid extraction was performed by the method previously described (Nagoth et al., 2014). The dry sample was mixed with ethanol in a 1:9 ratio (sample: solvent), and kept in a shaking bath (LabTech, LSB-015S, Mexico) at 60 rpm and 65 ºC for 15 min, subsequently cooled and centrifuged (Beckman Coulter, Allegra 25R, USA) at 10,000 rpm during 20 min at 4 °C and the supernatant was brought to dryness by rotoevaporation (Büchi, R-200, USA) at 34 ºC obtaining an oleoresin. Habanero pepper oleoresin was stored in an amber glass container at 4ºC and used to evaluate the capsaicinoids, total phenolic and antioxidant activity content during 0, 15, 35 and 45 days of storage.

High-performance liquid chromatography

The identification and quantification of CAP and DHO were performed by High-performance Liquid Chromatography (HPLC) (Davis et al., 2007). For quantification, natural Capsaicin (65% capsaicin and 35% dihydrocapsaicin, Sigma-Aldrich, USA) was used as the external standard. The oleoresin samples were resuspended in anhydrous ethanol (25 mg/mL) and filtered on 0.45 µm PVDF Millex acrodisks prior injection. A sample volume of 10 µL were chromatographed on a HPLC system with a symmetry C18 (5 µm 4.6 x 250 mm) column (22°C) and equipped with a controller (Waters, 600, USA), autosampler (Waters, 717 plus, USA), and with a diode-array detector (Waters, 2996, USA) at 284 nm. The gradient elution system was binary using acetonitrile (A) and 0.1% (v/v) formic acid (B). Separation was achieved using a non-linear gradient: 5 min 5% A-95% B, 9 min 50% A-50% B, 6 min 80% A-20% B, 2 min 50% A-50% B, and 3 min 5% A-95% B (25 min in total) the flow rate was 1 mL/min. Data was analyzed using Waters Empower v.2 software. The

capsaicin and dihydrocapsaicin were expressed as milligrams of capsaicin per gram and milligrams of dihydrocapsaicin per gram of Habanero pepper oleoresin (mg CAP/g and mg DHC/g, respectively).

Total phenolics content

Total phenolics were measured according to the Folin-Ciocalteu method (Stintzing et al., 2005). Absorption at 765 nm was measured in a spectrometer (Power Wave XS UV-Biotek, USA), and the total phenolics was expressed as milligrams of gallic acid equivalents per milliliter (mg GAE/mL) of HPO.

Determination of antioxidant activity

The antioxidant activity of HPO was evaluated by two methods, quantifying the discoloration of radical 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS•+) (Kuskoski et al., 2005) and by the activity of free radical capture 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Morales and Jiménez-Pérez. 2001). The absorbance to ABTS was measured at 754 nm after 7 minutes, while to DPPH was measured at 520 nm using a microplate reader (Power Wave XS UV-Biotek, USA). Both results were expressed in micromoles of Trolox Equivalent per milliliter (μmol TE/mL) of HPO.

Statistical analysis

All the analyses were performed by triplicate. The one way analysis of variance (ANOVA) test was used and Duncan's multiple range test was applied to compare means at a level significance of $p<0.05$ using SPSS 7.0 statistical package (IBM, US). Multivariate analysis was carried out using the Principal Component Analysis (PCA) based on the correlation matrix to determine which variables contributed the most to making distinctions within the data sets with the software SPSS 7.0 statistical package (IBM, USA).

RESULTS AND DISCUSSION

Capsaicinoids identification by HPLC-DAD

Capsaicinoids analysis by high performance liquid chromatography with a diode-array detector (HPLC-DAD) currently allows a precise identification of the nature and quantification of these alkaloid compounds (Fig. 2).

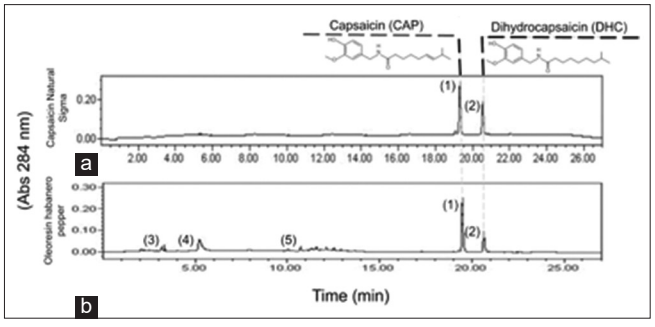

Fig 2. Chromatograms of capsaicin and dihydrocapsaicin identification. a) standard curve, b) HPO sample

Typical chromatograms to capsaicinoids identification are shown in Figure 1, where was possible identify two main capsaicinoids in the standard solutions: capsaicin and dihydrocapsaicin (Fig. 1a). HPLC chromatogram from HPO (Habanero pepper oleoresin) is shown in Fig. 1b. Under these conditions, retention times (Rt) were 19.23 (1) and 20.47 min (2) for capsaicin and dihydrocapsaicin, respectively. Peaks that elute before 4 minutes (3 and 4) were probably carotenoids and other pigments vegetables (Davis et al., 2007), the peak 5, could belong to other capsaicinoids like nordihydrocapsaicin (Othman et al., 2011).

Capsaicin (CAP) and dihydrocapsaicin (DHC) content

Quantification of CAP and DHC from HPO and the changes established during 45 storage days are presented in Fig. 3. The initial concentration of capsaicin was 14.37 mg CAP/g (Fig. 3a) and 10.49 mg DHC/g to dihydrocapsaicin (Fig. 3b).

Some authors have reported lower capsaicinoid content in ethanolic extracts of *Capsicum chinense* Jacq. (0.12 mg CAP and DHC/g of extract) and in oleoresins of *Capsicum* spp. of 4.9 mg CAP/g and 1.7 mg DHC/of oleoresin (Davis et al., 2007; Hoyos et al., 2011). However, Yañez et al., (2015) in an oleoresin from *Capsicum chinense* Jacq. had 11.33 mg CAP/g HPO, similar to present study. These differences can be due to the genetic composition of the plant and maturation of the product (Zewdie and Bosland, 2001), as well as the affinity of the components and disolvent used in the extraction method (Restrepo et al., 2007). During storage at 4ºC, capsaicin remained stable until day 35 ($p>0.05$), while DHC values were similar during 45 days.

These results are in agreement with Domínguez-Cañedo et al., (2015) who established that the deterioration of capsaicin in an oleoresin from habanero pepper depends on its storage temperature, because they studied the oleoresin of habanero pepper at different temperatures (35 to 45ºC) and observed that when the temperature increased, the loss of capsaicin accelerated. Guiffrida et al., (2014), determined that the storage of pepper powder at room temperature caused losses of the capsaicinoid content at 6 months. The decrease is by an isomeric transformation from dihydrocapsaicin to isodihydrocapsaicin, with respect to capsaicin, fragmentation occurs of the alkyl groups in the molecule of capsaicin (Henderson and Henderson, 1992). According to these results, oleoresins can be used in some food with the doses that have been seen benefits and used as an adjunct in obesity, diabetes or cancer (Janssesns et al., 2013; Kang et al., 2011; Liu et al., 2016).

Total phenolic content

The initial concentration of total phenolics in HPO was of 29.13 ± 0.61 mg GAE/mL (Table 1), similar result was presented by Sora et al., (2015) in aqueous ethanolic

extracts of Habanero pepper (26.6 mg GAE/mL). The total phenolic content did not show significant differences (*p>0.05*) from 0 to 35 days, while a decrease at 45 days (28.31 ± 0.17 mg GAE/mL) was observed. Another study reported stability in a microencapsulated extract at different storage conditions during 8 week (56 days) (Domínguez-Cañedo and Beristain-Guevara, 2011).

The stability behavior may be due to the modifications that occur in the compounds, synthesis of new compounds, that help maintain, even improve antioxidant activity during storage became present (Manzocco et al., 1998). Phenolic compounds due to their biological activity are attributed to health improvement as vasodilators, anticancer, anti-inflammation, anti-allergies (Martínez-Valverde et al., 2000).

Antioxidant activity

Respecting antioxidant activity, the results by ABTS in the initial concentration was of 7.78 ± 0.03 μmol TE/mL (Table 1). In previous studies presented higher concentrations in extracts of *Capsicum chinense* Jacq. and *Capsicum annum* (92 and 38 μmol/mL of extract, respectively) (Meckelmann et al., 2013; Hervert-Hernández et al., 2010). During storage, significantly decreased (*p<0.05)* for day 15, remaining stable until 35 days, for day 45 an increase was observed (7.40 ± 0.01 μmol TE/mL). The decrease on day 15 can be due to the oxidation of the components present (Cerecedo-Cruz et al., 2018) and the increase in the last day of storage could be attributed to the probable release or appearance of new components that improve antioxidant activity (Manzocco et al., 1998) as vanillin resulted from the oxidation of capsaicin, subsequent formation of phenols by a second breakdown and finally alkylamide (Domínguez-Cañedo et al., 2015; Henderson and Henderson, 1992). The antioxidant activity measured by DPPH was 11.30 ± 1.73 μmol TE mL at day 0 (Table 1). Value from *Capsicum annum* var. cacho de cabra oleoresin was reported at 1.4 x 10^{-3} μmol TE/mL (Riquelme and Matiacevich, 2015), it was a very low value than those obtained in this study. In this sense, the differences in antioxidant activity of extracts are dependent on the amount of capsaicin that the oleoresin contains, that is, a higher content of capsaicin can contribute to an increase in its antioxidant activity, from day 35 a decrease (*p<0.05*) was observed, possibly due to the reduction in bioactive compounds such as vitamins, phenols, carotenoids, etc. (Santander et al., 2017). This behavior may also be related to the decrease (21.89% in day 45 with respect to 35 day) in capsaicin in this day (Figure 2).

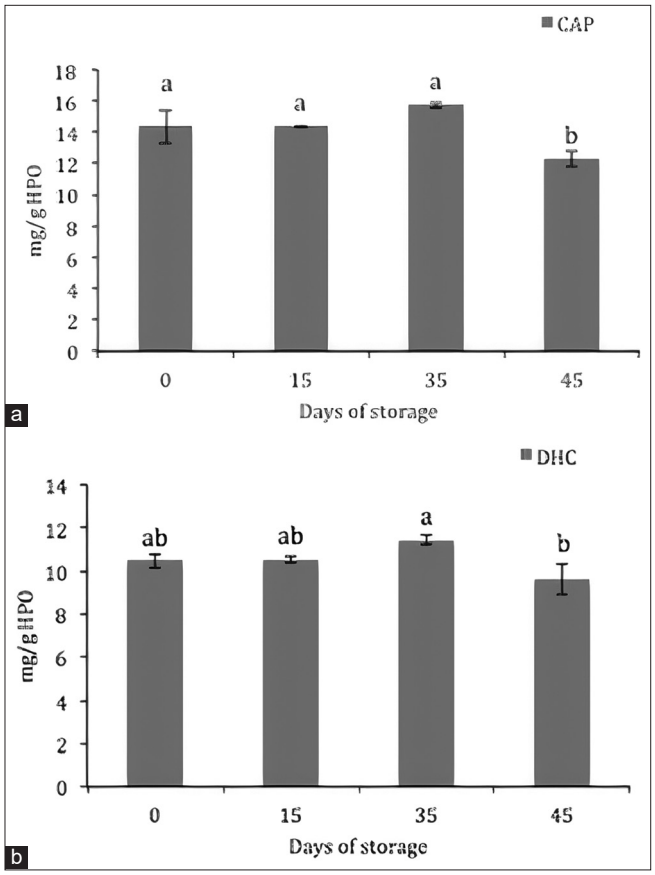

Fig 3. Capsaicin (a) and dihydrocapsaicin (b) of Habanero pepper oleoresin during storage.a-b Different letter indicate significant differences (p<0.05)

Principal component analysis

Two principal components from the data set of CAP, DHC, TPC and antioxidant activity (ABTS and DPPH) of habanero pepper oleoresin were obtained from PCA, which represented the 90.41% of the cumulative total percentage of variations accumulated percentage of total variations, where the PC1 and PC2 corresponded the 70.47 and 19.93%, respectively (Table 2).

In the Fig. 4, it can be observed that the content of CAP, DHC, TPC and antioxidant activity by DPPH were grouped, explaining the closely relationship between them, while the antioxidant activity by ABTS is scattered. This is confirmed in Table 3, where ABTS presented

Table 1: TPC[A] and antioxidant activity (ABTS and DPPH) of HPO[B] during storage

Storage (days)	TPC[A] mg GAE[C]/mL[B]	ABT[S] μmol TED/mL[B]	DPPH μmol TE[D]/mL[B]
0	29.13 ± 0.61[a]	7.78 ± 0.03[a]	11.30 ±1.73[a]
15	28.31 ± 0.17[a]	6.08 ± 0.07[c]	11.19 ± 0.51[a]
35	28.31 ± 0.17[a]	6.08 ± 0.07[c]	10.18 ± 0.67[b]
45	25.46 ± 0.92[b]	7.40 ± 0.01[b]	7.49 ± 1.28[c]

[a–c] Different letters in the column indicate significant differences (*p<0.05*).
A: Total phenolic content, B: Habanero pepper oleoresin, C: Gallic Acid Equivalent, D: Trolox Equivalent

Table 2: Principal component analysis

Component	Eigenvalue	Variance percentage	Accumulated percentage
1	3.52	70.47	70.47
2	0.99	19.93	90.41

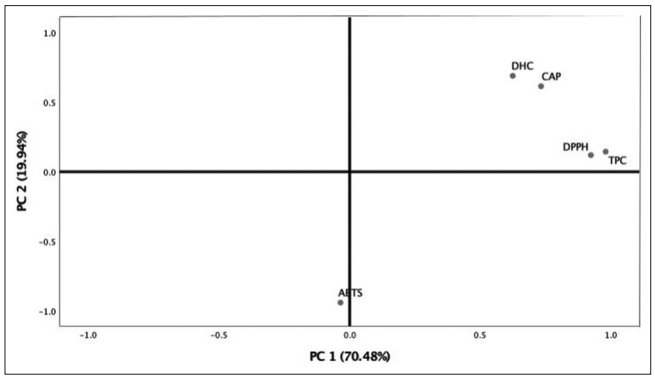

Fig 4. PCA biplot in the content of capsaicin (CAP), dihydrocapsaicin (DHC), total phenolic content (TPC) and antioxidant activity (ABTS and DPPH) in Habanero pepper oleoresin

Table 3: Component matrix PCA

	PC 1	PC 2
CAP	0.733	0.615
DHC	0.623	0.690
TPC	0.981	0.144
ABTS	-0.035	-0.942
DPPH	0.924	0.120

Principal component analysis (PCA), capsaicin (CAP), dihydrocapsaicin (DHC), total phenolic content (TPC)

negative values. The relationship between capsaicinoids, total phenolic content and antioxidant activity by DPPH has been previously demonstrated by other authors (Sora et al., 2015; Materska and Perucka, 2005).

Principal component analysis (PCA), capsaicin (CAP), dihydrocapsaicin (DHC), total phenolic content (TPC).

CONCLUSIONS

- The extraction of oleoresin from Habanero peppers (*Capsicum chinense* Jacq.) is of great interest, due to contain amounts appreciable of capsaicinoids (capsaicin and dihydrocapsaicin), as well as phenolic compounds.
- The results obtained from the present study, during the storage of the stability of dihydrocapsaicin and total phenolic content is at least 35 days was observed.
- In antioxidant activity, oleoresin showed stability by DPPH until day 35, while by ABTS only 15 days.
- This oleoresin can be used in some foods to obtain the previously reported health benefits, it must be borne in mind that its correct storage in an amber bottle and at low temperatures (4ºC) is necessary.

CONFLICT OF INTEREST DECLARATION

The authors have declared no conflicts of interest for his article.

Authors´ contributions

Mariel Guadalupe Valencia-Cordova M. G., carried out the research development, wrote the manuscript and received her Bachelor's degree in Nutrition, in the Universidad Autónoma del Estado de Hidalgo, México (Folio C34761 and act number 658/2020), under the direction of Alanís-García E. and Cruz-Cansino N. S., responsible for the general project and reviewers for the writing of the initial manuscript; Ángela Suárez-Jacobo A., provided reagents and trained team management; Ramírez-Moreno E., supervised statistical analysis and the visualization and presentation of manuscript data; Zafra-Rojas Q. Y., verified the general reproducibility of the results and experiments; José Alberto Ariza-Ortega J. A., verified the analytical methods. All authors participated in the final edition of the manuscript.

REFERENCES

Castellón-Najeta, G., L. Latournerie-Moreno, J. M. Lesher-Gordillo, E. De la Cruz-Lázaro and M. Mendoza-Elos. 2010. Identificación de variables para caracterizar morfológicamente colectas de chile (*Capsicum* spp.) en Tabasco, México. Univ. Cienc. 26: 225-234.

Castellón-Martínez, E., J. Luis Chávez-Servia, J. C. Carrillo-Rodríguez and A. M. Vera-Guzmán. 2012. Preferencias de consumo de chiles (*Capsicum annuum* L.) nativos en los valles centrales de Oaxaca, México. Rev. Fitotec. Mex. 35: 27-35.

Cerecedo-Cruz, L., E. Azuara-Nieto, A. Hernández-Álvarez, C. González-González and G. Melgar-Lalanne. 2018. Evaluation of the oxidative stability of Chipotle chili (*Capsicum annuum* L.) oleoresins in avocado oil. Grasas Aceites. 69: 1-12.

Costanzo, M. T., R. A. Yost and P. W. Davenport. 2014. Standardized method for solubility and storage of capsaicin-based solutions for cough induction. Cough. 10: 6.

Davis, C., C. Markey, M. Busch and K. Busch. 2007. Determination of capsaicinoids in habanero peppers by chemometric analysis of UV spectral data. J. Agric. Food Chem. 55: 5925-5933.

Domínguez-Cañedo, I. and C. Beristain-Guevara. 2011. Microencapsulation of habanero chilli (*Capsicum chinense*) oleoresin in β-ciclodextrin and antioxidant activity during storage. In: European Drying Conference-Euro Drying: 2011 Palma Balearic Island Spain, pp. 26-28.

Domínguez-Cañedo, I., C. Beristain-Guevara, R. Díaz-Sobac and A. Vázquez-Luna. 2015. Degradation of carotenoids and capsaicin in the molecular inclusion complex of habanero chili oleoresin (*Capsicum chinense*) in β-cyclodextrin. CyT J. Food. 13: 151-158.

Estrada-García, T., J. F. Cerna, M. R. Thompson and C. López-Saucedo. 2002. Faecal contamination and enterotoxigenic *Escherichia coli* in street-vended chili sauces in Mexico and its public health relevance. Epidemiol. Infect. 129: 223-226.

Giordano, C., L. Cristino, L. Luongo, D. Siniscalco, S. Petrosino, F. Piscitelli, I. Marabese, L. Gatta, F. Rossi, R. Imperatore, E. Palazzo, V. de Novellis, V. Di Marzo and S. Maione. 2011. TRPV1-dependent and-independent alterations in the limbic cortex of neuropathic mice: Impact on glial caspases and pain perception. Cereb. Cortex. 22: 2495-2518.

Giuffrida, D., P. Dugo, G. Torre, C. Bidnardi, A. Cavazza, C. Corradini and G. Dugo. 2014. Evaluation of carotenoid and capsaicinoid

contents in powder of red chili peppers during one year of storage. Food Res. Int. 65: 167-170.

Henderson, D. and S. Henderson. 1992. Thermal decomposition of capsaicin. 1. Interactions with oleic acid at high temperatures. J. Agric. Food Chem. 49: 2263-2268.

Hervert-Hernández, D., S. G. Sáyago-Ayerdi and I. Goni. 2010. Bioactive compounds of four hot pepper varieties (*Capsicum annuum* L.), antioxidant capacity, and intestinal bioaccessibility. J. Agric. Food Chem. 58: 3399-3406.

Hoyos, O., J. Martín, C. Solarte and M. Orozco. 2007. Extracción y cuantificación de capsaicina a partir de fruto del Ají (*Capsicum* spp). Sci. Tech. 33: 33-35.

Janssesns, P., R. Hursel, E. Martens and M. Westerterp-Plantega. 2013. Acute effects of capsaicin on energy expenditure and fat oxidation in negative energy balance. PLoS One. 8: 1-7.

Kang, J., G. Tsuyoshi, H. Ngon, H. M. Kim, T. H. Tu, H. J. Noh, C. S. Kim, S. Y. Choe, T. Kawada, H. Yoo and R. Yu. 2011. Dietary capsaicin attenuates metabolic dysregulation in genetically obese diabetic mice. J. Med. Food. 14: 310-315.

Kopec, S., R. DeBellis and R. Irwin. 2002. Chemical analysis of freshly prepared and stored capsaicin solutions: Implications for tussigenic challenges. Pulm. Pharmacol. Ther. 15: 529-534.

Kuskoski, E. M., A. G. Asuero, A. M. Troncoso, J. Mancini-Filho and R. Fett. 2005. Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. Ciênc. Tecnol. Aliment. Campinas. 25: 726-732.

Liu, T., G. Wang, H. Tao, Z. Yang, Y. Wang, Z. Meng, R. Cao, Y. Xiao, X. Wang and J. Zhou. 2016. Capsaicin mediates caspases activation and induces apoptosis through P38 and JNK MAPK pathways in human real carcinoma. BMC Cancer. 16: 790.

Manzocco, L., M. Anese and M. Nicoli. 1998. Antioxidant properties of tea extracts as affected by processing. LWT. 31: 694-698.

Martínez-Valverde, I., M. Periago and G. Ros. 2000. Significado nutricional de los compuestos fenólicos de la dieta. Arch Latinoam Nutr. 50: 5-18.

Materska, M. and I. Perucka. 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L). J. Agric. Food Chem. 53: 1750-1756.

Meckelmann, S., D. Riegel, M. Zonneveld, L. Ríos, K. Peña, R. Ugas, L. Quinonez, E. Mueller-Seitz and M. Petz. 2013. Compositional characterization of native Peruvian chili peppers (*Capsicum* spp.). J. Agric. Food Chem. 61: 2530-2533.

Mendoza-López, L., M. Mendoza-López, O. García-Barradas, E. Azuara-Nieto, L. Pascual-Pineda and M. Jiménez-Gernández. 2015. Physicochemical and antioxidant properties of Jalapeño pepper (*Capsicum annuum* val. annuum) during storage. Rev. Chapingo Hoertic. 21: 229-241.

Morales, F. and S. Jiménez-Pérez. 2001. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescense. Food Chem. 44: 1244-1253.

Nagoth, J., R. Preetam and L. Antoine. 2014. Impact of organic solvents in the extraction efficiency of therapeutic analogue capsaicin from *Capsicum chinense* Bhut Jolokia Fruits. Int. J. Pharm. Clin. Res. 6: 159-164.

Orellana-Escobedo, L., L. G. Amezquita, G. Olivas, J. Ornelas-Paz and D. Sepulveda. 2013. Capsaicinoids content and proximate composition of Mexican chili peppers (*Capsicum* spp.) cultivates in the state of Chihuahua. CyTA J. Food. 11: 179-184.

Othman, Z. A. A., Y. B. H. Ahmed, M. A. Habila and A. A. Ghafar. 2011. Determination of capsaicin and dihydrocapsaicin in *Capsicum* fruit samples using high performance liquid chromatography. Molecules. 16: 8919-8929.

Restrepo, M. 2006. Oleorresinas de *Capsicum* en la industria alimentaria. Rev. Lasallista Investig. 2: 43-4.

Restrepo, M., N. Llanos and C. Fonseca. 2007. Composición de las oleorresinas de dos variedades de ají picante (habanero y tabasco) obtenidas mediante lixiviación con solventes orgánicos. Rev. Lasallista Investig. 4: 14-19.

Riquelme, N. and S. Matiacevich. 2016. Characterization and evaluation of some properties of oleoresin from *Capsicum annuum* var. cacho de cabra. CYTA J. Food. 15: 344-351.

Sanchez, L. and F. Gutiérrez. 2016. El picor del chile, biosíntesis de la capsaicina y diferenciación morfológica. Ciencias. 118: 34-37.

Santander, M., O. Osorio and D. Mejía. 2017. Antioxidant and physicochemical properties evaluation of a mixed beverage during refrigerated storage. Rev. Mex. Cienc. Agríc. 34: 84-97.

Sora, G. T. S., C. W. I. Haminiuk, M. V. Silva, A. F. Z. Zielinski, G. A. Goncalves, A. Bracht and R. M. Peralta. 2015. A comparative study of the capsaicinoid and phenolic contents and *in vitro* antioxidant activities of the peppers of the genus *Capsicum*: An application of chemometrics. Food Sci. Technol. 52: 8086-8094.

Stintzing, F., K. Herbach, M. Mosshammer, R. Carle, W. Yi, A. Sellappan and P. Felker. 2005. Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. J. Agric. Food Chem. 53: 442-451.

Tapia-Vargas, M., A. Larios-Guzmán, D. Díaz-Sánchez, G. Ramírez-Ojeda, A. Hernández-Pérez and I. Vidales-Fernández. 2016. Producción hidropónica de chile hananero negro (*Capsicum chinense* Jacq.). Rev. Fitotec. Mex. 39: 241-245.

Vázquez-Flota, F., M. De Lourdes Miranda-Ham, M. Monforte-González, G. Gutiérrez-Carbajal, C. Velázquez-García and Y. Nieto-Pelayo. 2007. La biosíntesis de capsaicinoides, el principio picante del chile. Rev. Fitotec. Mex. 30: 353-36.

Yañez, P., D. Balseca, L. Rivadeneira and C. Larenas. 2015. Características morfológicas de capsaicina en cinco especies nativas del género *Capsicum* cultivadas en Ecuador. La Granja. 22: 12-32.

Zewdie, Y. and W. Bosland. 2001. Capsaicinoid profiles are no good chemotaxonomic indicators for *Capsicum* species. Biochem. Systems. 29: 161-169.