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ARTICULO DE INVESTIGACION

DISTRIBUTION OF Ω-1-METHYL- FATTY ACIDS REGARDING TO CAPSAICINOIDS CONTENT IN FRUITS AND PLANTS OF *CAPSICUM*

DISTRIBUCIÓN DE ÁCIDOS GRASOS Ω-1-METIL-CON RESPECTO AL CONTENIDO DE CAPSAICINOIDES EN FRUTOS Y PLANTAS DE CAPSICUM

TRABAJO PRESENTADO EN EL EVENTO CNIC PRONAT 2022

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ABSTRACT

Capsaicinoids, the pungent compounds of Capsicum (Chili) fruits, are amides of vanillylamine with ω-1-methyl-fatty acids of nine to eleven carbons. Isotopic studies have revealed that vanillylamine come from phenylalanine via the phenylpropanoid pathway, while the ω-1-methyl-fatty acids come from valine or leucine via isobutyrate or isovalerate, but the intermediate biochemical steps of the biosynthesis of these fatty acids are still obscure. Furthermore, there is no clear experimental evidence for the presence of these ω-1-methylated fatty acids precursors of capsaicinoids in fruits or other tissues of *Capsicum* plants. The objective of this study was to investigate by HPLC and GC/MS the presence and distribution of these branched fatty acids in fruits and biomass of cell cultures from several species of *Capsicum* in relation to the content of capsaicinoids. Capsaicinoids were detected exclusively in placental tissue and in biomass of cell suspension cultures. Its content regarding to the ripening stage of the fruits showed a typical growth pattern, reaching a stationary phase of accumulation during its transition from green to the final color, characteristic of each species. Interestingly, ω-1-methylated fatty acids were positively detected in tiny amounts in the placental tissue, but also in other parts of the fruit, and putatively in leaves adjacent to the peduncle of fruits.

Keywords: Chili, secondary metabolism, turnover, carotenes, phenols, non-climacteric fruits.

RESUMEN

Los capsaicinoides, compuestos pungentes los frutas de *Capsicum* (Chili), son amidas de vainililamina y ácidos grasos de nueve a once carbonos ω-1-metilados. Estudios isotópicos han revelado que la vainililamina proviene de la fenilalanina a través de la vía de los fenilpropanoides, mientras que los ω-1-metil-ácidos grasos provienen de la valina o de la leucina vía isobutirato o isovalerato, sin embargo, las etapas bioquímicas intermediarias de la biosíntesis de esos ácidos aún son obscuros. Aún más, no hay evidencia experimental clara de la presencia de estos ácidos grasos ω-1-metilados, precursores de los capsaicinoides, en frutos u otros tejidos de las plantas de *Capsicum*. El objetivo de este estudio fue investigar por HPLC y GC/MS la presencia y distribución de esos ácidos grasos ramificados en frutos y biomasa de cultivos celulares de varias especies de *Capsicum* en relación con el contenido de capsaicinoides. Los capsaicinoides se detectaron exclusivamente en el tejido placentario y en la biomasa de cultivos de células in vitro. Su contenido con respecto a la etapa de maduración de los frutos mostró un patrón de crecimiento típico, alcanzando una fase estacionaria de acumulación durante su transición de color verde al color final característico de cada especie. Interesantemente, los ácidos grasos ω-1-metilados se detectaron positivamente en muy pequeñas cantidades en el tejido placentario, pero también en otros órganos de los frutos y putativamente en las hojas adyacentes al pedúnculo de los frutos.

Palabras claves: Chile, metabolismo secundario, recambio, carotenos, fenoles, frutos no climatéricos.

INTRODUCTION

Capsaicinoids (Figure 1), the pungent compounds of the *Capsicum* fruits (Chili pepper), are pseudoalkaloid amides biosynthesized in the placental tissue of *Capsicum* by condensation of vainillilamina and nine to eleven carbons ω-1-methyl-fatty acids by the enzyme capsaicinoid synthetase (Suzuki et al., 1981a). They have been classified by Suzuki et al., (1981b), on the bases that the chain length of their branched chain fatty acyl moiety in two groups: CAP and DC. with even number (C10) branched· chain fatty acyl moieties (group 1); NDC, HDC and HC with odd-number (C9-C11) branched chain fatty acyl moieties (group, 2). Isotopic studies revealed that vanillylamine come from phenylalanine via the phenylpropanoid pathway, while the medium-length branched-chain fatty acids come from valine or leucine via isobutyrate or isovalerate (Suzuki et al., 1981a, b, Fujiwake et al., 1980, 1982). It has been speculated that the difference in the profile and content of capsaicinoids in fruits seems to be regulated by the specificity of the capsaicinoid synthetase enzyme (Suzuki et al., 1981b, Fujiwake et al., 1980, 1982), and by the state of maturation and the metabolic pull of their ω-1-methyl-fatty acid precursors (Suszuki et al., 1981b, Vazquez et al., 2007., Aza et al., 2011), but the biochemical steps of biosynthesis pathway of the ω-1-methyl-fatty remains obscure (Calva y Rios, 1999, Aza 2011). There are evidences that biosynthesis of these fatty acids occurs by conversion of isobutyrate or isovalerate through a still uncharacterized dehydrogenase enzyme (Suzuki and Iwai, 1984), and elongation of the carbon chain by successive addition of two carbons to the acyl intermediates bonded to an acyl carrier protein (ACP), and condensation catalyzed by a 3-ketoacyl-ACP synthase II (KAS II), process analogous to the general biosynthesis of fatty acids (Markai et al., 2003), which also involves enzymes such as keto-acyl-synthase (KAS), and a thioesterase (Curry et al., 1999; Aluru et al., 2003). Even more, there is not clear evidence for the presence of the ω-1-methyl-fatty acids, precursors of capsaicinoids, in fruits or plants of *Capsicum*. With the aim of stressing the knowledge on this topic, the objective of this study was to investigate by HPLC and GC/FI or GC/MS, the presence and distribution of these ω-1-methyl- fatty acids regarding to capsaicinoids content in fruits, plants, and biomass from cell cultures of several *Capsicum* species, regarding their capsaicinoids content.

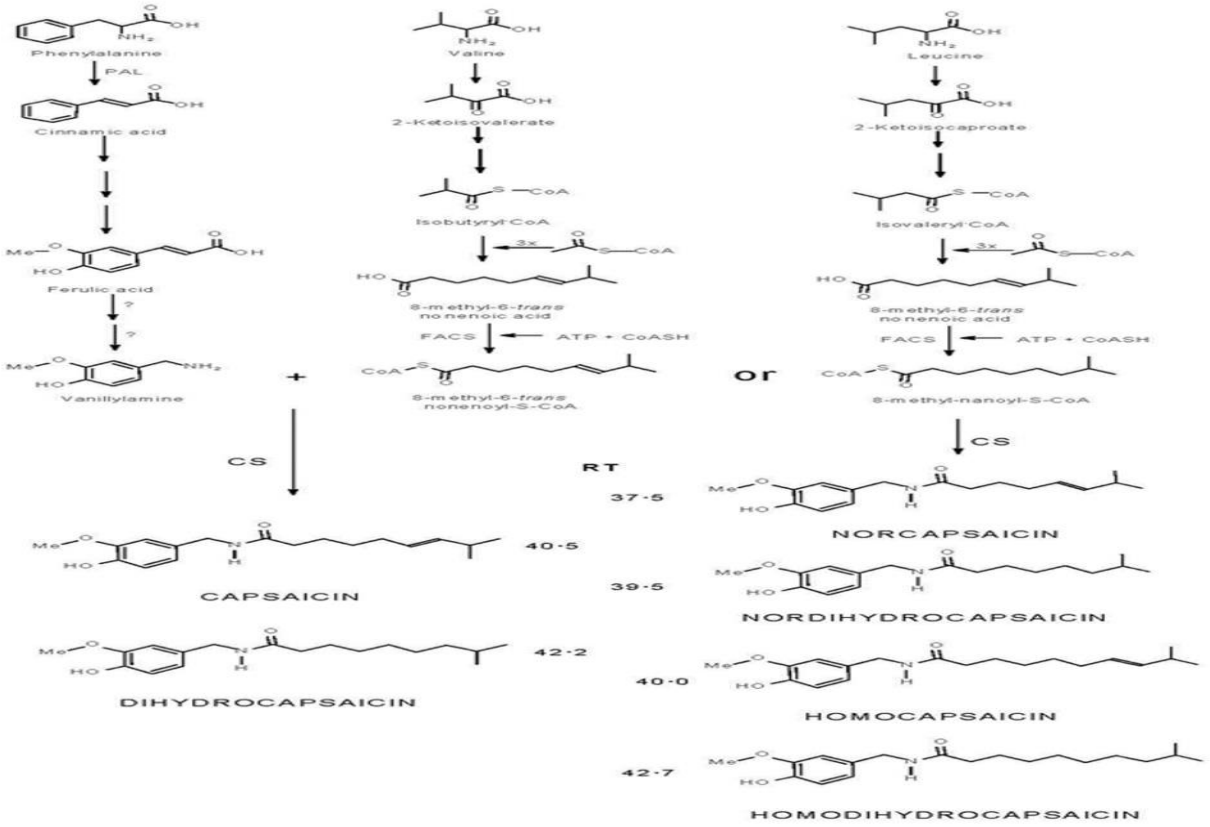


Fig. 1. *Proposed biochemical pathway leading to the formation of capsaicinoids (Fujiwake et al., 1982; Yeoman et al.,1989). PAL, phenylalanine ammonia lyase; CA4H, cinnamic acid 4-hydroxylase; CA3H, coumaric acid 3-hydroxylase; CAOMT, caffeic acid O-methyltransferase; FACS, fatty acyl-CoA synthetase; CS, capsaicinoid synthase.*

MATERIALS AND METHODS

Plant material *in vitro* and *in vivo*. Fruits from several *Capsicum* spp plants grown in greenhouse as reported in Calva and Rios (1999), Calva-Calva (1995), were used. The in vitro cultures of *Capsicum chinense*, were stablished from hypocotyl explants cultivated on MS medium supplemented with 30 g/L sucrose, 1.8 g/L phytagel, 12.5 µM 2,4- (2,4-D). and 0.5 µM kinetin (K). Cell suspension cultures were stablished from 3g of callus tissue transferred into 50 mL MS medium culture supplemented with 30g/L sucrose, 12.5 µM 2,4-D, and 0.5 µM K.

Capsaicinoids. Samples of biomass (500 mg) were ground in the presence of liquid N, extracted 3x with absolute ethanol and HPLC analysed as previously reported (Calva and Rios, 1999, Calva-Calva 1995).

Fatty acid analysis. The fatty acid fraction of the total lipids extracts from 2 g of fresh tissue ground in liquid N was trans esterified with methanol (FAME), and GC or GC-MS analyzed (Perkin Elmer Gas Chromatograph, INNOWAX column; 30m*0.32mm; the gradient used is 100°C→5°C/min→220°C-3°C min- 2°C/min →250°C-10min-end; Td 280°C, Ti. 250°C, MS or FI detector and 3 µL injection volume), as reported previously by Rivera Casado et al., (22015).

RESULTS

Growth pattern and capsaicinoids accumulation in cell suspension cultures and fruits of *Capsicum* spp. The growth pattern of cell suspension cultures of *Capsicum* spp., as that showed by the cell suspension cultures of *Capsicum chinense* (Figure 2A), and that reported for other *Capsicum* varieties, such as *Capsicum frutescens* (Sudha and Ravishankar, 2003a, 2003b), *Capsicum annuum* (Martínez Juarez et al., 2004), and *Capsicum chinense* (Arroyo et al., 2017), usually yield the typical sigmoid curve for the kinetic of growth of suspended plant cells (Mohamad 218). In contrast, the profile pattern for accumulation of capsaicinoids was usually erratic, showing presence occasionally throughout the end of the lag phase and/or the stationary phase, but most of the time these compounds are not detected along the cell suspensions growth cycle. These observations agree with previous reports for cell suspension cultures of other *Capsicum* varieties (Calva et al., 1995, Sudha and Ravishankar, 2003b, Martínez Juarez et al., 2004). However, in fruits, the profile pattern for capsaicinoids accumulation showed a continuous raise (Figure 2B), with a clear direct relationship with the fruit elongation and the placental development, entering to the stationary phase of accumulation when both the increment of the fruit's length and the placental tissue growth stop, once the transition of the green color to the red, orange, or yellow mature color, characteristic of each cultivar starts (Figure 3). So, it was expected to observe the presence of the ω-1-methyl-fatty acids along with the accumulation pattern of capsaicinoids.

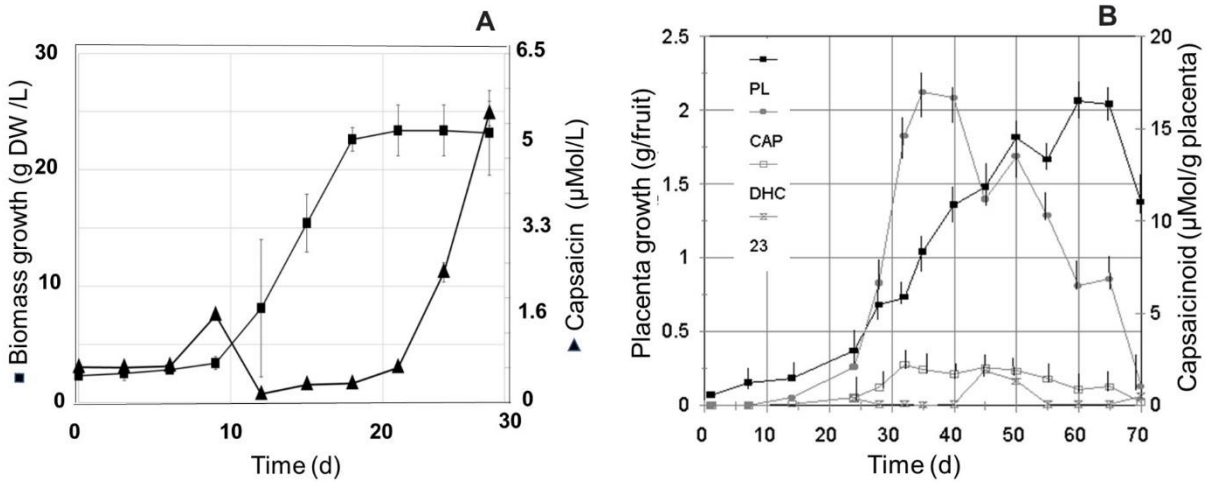


Fig. 2. A, *Kinetic of growth (■) of cell suspension cultures of C. chinense (Habanero) cultivated in 125 mL Erlenmeyer flasks with 30 mL of MS medium added with 2.4D (12.5 µM), and K (0.5 µM), on an orbital shaker at 90-100 rpm and 25°C, and accumulation pattern of capsaicin (▲), in comparison with* ***B,*** *the profile of capsaicin (CAP), dihydrocapsaicin (DHC), and an unidentified capsaicinoid (23), accumulation in the placental tissue (PL) of Capsicum annuum Var annuum (Chigol) fruits.*

Presence of ω-1-methyl-fatty acids regarding the capsaicinoids accumulation pattern. The GC/MS chromatograms of the fatty acid fraction from the lipidic extracts of placental tissue of several *Capsicum* species, such as *Capsicum frutescens* (Piquín), and *Capsicum Chinense* (Habanero Kahui), and *Capsicum annum* (Jalapeño), were analyzed along the corresponding capsaicinoids accumulation pattern, however the presence of the ω-1-

methyl-fatty acids, were not detected. But, interestingly, in the chromatograms for the extracts from immature fruits of *Capsicum pubescens* (Manzano), tiny amounts but showing clear signals at the retention time and molecular weight for some of the ω- 1-methyl-fatty acids precursors of capsaicinoids (7-7.5 min) were detected (inset in Figure 4A); and, the fragmentation pattern of the signal at the retention time of 7.4 minutes, corresponding to 8-methyl-6-transnonenoic acid (Figure 4A), precursor of capsaicin, produced a correlation with the fragmentation pattern of the standard fatty acid (Figure 4B), in more than 99%. Similar signals were putatively detected in extracts from leaves adjacent to the peduncle of fruits of *Capsicum pubescens* plants, however these results deserve further confirmation. Nevertheless, these results clearly prove the presence of the fatty acids precursors of capsaicinoids in the placental tissue of *Capsicum pubescens* fruits. Unfortunately, no similar results were observed for the extracts from other *Capsicum* species explored (*Capsicum annuum, Capsicum chinense,* and *Capsicum frutescens*), and neither for lipidic extracts from their respective cell suspension cultures.

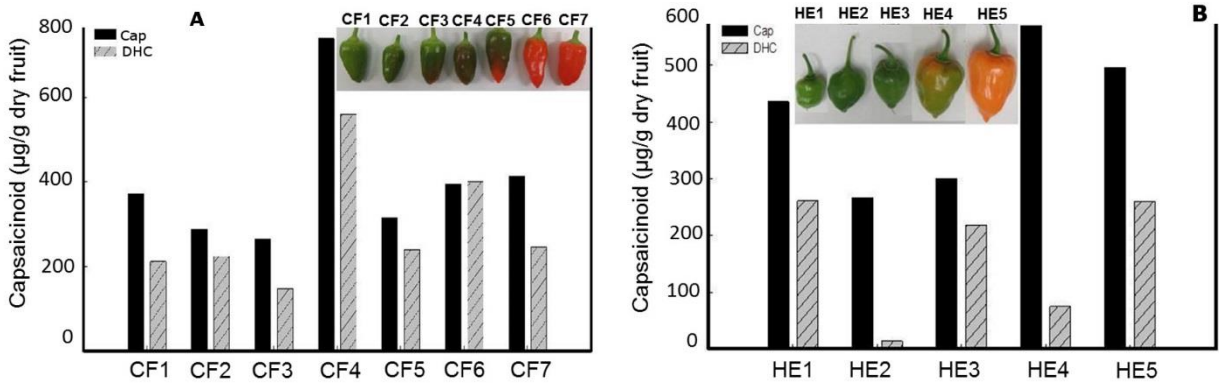


Fig. 3. *Capsaicin (Cap) and dihydrocapsaicin (DHC) content in dry fruits of Capsicum frutescens (A, Piquín), and Capsicum Chinense (B, Habanero Kahui), at different stages of maturation and color.*

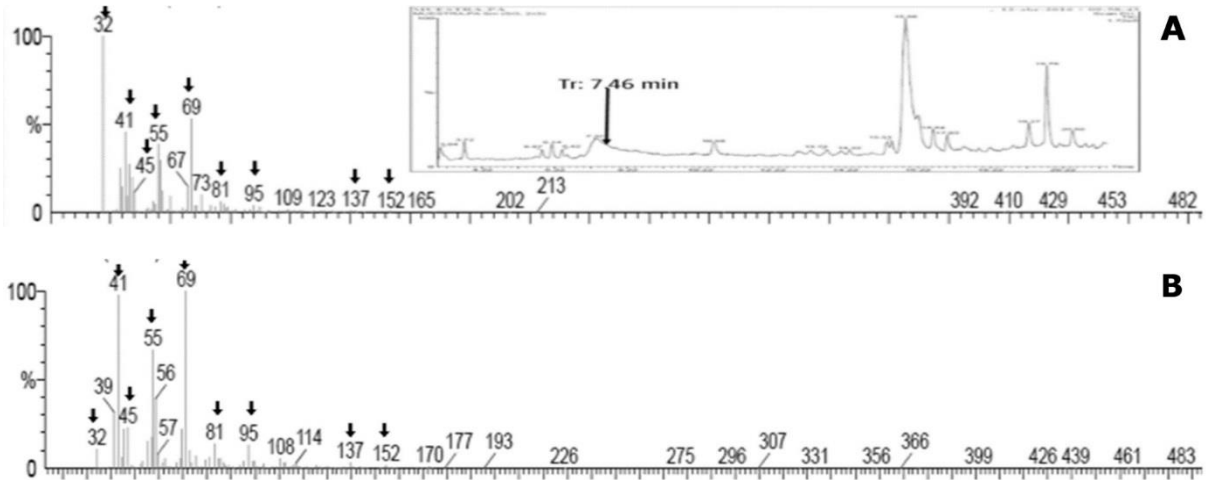


Fig. 4. A, *fragmentation pattern of the signal observed at 7.46 minutes in the GC/MS chromatograms of the fatty acid fraction from the lipidic extracts of placental tissue of Capsicum pubescens fruits (inset in a), in comparison with* ***B,*** *the fragmentation pattern of the true 8-methyl-6-trans-nonenoic acid with m/z 170 Uma, eluting at the same retention time as the selected peak.*

CONCLUSIONS

The highest accumulation of capsaicinoids occurred along the color transition of fruits: from green to the final color of the fruit, according to each specific *Capsicum* species. The clear presence of 8-methyl-6-trans-fatty acid detected in the placental tissue of *Capsicum pubescens* fruits provide evidence for these fatty acids to be the

capsaicinoids precursors. However, the biochemical reaction for the biotransformation of valine and leucine into the respective fatty acids is still pending of further research.

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BIBLIOGRAPHIC REFERENCES

Aluru, M. R., Mazourek, M., Landry, L. G., Curry, L., Lahn M., & O'Connell M. A. (2003). Differential expression of fatty acid synthase genes, Acl, Fat and Kas, in Capsicum fruit. *J. Exp. Bot., 54, 1655-1664.*

Arroyo Orbegoso, A.G., Pérez Vargas, J., Zamudio Moreno, E., Gómez Guzmán, O., & Calva-Calva, G. (2017). Actividad de deshidrogenasa en relación a omega-1-metil-ácidos grasos y capsaicinoides en frutos y cultivos in vitro de *Capsicum*. *Revista CiBIyT, Ciencias Básicas, Ingeniería y Tecnología. 12(35)*: 86-91.

Aza-González, C., Núñez-Palenius, H. G., & Ochoa-Alejo, N. (2011). Molecular biology of capsaicinoid biosynthesis in chili pepper (Capsicum spp.). *Plant cell reports*, *30*(5), 695-706.

Calva C. G., Ríos L. E., 1999. Cultivo de callos y su acumulación de Metabolitos secundarios. En: *Aspectos Aplicados de la Biotecnología* (Rodríguez V. R., Calva C. G., Salazar M. A., & Ramos R. E. G. Eds.) *pags. 267-301 Cap. 6.* Coedición SEP-IPN. ISBN 970-18-2172-6.

Calva-Calva G., Narbard A., Eagles J., Parr A. J., Rhodes M. J. C., Walton N. J., Amiot M-J., & Robins R. J., (1995). Phenolic transformation by Capsicum spp. In: *Current trends in fruit and vegetables phytochemistry*. En M. C.-B. Garcia-Viguera C., Consejo nacional de investigaciones científicas (*págs. 205-209*). Madrid, España. et al., (5) 199.

Curry J, Aluru M, Mendoza M, Nevarez J, Melendrez M, & O'Connell MA (1999) Transcripts for possible capsaicinoid biosynthetic genes are differentially accumulated in pungent and non-pungent Capsicum spp. Plant Sci 148: 47–57.

Fujiwake, H., Suzuki T., & K. Iwai (1982). Intracellular distribution of enzymes and intermediates involved in the biosynthesis of capsaicin and its analogues in Capsicum fruits. *Agric. Biol. Chem. 46(11)*: 2685 2689.

Fujiwake, H., T. Suzuki, S. Oka, & K. Iwai (1980). Enzymatic formation of capsaicinoid from vanillylamine and Iso type fatty acids by cell free extracts of Capsicum annuum var. annuum cv. Karayatsubusa. *Agric. Biol. Chem. 44(12):*2907 2912.

Markai S, Marchand Patrice A, Mabon F, Baguet E, Billault I, & Robins RJ. (2002) Natural deuterium distribution in branched-chain medium-length fatty acids is nonstatistical: a site-specific study by quantitative 2H NMR spectroscopy of the fatty acids of capsaicinoids. ChemBioChem 3: 212–218.

Martínez-Juárez V., Ochoa-Alejo N, Lozoya-Gloria E, Villareal-Ortega M, Ariza-Castolo A, Esparza-García F., & Calva-Calva G (2004). Specific synthesis of 5,5´-dicapsaicin by cell suspension cultures of *Capsicum annuum* var. *annuum* (Chili Jalapeño Chigol) and their soluble and NaCl-extracted cell wall protein fractions. J Agr Food Chem. 52 (4): 972─979.

Mohamad Puad, N. I., & Abdullah, T. A. (2018). Monitoring the growth of plant cells in suspension culture. In *Multifaceted Protocol in Biotechnology* (pp. 203-214). Springer, Singapur.

Rivera Casado NA, Montes Horcasitas MC, Rodriguez Vazquez R, Esparza Garcia FJ, Perez Vargas J, Ariza Castolo A., & Calva Calva G. (2015). The Fatty Acid Profile Analysis of Cyperus laxus Used for Phytoremediation of Soils from Aged Oil Spill-Impacted Sites Revealed That This Is a C18:3 Plant Species. *PLoS ONE 10(10):* e0140103.doi:10.1371/journal.pone.0140103.

Sudha G, & Ravishankar G. A. (2003a) Putrescine facilitated enhancement of capsaicin production in cell suspension cultures of *Capsicum frutescens*. J. Plant Physiol. 160: 339–346.

Sudha G, & Ravishankar GA. (2003b). Influence of methyl jasmonato and salicylic acid in the enhancement of capsaicin production in cell suspension cultures of *Capsicum frutescens* Mill. *Curr. Sci. 85:* 1212–1217.

Suzuki, T., & K. Iwai (1984). Constituents of red pepper species: Chemistry, biochemistry, pharmacology, and food science of the pungent principle of Capsicum species. In: "The alkaloids" (Brassi A., Ed.) 23:227 299. Academic Press. New York.

Suzuki, T., Kawada, T., & Iwai, K. (1981a). The Precursors Affecting the Composition of Capsaicin and Its Analogues in the Fruits of Capsicum annuum var. annuum cv. Karayatsubusa. *Agric. Bioi. Chem., 45 (2)*, 535", 537.

Suzuki, T., Kawada, T., & Iwai, K. (1981b). Biosynthesis of acyl moieties of capsaicin and its analogues from valine and leucine in Capsicum fruits. *Plant and Cell Physiology, 22(1)*, 23-32.

Vazquez Flota, F., Miranda Ham, M. d., Monforte Gonzalez, M., Gutierrez Carbajal, G., Velazquez Garcia, C., & Nieto Pelayo, Y. (2007). Biosynthesis of Capsaicinoids, The Pungent Principle of Peppers. *Fitotec, 30(4),* 353-360.

Yeoman M. M., M. A. Holden, R. D. Hall, P. R. Holden, & S. S. Holland (1989). Regulation of capsaicin synthesis in cultured cells of *Capsicum frutescens.* In: "Primary and secondary metabolism of plant cell cultures" (Kurz W. G. W., Ed.) pages 162-174. Springer-Verlag, Berlin Heidelberg.

Este artículo no presenta conflicto de interés

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Pérez Vargas, J: conceptualización, curación de datos, análisis formal.
Ariza Castolo, A: conceptualización, curación de datos, análisis formal.
Martínez Estévez, M: conceptualización, curación de datos, análisis formal.
Gómez Guzmán, O: conceptualización, curación de datos, análisis formal.
Calva-Calva, G: conceptualización, curación de datos, análisis formal, revisión final.