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Capsaicinoids quantification in chili peppers cultivated in the state of Yucatan, Mexico

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Abstract

Capsaicinoids are a group of 12 or more related alkaloids responsible of the pungent sensation in fruits of the genus *Capsicum*. Capsaicin [(*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide] and dihydrocapsaicin are responsible for more than 90% of the pungency. This work describes the quantitative analyses by gas chromatography of the content of capsaicin and dihydrocapsaicin in the pericarp, placenta, and seeds of seven cultivars of chili peppers cultivated in the state of Yucatan, Mexico [chawa, dulce, sukurre, xcat'ik (*Capsicum annuum* L. var. annuum), maax (*Capsicum annuum* L. var. aviculare), and habanero orange and habanero white (*Capsicum chinense* Jacq.)]. Capsaicin content was higher, as expected, in the fruits of habanero orange and habanero white, followed by sukurre, chawa, xkat'ik, and maax. Dihydrocapsaicin content did not follow the same scheme, being higher in the fruits of sukurre, followed by chawa, habanero white, habanero orange, and maax. Xcat'ik showed minor quantities of dihydrocapsaicin, while dulce chili contained only traces of these two alkaloids.

Keywords: Chili peppers; *Capsicum annuum*; *Capsicum chinense*; Chawa; Dulce; Habanero orange; Habanero white; Maax; Sukurre; Xkat'ik; GC quantification; Capsaicin; Dihydrocapsaicin; Yucatan

1. Introduction

Chili peppers (*Capsicum* spp.) are appreciated for their pungency, taste, and aroma as food additives, pigments, and for their physiological and pharmaceutical uses. They are economically important to Mexico, not only for the preparation of regional dishes that have made the Mexican gastronomy worldwide famous, but also due to the vast quantity and genetic variability that are produced in its territory, mostly the spicy species (Pozo, Montes, & Redondo, 1991). The pungent metabolites in the fruits of *Capsicum* species are called capsaicinoids, which are a group of 12 or more alkaloids with a structure of vanillylamide of branched fatty acids with 9–11 carbons (Suzuki & Iwai, 1984, chapter 4), but capsaicin [(*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide] and dihydrocapsaicin are responsible for more than 90% of the pungency (Kosuge & Murata, 1970). *Capsicum* is the only genus known to produce capsaicinoids (Blum et al., 2002; Kirschbaum-Titze, Mueller-Seitz, & Petz, 2002). Capsaicinoids biosynthesis takes place in the placenta, where epidermal specialized cells accumulate them in vacuoles and eventually excrete these alkaloids and drip them on seeds and internal pericarp surface.

In the present work we have determined the content of capsaicin and dihydrocapsaicin (Fig. 1) by means of gas chromatography (GC) in the pericarp, placenta, and seeds of seven cultivars of chili peppers cultivated in the state of

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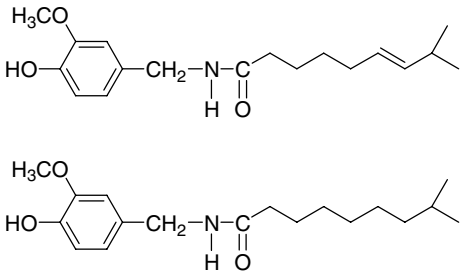

Fig. 1. Chemical structures of capsaicin and dihydrocapsaicin.

Yucatan: chawa, dulce, sukurre, xcat'ik (*Capsicum annuum* L. var. annuum), maax (*Capsicum annuum* L. var. aviculare), and habanero orange and habanero white (*Capsicum chinense* Jacq.). Despite of the Yucatan peninsula being one of the regions in Mexico with the largest cultivated *Capsicum* diversity, some cultivars, such as chawa, sukurre, and habanero white, are not well known to the general population in Yucatan and elsewhere.

2. Materials and methods

2.1. Plant material

Chili peppers chawa, sukurre, and habanero white were collected in October–November 2003 in Yaxcabá, a town situated southeast to Merida, the capital of the state of Yucatan; xcat'ik and dulce were obtained from Ixil, a small town located 25 km from the capital; habanero orange was provided by INIFAP-Uxmal; and maax was acquired from a market in downtown Merida. These four varieties were collected in January 2004. Fruits were obtained at different stages from immature green to senescent red and, according to the sellers, they were field-grown harvested. Voucher specimens are deposited in the herbarium of Unidad de Recursos Naturales at CICY. Fruits of each species, collected from at least two individual plants, were cut and their pericarp, placenta, and seeds separated, which were weighted fresh, then frozen at −20 °C, and finally dried by means of a freeze drier (Labconco, model 77540-00, Kansas City, Missouri, USA) for three days at −40 °C and 3.3×10^{-3} Mbar.

2.2. Extraction of capsaicinoids

Dried tissue (0.2–1.0 g) was added to a FastPrep instrument (model FP120, Bioselect, SA, Mexico City), in which it was ground for 20 s, extracted with acetone (HPLC grade, J.T. Baker, Phillipsburg, NJ, USA), and then centrifuged at 7826*g* (10,000 rpm) for 3 min (Hettich, model Micro20, Tuttlingen, Germany); this process was repeated three more times. The combined filtrates were adjusted to a final volume of 1 mL with fresh acetone. From this solution, 1.6 μL were used to inject into the GC. Area measurements of four injections were used as mean value for quantification.

2.3. GC analysis

Analyses were performed on a Hewlett-Packard 5890 gas chromatograph (Agilent Technologies Mexico, Mexico City) equipped with a flame ionization detector (FID) operated at 290 °C. The injection liner was a packed liner, with internal diameter of 4 mm, for a volume of 800 μL (Hewlett-Packard 18740–60840, Agilent Technologies Mexico, Mexico City). Samples (1.6 μL) were loaded into the injection port at 290 °C as split injections at a ratio of 1:100 with a 10-mL syringe (Hamilton). The column used was an Ultra 2 (crosslinked 5% Ph Me silicone; 25 m × 0.32 mm × 0.52 μm film thickness, Agilent Technologies Mexico, Mexico City). The column temperature program was as follows: 180 °C for 2 min, 15 °C/min to reach 280 °C for 6.7 min, 280 °C for 15 min (23.7 min total). The carrier gas was N_2 with a flow rate of 1.0 mL/min at 290 °C and a septum purge of 2 mL/min.

2.4. Standards and quantification

Identification of capsaicin in extracts was performed by comparison of retention time of its peak in the extract with that of commercial capsaicin (HPLC grade, min. 97%, Fluka Chemicals, Toluca, Mexico). A stock solution of capsaicin was prepared with acetone (HPLC grade, J.T. Baker, Phillipsburg, NJ, USA) at a concentration of 0.05 g/5 mL. From this stock, six 1-mL solutions were prepared at the final concentrations of 0.25, 0.50, 1.00, 1.50, 2.00, and 2.50 mg/mL to be used to obtain a standard curve of capsaicin ($R_T = 9.92$ min). Vanillin (98%, Sigma) was used as the internal standard at a concentration of 2.00 mg/mL ($R_T = 1.99$ min). Each point of the curve was obtained as an average of four injections. Quantification was performed from integrated FID peak area measurements observed in the chromatograms printed in a Shimadzu C-R6A Chromatopac integrator. Finally, the standard curve was produced using a linear regression program ($R^2 = 0.9883$). A standard curve of dihydrocapsaicin (90%, Sigma–Aldrich, Toluca, Mexico) ($R^2 = 0.9513$) to identify it in the extracts was prepared in the same manner as described above for capsaicin.

3. Results and discussion

Fruits of cultivars of *C. annuum* and *C. chinense* evaluated in this study showed variation in fresh and dry weight for pericarp, placenta and seeds as summarized in Table 1. Xcat'ik and dulce showed the highest fresh and dry weights for pericarp, placenta and seeds, representing the largest local chili cultivars analyzed in this study. Habanero orange and habanero white presented intermediate values, while chawa and sukurre showed the lowest fresh and dry biomass values. Because of its small size, maax cultivar was not included in Table 1 since it was not possible to separate its fruit components; however, it was the cultivar with the smallest fruit biomass. Differences in pericarp, placenta,