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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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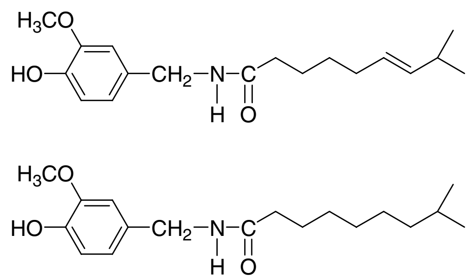

Fig. 1. Chemical structures of capsaicin and dihydrocapsaicin.

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,

Table 1
Fresh and dry weight of pericarp, placenta, and seeds of six chili pepper cultivars grown in the state of Yucatan, Mexico[a,b,c]

Cultivar	Fresh weight (g)			Dry weight (g)		
	Pericarp	Placenta	Seeds	Pericarp	Placenta	Seeds
Chawa	1.47 (±0.63)	0.21 (±0.15)	0.27 (±0.10)	0.33 (±0.10)	0.04 (±0.02)	0.14 (±0.03)
Dulce	34.43 (±7.69)	6.40 (±2.19)	2.00 (±0.59)	2.38 (±0.71)	0.48 (±0.18)	0.37 (±0.16)
Habanero orange	5.76 (±1.08)	0.53 (±0.14)	0.47 (±0.15)	0.84 (±0.39)	0.08 (±0.02)	0.23 (±0.07)
Habanero white	5.06 (±0.76)	0.73 (±0.41)	0.39 (±0.07)	0.47 (±0.21)	0.08 (±0.04)	0.15 (±0.03)
Sukurre	1.10 (±0.77)	0.10 (±0.12)	0.16 (±0.10)	0.15 (±0.09)	0.01 (±0.01)	0.07 (±0.06)
Xcat'ik	36.54 (±7.24)	7.69 (±1.83)	1.46 (±0.46)	2.54 (±0.50)	0.57 (±0.14)	0.48 (±0.20)

[a] Maax chili components were not possible to separate and therefore are not included.
[b] An ANOVA analysis was performed at a 95% confidence interval.
[c] Values in parentheses indicate standard deviation of the mean.

and seeds biomass may influence determination of capsaicinoids concentration as a consequence of the higher proportion of pericarp and seeds in relation to placenta and contribute to under- or overestimation of capsaicinoids content. To compare capsaicinoids concentration among the studied cultivars, analysis was carried out on dried separated pericarp, placenta, and seeds tissues instead of using dried ground whole fruits.

The gas chromatographic method used for the analysis of capsaicinoids in the present work was an adaptation of those reported in the literature (Thomas, Schreiber, & Weisskopf, 1998; Wooderck, Jaeho, Jinbong, & Youngjung, 1994). Basically, an Ultra 2 column (Agilent Technologies Mexico, Mexico City) was used instead of an AT-1701 column, and the temperature program was modified as described in Section 2. Capsaicin content observed in the pericarp, placenta, and seeds of the dried fruits of the seven cultivars under study is shown in Table 2. Higher capsaicin amounts were found in placental tissues and contributed for most of the total capsaicin fruit content of the pungent chilies. Worldwide known for its highest capsaicin content, habanero orange (Fig. 2A) was included in this study as a reference for comparison with other chili cultivars. In our analysis, the dried placenta of this pepper showed to have 62,886 μg/g of capsaicin. Habanero orange is, among local chili cultivars, the most popular chili pepper eaten fresh in almost every meal, or used, green or aged, to prepare hot sauces. Although habanero is not a native plant to Yucatan (Andrews, 1999; Long-Solıs, 2004; Pickersgill, Heiser, & McNeill, 1979), it is well adapted to its environment and fruit quality regarding pungency, aroma, and flavour achieved with its local cultivars are highly appreciated regionally and abroad. Therefore, most of the habanero orange's local production is for export, 70% of which is consumed fresh in meals, 25% is used industrially, and the rest is used to extract the seeds (Piña-Razo, 1982). Habanero white (Fig. 2B), less pungent than habanero orange (29,483 μg/g), but still considered strong, is the fruit of a shrub with green leaves, 50–80 cm high, that presents usually four white flowers per axil. The peel colour of the immature fruit is remarkably light-green as compared to its counterpart, habanero orange, which is dark-green. The fruit is composed of three locules in a similar fashion to habanero orange, although more locules are not uncommon (data not shown). As it matures, the colour turns to a dark red. This pepper, not previously described in the literature, is not well known among people and plant individuals tend to be scarce, which are grown in backyard gardens for self consumption. Local farmers consider this cultivar more susceptible to fungal and bacterial diseases than habanero orange.

On the other end of the scale, with only traces of capsaicin, there is dulce chili, a green pepper with great taste (dulce = sweet) and no pungency, appreciated for salads

Table 2
Capsaicin content in the pericarp, placenta, and seeds of seven chili pepper cultivars grown in the state of Yucatan, Mexico[a,b]

Cultivar	μg/g of dried pericarp	μg/g of dried placenta	μg/g of dried seeds
Chawa (unripe)	220 (±16)	11,698 (±1208)	897 (±43)
Chawa (ripe)	222 (±34)	5689 (±475)	tr
Dulce	tr	tr	tr
Habanero orange	3914 (±292)	62,886 (±3497)	2280 (±139)
Habanero white	2592 (±85)	29,483 (±1854)	3195 (±170)
Maax (unripe)[c]		640 (±58)	
Maax (ripe)[c]		750 (±34)	
Sukurre (unripe)	tr	26,945 (±2499)	tr
Sukurre (ripe)	tr	18,995 (±438)	tr
Xkat'ik	tr	3189 (±43)	tr

[a] An ANOVA analysis was performed at a 95% confidence interval.
[b] Values in parentheses indicate standard deviation of the mean.
[c] Analyzed as entire fruit. tr = traces.

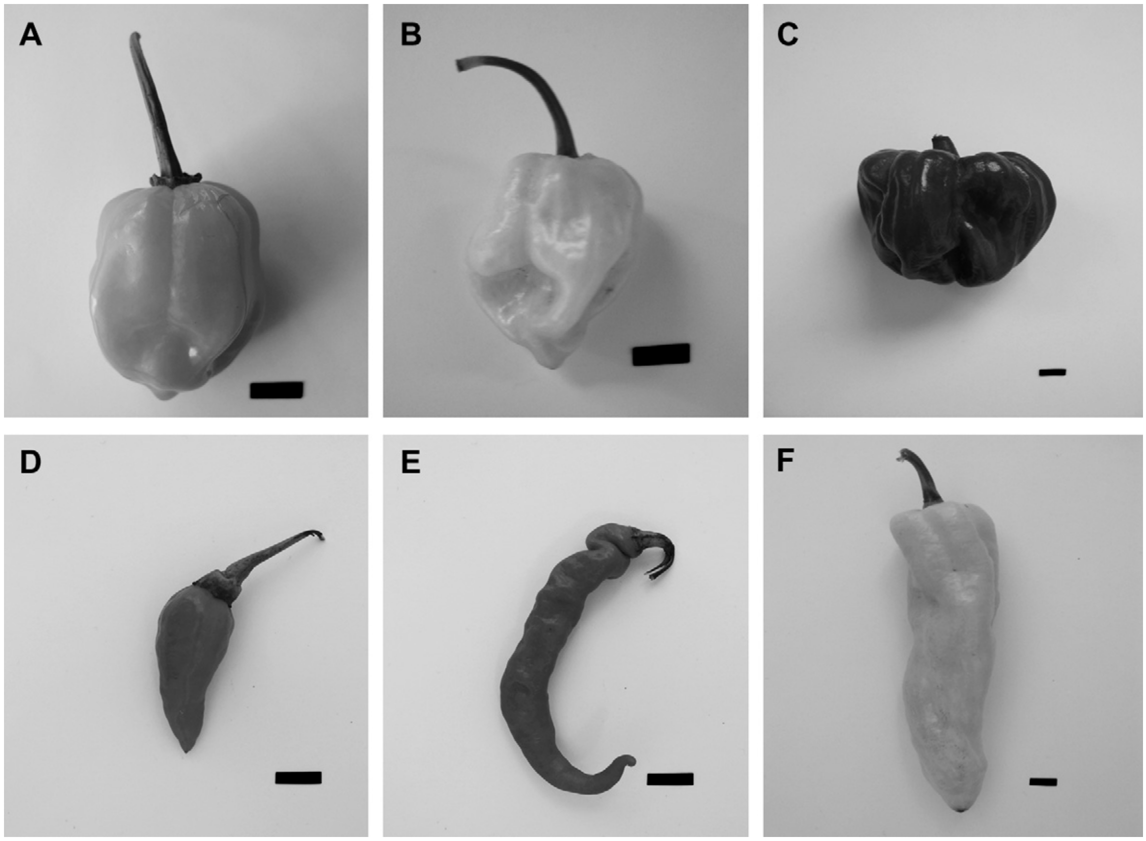

Fig. 2. Fruit morphology of *Capsicum annuum* and *Capsicum chinense* cultivated in the state of Yucatan, Mexico. Habanero orange (A) and habanero white (B) (*Capsicum chinense* Jacq.). Dulce (C), sukurre (D), chawa (E) and xcat'ik (F) (*Capsicum annuum* L. var. annuum).

and cooking. Also known in Maya language as *ch'uhuk ik*, it is a shrub with dense foliage and dark-green, oval leaves. The fruit is bell-shaped (Fig. 2C), with a green pericarp, when unripe, and red in plain maturity with abundant seeds on its large placenta (Terán, Rasmussen, & May-Cauich, 1998).

The capsaicin content of sukurre chili (Fig. 2D) when the fruit is immature is comparable to habanero white (26,945 vs. 29,483 μg/g), but this amount is partially lost when it is ripen and turns red. Plant species reach a maximum height of 86 cm with dense foliage, scarce branches, and light-green or yellowish-green leaves. Individuals present one white flower per axil that lasts for more than three months. The erect fruit is pale yellow to light green with a medium corrugated surface when unripe, and it lacks of a constriction at the peduncle base (Velasco-Morales, 2003). Seeds are abundant and occupy most of the internal locules space. Sukurre chili is difficult to find in local markets and we uncovered it as a backyard crop with very few local farmers growing this cultivar.

Chawa chili is also known in Maya language as *chak ik*, *chawa'ik*, *chowak*, and *sak ik* (Herrera, 1994). The plant is an herb or small shrub with intermediate or dense foliage, oval leaves, and white flowers. Its elongated fruits (Fig. 2E) present a corrugated epidermis, sometimes plain, which is yellow as they age and red when in full ripeness (Velasco-Morales, 2003). Fruit anatomy features are very similar to sukurre chili. GC analyses showed that more than 50% of the content of capsaicin (11,698 μg/g) is lost as the fruit matures.

Plants of xkat'ik (blond) chili reach a height of 70 cm, depending on seasonal and seedtime conditions. The leaves present diverse tonalities of green colour. The thin fruit is pale-yellow, conic elongated, sharp-pointed, and a little undulated, reaching a length of 9–17 cm (Fig. 2F). Fruit flesh is thick and tasty and placenta sets many seeds. It is moderately hot (3189 μg/g of capsaicin), but because of its pleasant taste is highly appreciated in Yucatan to consume fresh or grilled, and it is usually accompanying traditional souse dishes of fish, poultry, or turkey (Velasco-Morales, 2003).

Chili maax is also known as *piquín* or *chiltepín*, although it might be mistaken by other pepper similar in size and pungency that grows in the northern-side plateau of Mexico (Velasco-Morales, 2003). This variety (aviculare) has been proposed as the wild progenitor of domesticated *C. annuum* (D'Arcy & Eshbaugh, 1984; Pickersgill, 1971). In Mexico, it is distributed along the Pacific coast, from Sonora to Chiapas, and in the Atlantic side, from Tamaulipas to the Yucatan peninsula. Generally, the plant possesses small fruits (0.6–2.0 cm in length with an average fresh weight of 0.13 ± 0.02 g) that come in great varieties of forms, usually ovoid or rounded (Fig. 3A). Tender fruits are green, but as they mature, they acquire a red, orange,

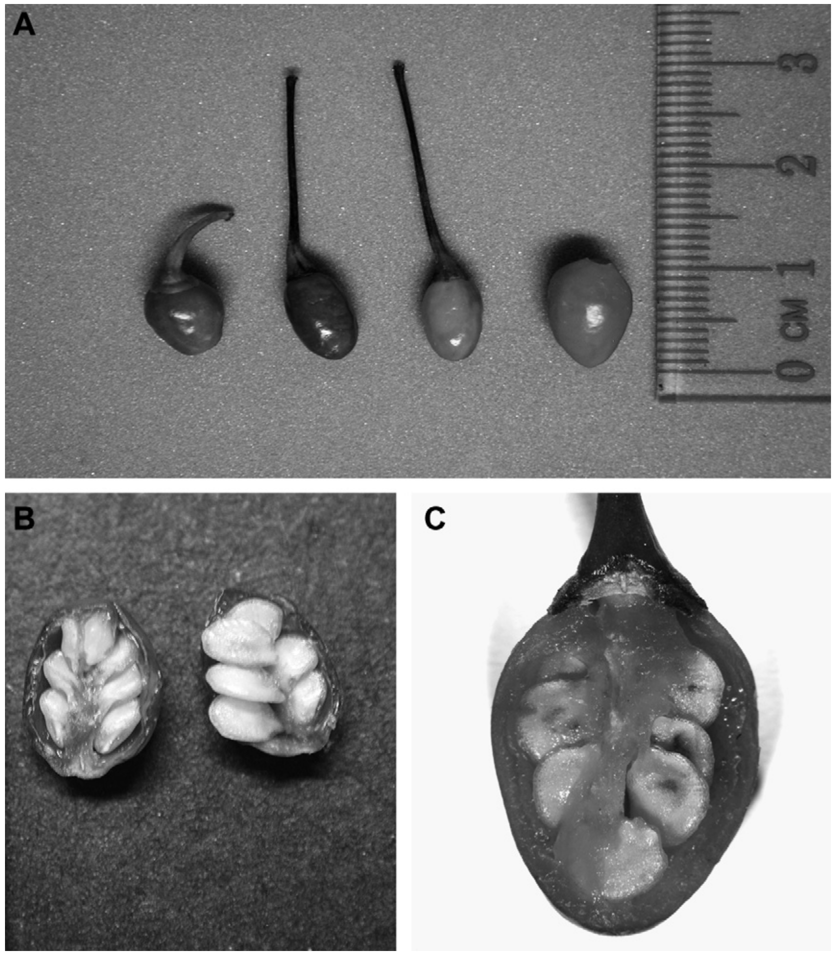

Fig. 3. Fruits of maax chili (*Capsicum annuum* L. var. aviculare). Fruit morphology (A) and cross sections (B, C) showing fruit anatomy. Note the relatively large size of seeds compared to the fruit size, a thin pericarp and a small placenta.

or yellow colour (Arzudia & González, 1986). Internal fruit cavities (locules) are fully occupied by seeds surrounded by a thin pericarp (Fig. 3B and C). In our analysis, in comparison to the other peppers, maax chili showed the lowest content of capsaicin (640 μg/g), which becomes slightly larger as it matures.

Our results indicated that *C. annuum* cultivars present lower capsaicin content in its placental tissues compared to *C. chinense* cultivars. However, pungency is not the only organoleptic feature chosen for local cooking, since aroma and flavour are also appreciated, maintaining a demand for most of the cultivars analyzed in this study.

On the other hand, the contents of dihydrocapsaicin (Table 3) did not follow the same pattern in the seven chili cultivars. Unexpectedly, sukurre chili presented the highest quantity of this alkaloid, although not as high as that for

Table 3
Dihydrocapsaicin content in the pericarp, placenta, and seeds of seven chili pepper cultivars grown in the state of Yucatan, Mexico[a,b]

Cultivar	μg/g of dried pericarp	μg/g of dried placenta	μg/g of dried seeds
Chawa (unripe)	87 (±150)	2088 (±1394)	tr
Chawa (ripe)	652 (±106)	3214 (±253)	1689 (±157)
Dulce	tr	tr	tr
Habanero orange	663 (±69)	1607 (±79)	439 (±53)
Habanero white	602 (±14)	2349 (±272)	935 (±48)
Maax (unripe)[c]		370 (±40)	
Maax (ripe)[c]		tr	
Sukurre (unripe)	tr	3652 (±339)	tr
Sukurre (ripe)	tr	5043 (±211)	tr
Xkat'ik	tr	tr	tr

[a] An ANOVA analysis was performed at a 95% confidence interval.
[b] Values in parentheses indicate standard deviation of the mean.
[c] Analyzed as entire fruit. tr = traces.

capsaicin, followed by chawa, habanero white, and habanero orange, while maax chili showed the least amount of it. Xkat'ik and dulce chilies showed only traces. It seems that *C. chinense* cultivars contain less amounts of dihydrocapsaicin as compared to those of *C. annuum* cultivars.

Capsaicinoids all share a common aromatic moiety, vanillylamine, and differ in the length and degree of unsaturation of a fatty acid side chain. The two most common capsaicinoids, capsaicin and dihydrocapsaicin, differ in the degree of unsaturation of a 9-carbon fatty acid side chain (Curry et al., 1999). Differences in capsaicinoids concentration, such as capsaicin and dihydrocapsaicin, have been reported for different capsicum cultivars. However, it has also been reported that capsaicinoids concentration and profiles are not good chemotaxonomical indicators for *Capsicum* (Zewdie & Bosland, 2001). Variations in capsaicinoids quantity found in our study could be attributed to intrinsic genetic factors to each cultivar or alternatively to the environmental conditions where they were cultivated. Further research will be required to investigate the molecular genetic background of the chili pepper cultivars included in this study.

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