HortScience 43(5):1344–1349. 2008.

Capsaicinoids Content in Habanero Pepper (*Capsicum chinense* Jacq.): Hottest Known Cultivars

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Additional index words. pungency, capsaicinoids, HPLC, Scoville Units, germplasm, Habanero pepper, *Capsicum chinense*, Jacq.

***Abstract*. The aim of this study was to determine the pungency level of different accessions of Habanero peppers. The high-performance liquid chromatography (HPLC) technique was used to evaluate the content of total capsaicinoids in the whole fruit, placenta, and pericarp of 18 accessions of Habanero pepper from the germplasm bank of the *Capsicum chinense* species maintained in the Scientific Research Center of Yucatan [Centro de Investigación Científica de Yucatán (CICY)]. Thirteen of these accessions belonged to the "orange type", four to the "red type", and one to the "yellow type". During the study, the plants were cultivated and maintained under greenhouse conditions and the fruit was harvested only when it was completely ripe on the plant. The results show considerable intraspecific diversity for this characteristic as well as the existence of cultivars of this species that surpass the levels of pungency reported for Habanero peppers under the conditions evaluated.**

Peppers (*Capsicum* spp.) are well known for their ability to cause an intense organoleptic sensation of heat when consumed. Peppers probably originated in Bolivia, as this area contains many of the 20 to 27 recognized species of *Capsicum* (Andrews, 1999; Hunziker, 2001;Walsh and Hoot, 2001). In 2006, *C. chinense* cv. Red Savina was recognized as the hottest chili pepper on record with a heat level of 577,000 Scoville Units (SHU) (Guinness Book of World Records, 2006). However, Bosland and Baral (2007) recently reported that "Bhut Jolokia", a natural interspecific hybrid between *Capsicum chinense* and *Capsicum frutescens*, is in fact the world's hottest known chili pepper with a heat level of 879,953 to 1,001,304 SHU.

The heat sensation is incited by a group of capsaicinoids, alkaloids found only in chili pepper (Zewdie and Bosland, 2000). Capsaicin and dihydrocapsaicin are responsible for 90% of the pungency (Govidajaran, 1986; Iwai et al., 1979; Kawada et al., 1985; Kosuge and Furata, 1970). Capsaicinoids are unique to the genus *Capsicum* (Govidarajan et al., 1987 ; Govindarajan and Sathyanarayana, 1991). The level of capsaicinoids can be determined using chemical, instrumental, or sensorial methods. The Scoville organic test, invented by Scoville in 1912, is a subjective measure of chili pungency. Several different methods have been used for the quantification of capsaicinoids from peppers and oleoresins, including organoleptic methods (Govindarajan et al., 1977; Scoville, 1912), spectrophotometry (Anan et al., 1996; Awasthi and Singh, 1973; Bajaj, 1980; Bajaj and Kaur, 1979; Mori, 1976), thin-layer chromatography (Sankarikutty et al., 1978), gas–liquid chromatography (Todd et al., 1977), and high-performance liquid chromatography (Weaver and Awde, 1986). Of these, high-pressure liquid chromatography (HPLC) is considered the most reliable and rapid method (Yao et al., 1994) available for the identification and quantification of capsaicinoids. The aim of this study was to determine the content of total capsaicinoids in the whole fruit, placenta, and pericarp of 18 accessions of Habanero pepper collected in the Peninsula of Yucatan using the HPLC technique.

Received for publication 13 Nov. 2007. Accepted for publication 5 Mar. 2008.
We thank the Yucatán-Produce Foundation and SINAREFI for funding our research, M.C. Rosa María Galaz Avalos for technical assistance, and Ing. Eleazar Xool for the use of his greenhouse.
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Materials and Methods

For this study, 18 accessions of Habanero pepper (*Capsicum chinense*) (Table 1) from a germplasm bank established in the Centro de Investigación Científica de Yucatán (Scientific Research Center of Yucatan) were used. Of these, 13 accessions were of the "orange type", four of the "red type", and one "yellow type". The plants were cultivated and maintained under protected conditions and the fruit was harvested when it had ripened on the plant. Thirty fruits per plant were taken from 10 plants of each accession.

This work was carried out in the municipality of Ixil, Yucatan, located between the parallels 21°09′ and 21°19′ lat. north and the meridians 89°25′ and 89°34′ long. west at an average height of 9 m above sea level. The climate of the region is hot, subhumid with rain in the summer and a dry season from January to May. The average annual temperature fluctuates between 24.6 and 27.7 °C and annual rainfall between 600 and 900 mm. The predominant soil type in this region is litosols or superficial stony soil in accordance with the classification by the Food and Agriculture Organization of the United Nations.

Determination of capsaicinoid content. The capsaicinoid content in placenta, pericarp, and whole fruit of each selected accession was evaluated. They were frozen immediately in liquid N_2 and lyophilized to carry out the capsaicinoid analysis. Capsaicinoids were quantified with HPLC according to the method reported by Collins et al. (1995) but slightly modified. Three repetitions of 1 g (fresh weight) lyophilized samples of whole fruit, placenta, and pericarp were evaluated. These samples were mixed with acetonitrile (1 : 40) and maintained at 80 °C for 4 h with constant shaking before they were allowed to cool to room temperature. The mixtures were then filtered through a Wathman filter (Wathman Intl LTD., Maidstone, England) and subsequently centrifuged for 10 min at 10,000 *g*. Supernatant was extracted and filtered through a nylon membrane (0.45 μm) into a 5-mL glass sample vial covered from the light and stored at 5 °C until analysis. A 20-μL aliquot was used for each HPLC injection. An HPLC instrument, Agilent 1100 (Agilent Technologies, Germany) series, equipped with a Zorbax (ODS)-C18 reversed-phase column of 4.6 mm i.d. × 250 mm, with a detector set at 280 nm was used. The mobile phase was

Table 1. Accessions of Habanero pepper used for the analysis of pungency by high-performance liquid chromatography.

Accession	Color	Accession	Color
RUX	Red	NP1EG	Yellow
Xaman	Orange	AL	Orange
Cuza	Orange	AJ	Orange
Campn	Orange	AI	Orange
REX	Red	AG	Orange
Btz	Red	AF	Red
SiQR	Orange	AC	Orange
NP4EC	Orange	Csc	Orange
NP3EC	Orange	AK	Orange

isocratic with 70% solvent B (100% methanol) and 30% solvent A (10% methanol solution v/v). HPLC operating conditions to determine total capsaicinoids included: ambient temperature, flow rate of 1 mL·min, and run duration of 10 min. All solvents were filtered and degassed using a 47-mm, all glass filter holder. Capsaicin and dihydrocapsaicin were identified and quantified using standards of both compounds (Fluka, purity was 98% for capsaicin and 90% for dihydrocapsaicin). Standard curves were prepared in 100% methanol using serial dilution of 10, 25, 50, 100, 500, and 1000 ppm by a dilution of a 2000 pm stock solution.(r^2 = 0.996). Quantification of unknown capsaicinoids was registered by the external standard method.

The content of capasaicinoids was converted from parts per million (ppm) to SHU by multiplying the parts per million by 16 (Helrich, 1998). The collected data were processed with the program STATISTICA (version 6; Statsoft, 2001). An analysis of variance (ANDEVA) of simple classification was done based on a linear model corresponding to the design completely randomized with two repetitions by agreement. The differences between the agreements were determined by Tukey test ($P < 0.05$) (Steel and Torrie, 1980). The results graphically appeared in bar form using the Microsoft Excel program (Microsoft, Redmond, WA). A conglomerated analysis hierarchic of complete cluster analysis was also done using Manhattan distance index to classify the agreements with base to their degree of heat in three analyzed weaves.

Results

The determination of capsaicinoid content in the fruit of peppers, using HPLC, is based only on the period of retention and on the size of the peak of each capsaicinoid present, which was identified by comparison with the retention periods of the commercial standards for each composite. The chromatograms obtained for whole fruit, placenta, and pericarp of Habanero pepper showed two major peaks, identified as capsaicin (Cap) and dihydrocapsaicin (Dhc), which registered a difference of 2.03 min between the retention periods of the capsaicin (6.83 min) and the dihydrocapsaicin (8.86 min). With these results, we can assume that the separation of the major capsaicinoids was efficient. Figure 1 shows the chromatograms of the RUX accession, red-type Habanero. Similarly, it was possible to detect minor capsaicinoids such as norhydrocapsaicin (Ndc) and homodihydrocapsaicin (Hc) as well as other composites with shorter retention periods, which probably correspond to pigments. These results corroborate with what has been described by Attuquayefio and Bucle (1987) and Collins et al. (1995) who, while working with other species of the genus Capsicum, detected a peak corresponding to pigments in approximately the first minute. A second peak registered immediately after the dihydrocapsaicin, which apparently corresponded to the homodihydrocapsaicin, and another peak, which was detected at a retention period very close to that of the capsaicin and which probably corresponded to the norhydrocapsaicin. The results obtained in this study also highlighted the considerable difference in capsaicinoid content among the different parts of the fruit. The placenta produced the greatest number of peaks corresponding to capsaicinoids, whereas the fewest were detected in the pericarp. However, the pericarp apparently registered the highest number of peaks corresponding to pigments. These results corroborate those presented by Iwai et al. (1979), Susuki and Iwai (1984), and Bagdathoglu (2002), who reported that capsaicinoids synthesize and accumulate in the vesicles of placenta tissue in the fruit.

Pungency levels obtained for whole fruit generally varied ranging from 405,228 to 892,719 SHU (Table 1). In this study, besides detecting a considerable variation in pungency levels for the cultivars included in the collection evaluated, it was also possible to observe that these levels were superior to those already reported for Habanero pepper (Bosland and Baral, 2007). This response could be attributable to a genotype-ambient (environment) interaction, in which the cultivars of Habanero pepper might have found soil conditions, temperature, and humidity, among other factors, favorable to capsaicinoid biosynthesis. In this study, the NP1EG accession differed significantly from the rest of the accessions evaluated with a pungency level of 892,719 SHU, a value very similar to that reported for the cultivar, Bhut Jolokia, placing it among the most pungent chili peppers in the world. From a morphological point of view, the accession NP1EG is characterized by an abundant flowering (four to five flowers per axilla), large, wide but short fruit, mainly tetraloculate, pale green when immature and bright yellow–orange when mature (Fig. 2).

Based on the variation observed in the capsaicinoid content, the accessions evaluated were classified into five groups. Group I comprise only the NP3EG accession; group II with six accessions, group III with the accession AL, group IV included six accessions, and group V with the remaining four accessions (Fig. 3).

The results obtained from the analysis of the capsaicinoid content in placenta, pericarp, and whole fruit (mg·g^{-1}), shown in Table 2, indicate the placenta to be the part of the fruit contributing most to the integration of the groups with the exception of group I comprising NP3EG. Although the pungency level in placenta of this accession did not differ significantly from those of NP4EC, NP1EG, and SiQR, the high degree of pungency in its pericarp (1,382,889 SHU) most likely influenced its independent placement in the first group (group I). In contrast, AL, which occupied group III alone, registered the lowest pungency degree in placenta (2,545,143 SHU) and proved to be one of the accessions with the lowest pungency degree in pericarp together with the accessions Xaman, Cuza, and Rex. Group II included the accessions with the most pungent placenta and pericarp (NP4EC, NP1EG, and SiQR), whereas the accessions registering

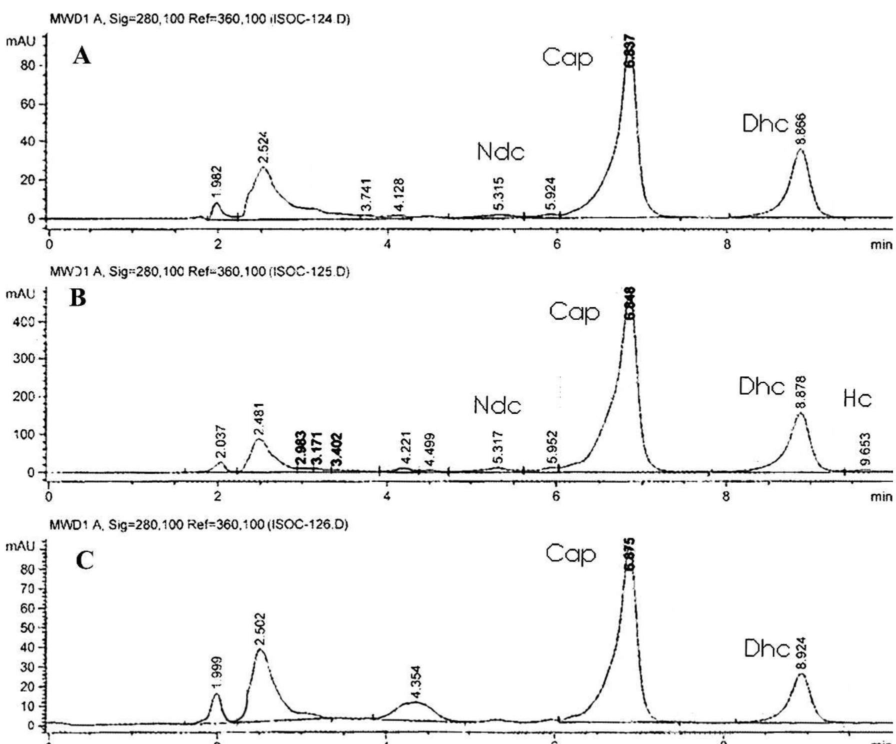

Fig. 1. Chromatograms of the capsaicinoids content in fruit from the RUX accession of Habanero pepper (**A**) whole fruit, (**B**) placenta, and (**C**) pericarp.

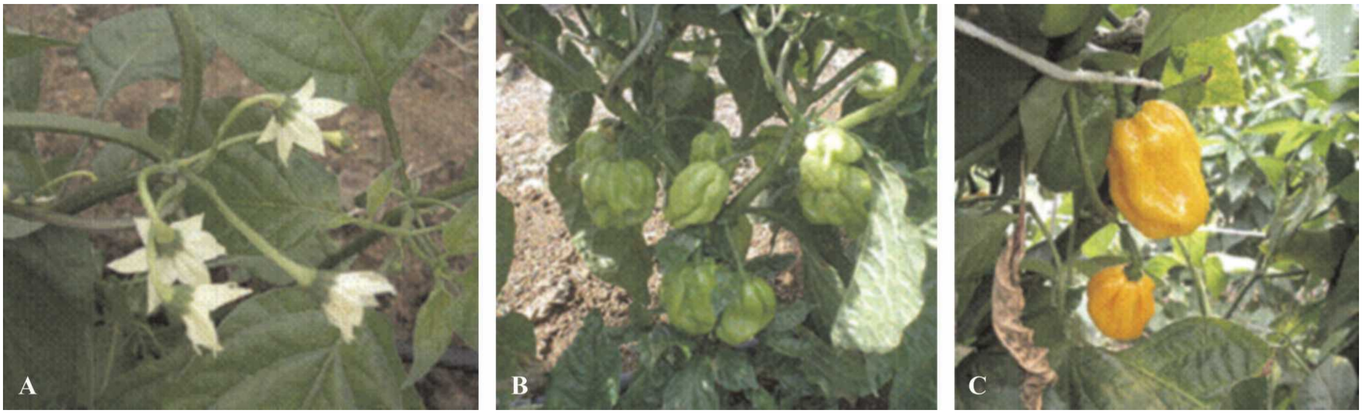

Fig. 2. Flowers and fruit of the NP1EG accession of Habanero pepper: (**A**) formation of four to five flowers per axilla, (**B**) pale green fruit in the immature stage, and (**C**) yellow–orange when ripe.

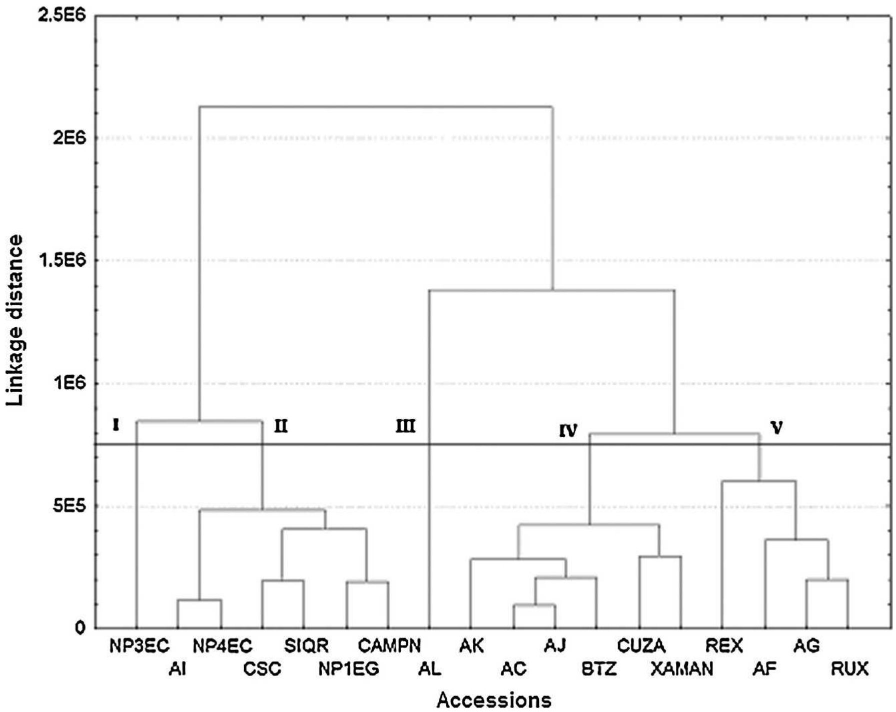

Fig. 3. Dendrogram of the distribution of 18 accessions of Habanero pepper, based on the capsaicinoid content (mg·g^{-1}), by means of the Manhattan distance analysis and the whole ligament method.

the lowest pungency values were placed in groups IV and V.

In a comparison of the capsaicinoid content (mg·g^{-1}), a considerable variation is evident not only among the parts of the fruit, but also among the fruit from different accessions (Fig. 4A–C). For example, the placenta of the accession NP4EC presented the highest capsaicinoid content (307.78 mg·g^{-1}), whereas the accession AL showed the lowest (169.68 mg·g^{-1}) (Fig. 4A). In whole fruit (Fig. 4C), the accession with the highest capsaicinoid content proved to be NP1EG, with 59.52 mg·g, whereas the accession Cuza registered the lowest (9.73 mg·g^{-1}). In pericarp (Fig. 4B), the accession showing the highest capsaicinoid content was NP3EC, with 92.198 mg·g^{-1}, whereas the accessions registering the lowest amounts of these composites were Cuza and REX, with 13.92 and 12.02 mg·g^{-1}, respectively.

The analysis of correlation showed a value of r = 1 between the different units used (SHU, mg·g^{-1}, and ppm) to express the pungency of whole fruit in each of the accessions studied; therefore, the data in SHU were used to perform the comparative analysis among the 18 accessions included in the collection evaluated. The results of this analysis (Fig. 4) showed that the accession with the most pungent whole fruit was NP1EG, with 892,719 SHU, differing significantly from the rest of the accessions, followed by Campn and SiQR, both of the ''orange type'' Habanero pepper, which, although significantly different from the NP1EG accession, also showed high pungency levels (739,557 and 705,159 SHU, respectively). The accessions NP3EC and NP4EC registered the most pungent pericarp and placenta, respectively, with no significant differences between the two. However, these were not precisely the accessions with the most pungent fruit. The least pungent fruits were those of the accession Cuza (145,950 SHU), a significant difference compared with the rest of the accessions, followed by the accessions Btz and AF, with 405,228 and 454,224 SHU, respectively; there were no significant differences between these two accessions, although there were differences in comparison with the accession Cuza. As shown in Figure 4, the majority of the accessions (83.3%) registered pungency levels superior to those reported for Habanero pepper, particularly the accession NP1EG (yellow–orange Habanero). Only 16% of the collection presented pungency levels close to those reported for Habanero pepper. Table 2 shows the mean values of capsaicinoid content for the 18 accessions of Habanero pepper, expressed in SHU and mg·g, as well as their corresponding standard error (SE).

Discussion

This study confirms that the *Capsicum chinense* species includes the most pungent chili peppers known to date. According to some reports, the pungency level of the Habanero pepper ranges between 100,000 and 350,000 SHU; Red Savina between 350,000 and 577,000 SHU; Naga Jolokia between 855,000 and 1,041,427; and Bhut Jolokia, recently reported to be the most pungent cultivar in the world, with a level ranging from 879,953 to 927,199 SHU (Bosland and Baral, 2007). All of these cultivars belong to the *Capsicum chinense* species. Pungency is an exceptional attribute that distinguishes the Habanero pepper from other vegetable species. This characteristic is identified by a sharp taste or sensation of heat caused by the fruit when consumed, and this sensation is a result of the capsaicinoids, alcamids only found in some species of the genus *Capsicum* (Zewdie and Bosland, 2000). It is generally accepted that capsaicinoids are produced solely in pepper fruits, although the location of the biosynthesis

Table 2. Capsaicinoid content in 18 accessions of Habanero pepper (*Capsicum chinense*) expressed in SHU and $mg \cdot g^{-1}$.[z]

	Whole fruit		Placenta		Pericarp	
Accession	SHU	$mg \cdot g^{-1}$	SHU	$mg \cdot g^{-1}$	SHU	$mg \cdot g^{-1}$
RUX	673,730 ± 2,033.4	44.922 ± 0.14	3,640,533 ± 107,077.1	242.7022 ± 7.14	6,090,84 ± 34.6	40.6056 ± 0.10
Xaman	477,144 ± 2,816.3	31.8096 ± 0.20	3,548,259 ± 9,763.6	236.5506 ± 0.67	388,359 ± 3469.3	25.8906 ± 0.23
Cuza	145,950 ± 2,591.1	9.73 ± 0.17	3,314,634 ± 4,5691.5	220.9756 ± 3.05	208,931 ± 2,062.9	13.9154 ± 0.14
Campn	739,557 ± 2,987.8	49.3038 ± 0.20	4,313,742 ± 106,223.2	287.5828 ± 7.08	814,692 ± 5,577.2	54.3128 ± 0.37
REX	580,329 ± 8,828.3	38.6886 ± 0.59	3,843,039 ± 19,573.9	256.2026 ± 1.30	180,228 ± 675.5	12.0152 ± 0.05
Btz	405,228 ± 17,511	27.0152 ± 1.17	3,352,071 ± 12,524.5	223.4714 ± 0.83	594,669 ± 3,815.7	39.6446 ± 0.25
SiQR	705,159 ± 213.04	47.0106 ± 0.01	4,282,221 ± 4,356.1	285.4814 ± 0.29	587,466 ± 3,575.0	39.1644 ± 0.24
NP4EC	626,919 ± 2,700.3	41.7946 ± 0.18	4,616,628 ± 18,252.4	307.7752 ± 1.22	708,114 ± 3,727.4	47.2076 ± 0.25
NP3EC	628,641 ± 3,711.8	41.9094 ± 0.25	4,436,796 ± 7,513.6	295.7864 ± 0.50	1,382,889 ± 4,137.9	92.1926 ± 0.28
NP1EG	892,719 ± 9,573	59.5146 ± 0.64	4,215,615 ± 19,127.0	281.041 ± 1.28	758,190 ± 9,200.7	50.546 ± 0.61
AL	536,499 ± 2,350.4	35.7666 ± 0.16	2,545,143 ± 472.8	169.6762 ± 0.03	410,766 ± 3,796.7	27.3844 ± 0.25
AJ	528,111 ± 6,441.5	35.2074 ± 0.43	3,397,218 ± 25,059.3	226.4812 ± 1.67	617,625 ± 396.6	41.175 ± 0.03
AI	608,970 ± 245.95	40.598 ± 0.02	4,504,524 ± 11,078.2	300.3016 ± 0.74	741,078 ± 5,646.5	49.4052 ± 0.38
AG	562,140 ± 103.25	37.476 ± 0.01	3,782,298 ± 350.2	252.1532 ± 0.01	517,986 ± 25.3	34.5324 ± 0.03
AF	454,224 ± 2,625.8	30.2816 ± 0.18	3,878,367 ± 16,650.2	258.5578 ± 1.11	767,735 ± 6361.8	51.189 ± 0.42
AC	613,566 ± 6,020.6	40.9044 ± 0.40	3,381,852 ± 25,828.3	225.4568 ± 1.72	573,996 ± 374.1	38.2664 ± 0.02
Csc	528,270 ± 201.23	35.218 ± 0.02	4,199,340 ± 300.2	279.956 ± 1.02	579,036 ± 15.2	38.6024 ± 0.10
AK	581,790 ± 165.23	38.786 ± 0.01	3,154,110 ± 402.6	210.274 ± 0.05	499,404 ± 10.5	33.2936 ± 0.22

[z]The data are shown with the mean value ± SE.
SHU = Scoville Units.

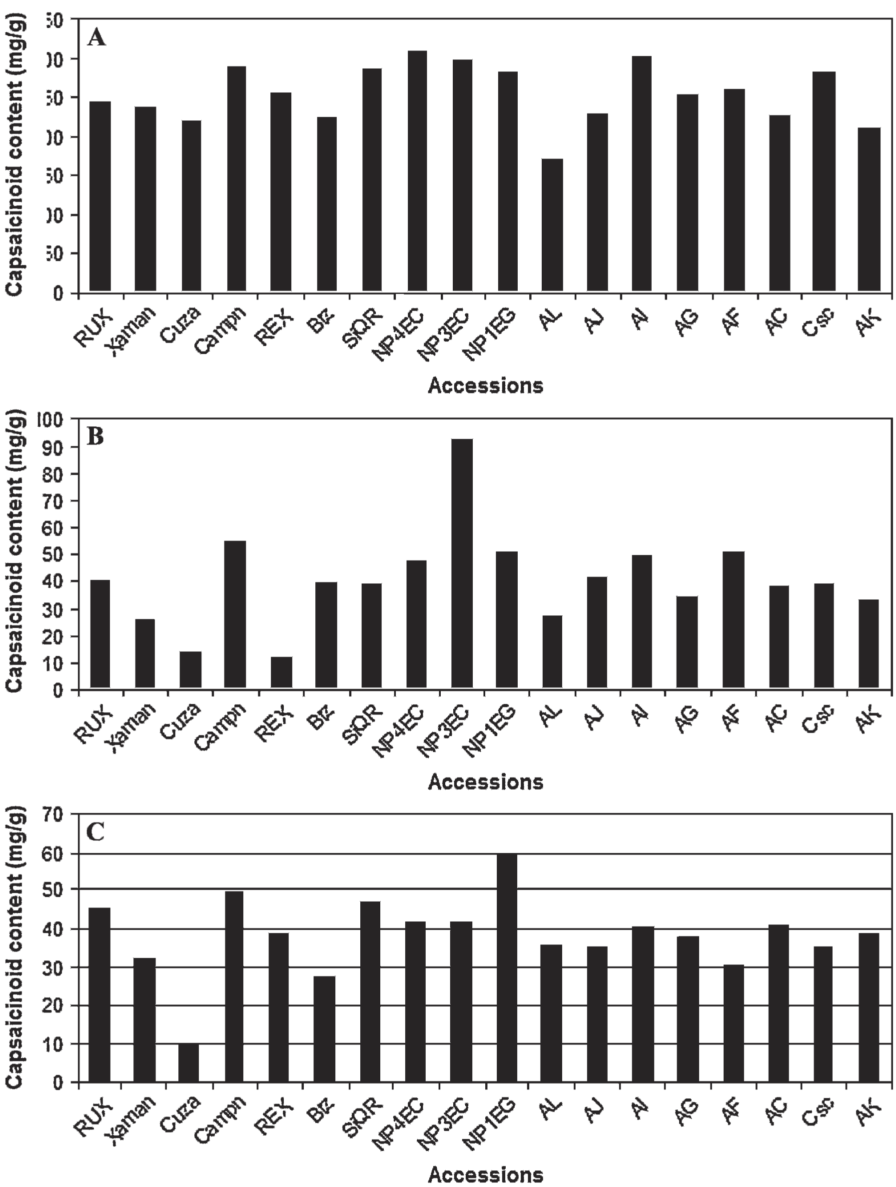

Fig. 4. Total capsaicinoids contained in: (**A**) placenta, (**B**) pericarp, and (**C**) whole fruit of the 18 accessions of Habanero pepper studied.

and accumulation of these alkaloids within the fruit has been debated. A recent report describes the detection of capsaicinoids in vegetative organs, and others have reported small amounts of capsaicinoids in seeds (Balbaa et al., 1986; Estrada et al., 2002; Ohta and Chuong, 1975). Within the pepper fruit, capsaicinoids mainly accumulate along the epidermal cells of the interlocular septum, which defines the fruit locules and is derived from the tissue connecting the placenta to the pericarp (Judd et al., 1999). The epidermal cells of the interlocular septum have been implicated in capsaicinoid biosynthesis based on morphological changes during fruit development and the existence of osmiophilic granules in these cells (Furuya and Hashimoto, 1954; Ohta and Chuong, 1975; Suzuki and Iwai, 1984). In pungent varieties, epidermal protrusions or blisters arise from the lifting of the cuticle layer from the cell wall during the filling of subcuticular cavities with capsaicinoids (Rowland et al., 1983; Zamski et al., 1987) ripening (Rao and Paran, 2003). Huffman et al. (1978) attributed the small amounts of capsaicinoids detected in the seeds to surface contamination during dissection. There has been persistent debate in the literature regarding the existence of glands that accumulate capsaicinoids (Balbaa et al., 1986).

Within the domesticated species of the genus *Capsicum*, *Capsicum chinense* is recognized as having the most pungent fruit. Since the publication of the Scoville organic test, a subjective measure of chili pungency invented by Scoville in 1912, the cultivars of Habanero pepper have occupied the highest values of this scale. As more pungent cultivars have become known (Red Savina Habanero 580,000 SHU), the number of levels in the scale has changed; however, the *Capsicum chinense* species continues to occupy the highest places in the scale. Recently, in a comparative study of pungency between the three most pungent cultivars reported (orange criolle Habanero, Red Savina Habanero, and the variety Bhut Jolokia), all of which belong

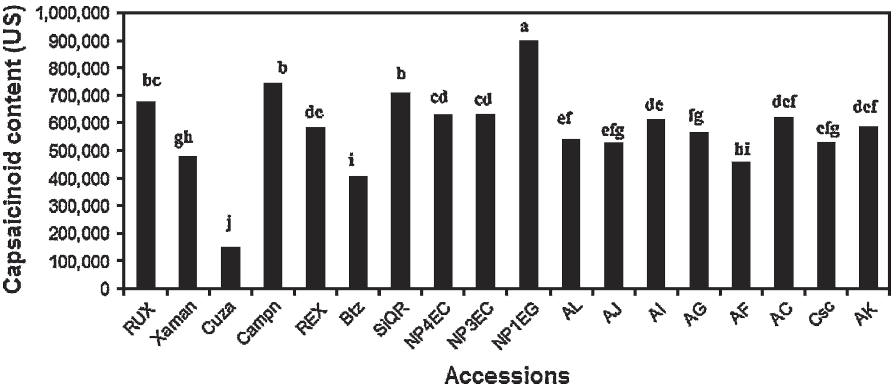

Fig. 5. Capsaicinoids content (Scoville Units) in whole fruit of 18 accessions of Habanero pepper. Letters differ in accordance with Tukey docima ($P < 0.05$).

to the species *Capsicum chinense*, Bosland and Baral (2007) found that Bhut Jolokia was significantly more pungent (1,001,304 SHU) making it the most pungent chili pepper known to date, only surpassed by pure capsaicin (16,000,000 SHU), whereas the variety Red Savina Habanero, despite expectations to the contrary, registered a pungency lower than that of the standard orange Habanero pepper (248,556 versus 357,729 SHU, respectively). In this study, the results of the evaluation of 18 accessions of Habanero pepper, comprising a great variety of colors, shapes, and sizes in the fruits of Habanero pepper, showed that 83.3% of the collection surpassed the pungency levels reported for Habanero pepper (Bosland and Baral, 2007; Scoville, 1912) in other regions of the world. Four accessions registered pungency levels close to those reported for the species, and although three of these registered the lowest pungency levels, they still surpassed 400,000 SHU. Only the accession Cuza registered a pungency lower than 200,000 SHU. However, it was interesting to observe that 33.3% of the accessions surpassed 500,000 SHU and 44.4% registered above 600,000 SHU. The accession NP1EG (yellow Habanero) registered a pungency level of 892,719 SHU in whole fruit, whereas the accession NP3EC (orange Habanero) registered 1,382,889 SHU in pericarp. These two cultivars (NP1EG and NP3EC) were similar to Bhut Jolokia in pungency level; however, they differed noticeably in shape and color; although the characteristics look like the Habanero pepper typical of the region (Fig. 5).

Our results demonstrate the existence of a considerable variability in this character (pungency) within the germplasm of the Habanero pepper, which may be attributable to genetic or environmental factors. There are reports in the literature indicating that the capsaicinoid profile differences within a given variety are well established and can be caused by variations in growing conditions or maturity (Cordell and Araujo, 1993; Govindarajan and Sathyanarayana, 1991; Huffman et al., 1978; Todd et al., 1977). There has also been some discussion regarding the influence of the environment on these composites (Harvell and Bosland, 1997; Zewdie and Bosland, 2000). Even peppers from the same plant can vary in their capsaicinoid profiles attributable merely to differences in postharvest ripening conditions (Iwai et al., 1979). Tewksbury and Nabhan (2001) have related the capsaicinoids in chili peppers to defense mechanisms of the plant against certain pathogens, all of which would indicate that the pungency characteristics can be extremely variable and highly sensitive to the growing conditions of the plant. However, there is one common criterion regarding pungency: it is the most important quality and the fundamental reason why hot chili peppers are widely consumed all over the world.

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