RESEARCH

Pungency and fruit quality in Mexican landraces of piquín pepper (*Capsicum annuum* var. *glabriusculum*) as affected by plant growth environment and postharvest handling

Deisy D. Díaz-Sánchez[1], Pedro A. López[2], Higinio López-Sánchez[2], Hilda V. Silva-Rojas[1], Alfonso A. Gardea-Béjar[3], Nicacio Cruz-Huerta[1], Iván Ramírez-Ramírez[1], and Víctor A. González-Hernández[1*]

[1]Colegio de Postgraduados, Campus Montecillo, Carr. México-Texcoco km 36.5, Montecillo 56230, Texcoco, Estado de México, México. [*]Corresponding author (vagh@colpos.mx).
[2]Colegio de Postgraduados, Campus Puebla, Carr. Federal México-Puebla km 125.5, Santiago Momoxpan 72760, Puebla, México.
[3]Centro de Investigación y Desarrollo, A.C. (CIAD), Carr. Gustavo E. Astiazarán Rosas N° 46, CP 83304, Hermosillo, Sonora, México.

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ABSTRACT

The variability in fruit pungency, size and quality was analyzed in 31 landraces of piquín pepper (*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill) collected from 10 Mexican states. The response of fruits collected *in situ* and harvested in greenhouse was compared. Pungency was estimated by measuring capsaicinoids content with HPLC. Additionally, pungency was recorded along fruit maturity stages in greenhouse-grown fruits. On average, greenhouse-produced fruits were markedly more pungent than fruit collected from field locations (29485 *vs*. 6114 SHU). Field-collected fruits averaged 5017 μg mL^{-1} capsaicin content, while dihydrocapsaicin averaged 7618 μg mL^{-1}. In contrast, greenhouse-harvested fruits contained substantially more capsaicin and dihydrocapsaicin (34762 and 26174 μg mL^{-1}, respectively), while the capsaicin:dihydrocapsaicin ratio inverted. The three most pungent field-collected landraces averaged 35% of the pungency measured in the three most pungent greenhouse-grown fruits. Pungency increased as maturity stage advanced: green (14813 SHU) < intermediate (24767 SHU) < red (29485 SHU). The fruits collected from 31 field locations had lower titratable acidity, higher content of total soluble solids, and higher maturity index than fruits harvested in greenhouse. Greenhouse harvested fruits were larger and heavier than those collected in the field.

Key words: Capsaicinoids, fruit quality, fruit size, landraces, maturity stages, piquín pepper, pungency.

INTRODUCTION

Piquín, chiltepín or bird pepper (*Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill) (Cag) is one of the preferred peppers in Mexico (Bañuelos et al., 2008). The growing zone for this pepper spans from southern USA to South America (Hernández-Verdugo et al., 2012). Cag is considered the wild ancestor of numerous cultivated peppers of the *C. annuum* species (Montoya-Ballesteros et al., 2010), such as jalapeño, serrano, ancho, pasilla, guajillo, árbol, and other commercial peppers. Cag fruits are frequently harvested from wild plants by uprooting the whole plant without being replanted (Bañuelos et al., 2008). Consequently, the *in situ* conservation of this plant genetic resource is at risk. Yet, commercial Cag plantings are scarce due mainly to the difficulty of germinating the seeds (Villalón et al., 2013; Cano-Vázquez et al., 2015) and to the lack of genetically bred commercial varieties. On the other hand, the Cag fruits harvested from plantations may show different changes compared to wild-harvested fruits (Sandoval-Rangel et al., 2018).

Cag fruit is small, turns red when ripe, and is often highly pungent. It is up to 40 times more pungent than popular peppers such as serrano and jalapeño, and it is consumed fresh or dried for food seasoning (Quintero Barrera, 2000). According to Chasing-Chilli (2020) the piquín/chiltepín pungency ranges between 50 000 and 100 000 SHU (Scoville heat units). However, González-Zamora et al. (2015) reported pungency levels from 324 924 to 1 765 283 SHU for piquín fruits at the green maturity stage harvested from plants cultivated in irrigated field conditions in northern México. Despite its high pungency, Cag is regarded as having a pleasant taste, and its hot sensation presumably fades quickly without irritating the stomach (Martínez et al., 2006; Bañuelos et al., 2008). Most studies on piquín pepper have focused on describing fruit morphological and genetic variability from wild local landraces (Hernández-Verdugo et al., 2001; Alonso et al., 2012; Narez-Jiménez et al., 2014; Murillo-Amador et al., 2015). However, fruit quality traits such as antioxidant content and taste, have been measured only in a few regional landraces (also called accessions, ecotypes, or morphotypes) (Montoya-Ballesteros et al., 2010; Rodríguez-Maturino et al., 2012; Flores-González et al., 2018). Other quality traits like fruit size, color, and flavor have scarcely been reported for piquín pepper, contrasting with the vast information available for the commercially cultivated chilis. For example, for nine wild piquín pepper landraces from northeast México (Moreno-Ramírez et al., 2018), the reported pungency ranged from 22 190 to 44 035 SHU. Phenolic compounds content and antioxidant capacities varied too. The changes in piquín pepper pungency when used for elaborating sauces and pickles, has also been reported (Montoya-Ballesteros et al., 2010).

Capsaicinoids synthesized in the placenta of *Capsicum* spp. fruits are responsible for pungency (Barchenger and Bosland, 2016). Barchenger and Bosland (2016) reported 20 different capsaicinoids in *C. annuum*, and two of them, capsaicin and dihydrocapsaicin, account for about 90% of the total capsaicinoid content in chili peppers (de Aguiar et al., 2016).

In this study, we explored the variation of piquín accessions in a wide sample of 31 Mexican landraces collected across 10 Mexican states, by measuring their capsaicinoids content, titratable acidity, total soluble solids content, and the palatability index. These traits were assessed both on fruits obtained from collected samples at different 31 sites and conditions, and on fruits harvested from plants of the same landraces grown under a greenhouse and supplied with water and nutrients with a drip irrigation system.

MATERIALS AND METHODS

Plant material and experimental management

The 31 piquín or bird pepper (*Capsicum annuum* L. var. *glabriusculum* [Dunal] Heiser & Pickersgill,) (Cag) fruit accessions were obtained from 10 Mexican states and had four different provenances: harvested from backyard plots, harvested from commercial field plots, harvested from wild plants, or bought in local markets (Table 1). Pungency and fruit quality was assessed in the collected fruits, as well as in fruits harvested from plants grown in a greenhouse fitted with a drip irrigation system at Texcoco (19°23’40” N, 99°01’45” W; 2250 m a.s.l.) In the greenhouse temperatures varied across the year from 8.9 to 26.3 °C, with a mean value of 16.3 °C. These values are very similar to State of México field conditions where mean annual temperature is 16 °C. Plants were grown from seeds extracted from collected fruits and germinated in polystyrene trays of 200 cavities filled with a 1:1 mixture of agrolite and peatmoss as substrate (Cano-Vázquez et al., 2015), under greenhouse conditions. When seedlings reached 7 cm tall, they were transplanted into 10 L black bags filled with volcanic, porous red-gravel, 2-4 mm diameter (locally called ‘tezontle’), commonly used as substrate for hydroponic cropping. Nutrients were supplied with a Steiner solution (Steiner, 1961) provided nine times a day for 5 min periods.

The greenhouse piquín plants were randomly distributed in two pot lines, 1 m apart, and individual plants were 40 cm apart along each line. When plants were between 14 and 18 mo-old (after transplant), fruits were harvested at three stages of maturity, according to the BBCH scale proposed by Feldmann and Rutikanga (2021) for chili fruits: green (growth stage 703), intermediate (beginning to acquire the red color, considered commercial harvest maturity; growth stage 801), and mature red (growth stage 809). The pre- and postharvest handling were identical for all landraces.

Table 1. Geographic locations of the 31 piquín pepper Mexican landraces evaluated in this study.

ID	Landrace	Latitude	Longitude	State	Location (city/town)	Origin
1	Ags01	22.145	-102.273	Aguascalientes	Aguascalientes	Local market
2	Ags02	22.887	-102.291	Aguascalientes	CONAGUA	Backyard plot
3	Ags03	22.865	-102.289	Aguascalientes	Aguascalientes	Cultivated plot
4	Dur01	24.027	-104.653	Durango	Durango	Local market
5	Hgo01	20.970	-98.508	Hidalgo	Tlamamala	Wild plants
6	Mich01	20.336	-102.022	Michoacán	La Piedad	Wild plants
7	Mich02	20.336	-102.022	Michoacán	Michoacán	Backyard plot
8	Nay02	21.726	-105.058	Nayarit	El Jicote	Wild plants
9	Oax01	17.332	-95.418	Oaxaca	San José de las Flores	Backyard plot
10	Oax03	16.948	-96.751	Oaxaca	Zaachila	Backyard plot
11	Oax05	16.948	-96.751	Oaxaca	Zaachila	Backyard plot
12	Oax07	16.612	-96.852	Oaxaca	San Martín Lachila	Local market
13	Oax10	16.612	-96.852	Oaxaca	San Martín Lachila	Backyard plot
14	Pue01	20.284	-97.964	Puebla	Xicotepec	Wild plants
15	Pue04	20.018	-97.523	Puebla	Cuetzalan	Cultivated plot
16	Qro01	20.907	-99.933	Querétaro	Tolimán Xiti	Wild plants
17	Qro02	20.689	-99.820	Querétaro	Higuerillas	Wild plants
18	Qro03	20.907	-99.933	Querétaro	El Patol	Wild plants
19	Qro04	20.778	-99.872	Querétaro	El Patol	Wild plants
20	Qro06	20.907	-99.933	Querétaro	San Antonio Tolimán	Wild plants
21	Qro07	21.219	-99.471	Querétaro	Jalpan de Serra	Wild plants
22	Qro09	20.786	-100.050	Querétaro	Colón	Wild plants
23	Qro10	21.219	-99.471	Querétaro	Jalpan de Serra	Wild plants
24	Sin05	28.681	-110.455	Sinaloa	El Porvenir	Wild plants
25	Ver01	20.979	-98.175	Veracruz	Chicontepec	Local market
26	Ver04	20.702	-97.310	Veracruz	Cerro Verde	Cultivated plot
27	Ver06	20.444	-97.325	Veracruz	Papantla	Cultivated plot
28	Ver08	20.956	-97.406	Veracruz	Tuxpan	Cultivated plot
29	Ver09	20.956	-97.406	Veracruz	Tuxpan	Local market
30	Ver11	20.702	-97.310	Veracruz	Cazones	Local market
31	Ver12	20.672	-97.206	Veracruz	Jilotepec	Wild plants

Capsaicinoids extraction

Four 10 g fruit samples (fresh weight) were taken from each landrace, both from field-collected and greenhouse-harvested, washed with distilled water, and freeze-dried (benchtop freeze dryer FreeZone 4.5 L, Labconco, Kansas City, Missouri, USA) for 3 d to preserve their integrity. The dried samples were ground in a 1 mm sieve mill (Thomas Model 4 Wiley mill, Thomas Scientific, Swedesboro, New Jersey, USA) to a fine powder. From each sample, 0.5 g powder were mixed with 5 mL acetonitrile into a 15 mL plastic centrifuge tube, and placed in a water bath for 5 h at 60 °C. Every hour the tubes were stirred vigorously for 1 min. Thereafter, the tubes were cooled to room temperature in the dark. In every tube, evaporated acetonitrile was replaced to 5 mL. Each resulting extract was filtered with polytetrafluoroethylene (PTFE) syringe filters (25 mm diameter, 0.45 μm pore) and stored at 4 °C until HPLC analysis.

Capsaicinoid quantification by HPLC

Capsaicin (C) and dihydrocapsaicin (D) contents were determined in 20 μL of each liquid extract. Samples were injected into an HPLC 1260 equipped with a DAD detector (Agilent, Santa Clara, California, USA). The running conditions were: Hypersil ODS MR column (25 cm, 4.6 mm, 5 μM), a mobile phase of a 45:55 acetonitrile-water mixture, flow of 1.5 mL min^{-1} and 20 min running time per injection. For automatic detection and quantification, calibration curves ($R^2 = 0.99$) were constructed with external standards of capsaicin (8-methyl-*n*-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-*n*-vanillyl-nonanamide) (Sigma-Aldrich, St. Louis, Missouri, USA) at 0.25, 0.5 and 1.0 mg mL^{-1} concentrations. In greenhouse-harvested fruits, capsaicinoid contents were measured at three different maturity fruit stages: green, intermediate, and red.

Peak areas obtained by HPLC were transformed to Scoville heat units (SHU) using the equations proposed by the AOAC Official Method 995.03 (AOAC, 1995):

$$C = (Pc/Ps) \times (Cs/Wt) \times (10/0.89) \times 16\,100$$
$$D = (Pd/Ps) \times (Cs/Wt) \times (10/0.93) \times 16\,100$$

where C is capsaicin content (SHU); D is dihydrocapsaicin content (SHU); Pc and Pd are peak areas for capsaicin and dihydrocapsaicin, respectively; Ps is peak area of the corresponding standard; Cs is concentration of the standard solution (mg mL^{-1}); Wt is sample weight (g). Conversion to SHU from mg mL^{-1} was calculated with the formula 1 μg capsaicinoids g^{-1} dry weight = 15 SHU (Wall et al., 2001).

Fruit size and quality traits

Fruit length and width (mm) were measured with a digital caliper (Truper, Mexico) in 20 randomly selected fruits per accession. Fruit dry-weight (mg) was measured with an analytical balance (Sartorius Handy H51, Sartorius, Gottingen, Germany) in the 20 previously selected fruits after they were oven dried to constant weight. The roundness index per fruit was calculated as the length to width ratio. The Manual of Descriptors for *Capsicum* spp. (IPGRI, AVRDC, CATIE, 1995) was used as a guide for these measurements.

Titratable acidity (TA), as percentage of citric acid was determined in a sample of 5 g, according to the AOAC technique (AOAC, 1995). The fruit pH was measured with a digital pH meter (HI 2211, Hanna Instruments, Leighton Buzzard, UK). Total soluble solids (TSS, in °Brix) were measured in a drop of juice obtained by fruit maceration, with a digital refractometer (PR-100, ATAGO, Tokyo, Japan). For each quality characteristic, three samples were measured, and the results averaged. The maturity index (MI) was calculated as the ratio MI = TSS/TA.

Experimental design and statistical analyses

Both for the field-collected fruits and for the greenhouse-harvested fruits, the experimental treatments were the 31 landraces randomly distributed under a completely randomized design with four replicates (4 plants). Capsaicin and dihydrocapsaicin contents, as well as total capsaicinoid content (Total = capsaicin + dihydrocapsaicin), were analyzed under a completely randomized design with three replicates. All data were statistically analyzed with ANOVA, and treatment means were ranked by multiple mean comparisons with the Tukey's test ($P \leq 0.05$) when needed, with InfoStat (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina). Based on the statistical analyses of morphological and quality fruit traits, the most significant variables were fed into a principal component analysis data array in SAS 9.4 (SAS Institute, Cary, North Carolina, USA). Landraces were classified into similarity groups with a cluster analysis by using Minitab v18 (Minitab, State College, Pennsylvania, USA).

RESULTS AND DISCUSION

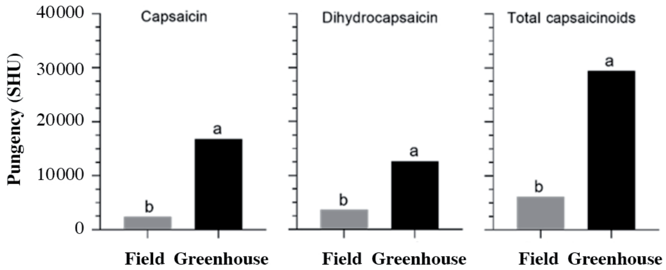
Capsaicinoid content as affected by growth environment and postharvest handling

The average total capsaicinoid content in field collected fruits from the 31 landraces was 6114 SHU. This content split into 40% for capsaicin content and 60% for dihydrocapsaicin content (Figure 1). Fruits harvested in the greenhouse had 29 485 SHU, of which capsaicin accounted for 57% while dihydrocapsaicin was the remaining 43%. The greenhouse fruits were almost five times more pungent ($P < 0.05$) than the field-collected fruits.

Pungency variation in field-collected fruits

The average content of total capsaicinoids for field-collected fruits was 408 μg mL^{-1} for the 31 landraces (Table 2). Dihydrocapsaicin content was higher than capsaicin content (246 *vs*. 162 μg mL^{-1}; i.e., 60% *vs*. 40%). Pungency varied greatly among the evaluated landraces, from low (2101 SHU) to high pungency (20 693 SHU), thus spanning a wide range of consumer preferences. These differences result from the combination of inherent genetic differences among landraces, and to the effects of multiple environmental factors associated with climate, soil, and postharvest handling. The environmental variation is associated to the contrasting soil and climate conditions inherent to the 10 geographic studied Mexican regions. Furthermore, different planting settings (i.e., wild plants, plants from commercial field plots, plants cultivated in small backyard plots), as well as unidentified differences in postharvest handling, most particularly from market-bought fruits, may have influenced the observed variability. Harvell and Bosland (1997) demonstrated that pungency was more affected by environment than by genotype, in several chili genotypes. Similarly, González-Zamora et al. (2013) reported that high temperatures above 40 °C interact significantly with genotype since hot weather increased pungency in some *Capsicum* species while in others it became decreased.

Figure 1. Average contents of capsaicin, dihydrocapsaicin, and total capsaicinoids in fruits from 31 field locations *vs*. greenhouse harvested plants. Total capsaicinoids content is the sum of capsaicin and dihydrocapsaicin contents.



Values with different letters are significantly different (Tukey, $p < 0.05$).

Table 2. Capsaicinoid content in fruits of 31 piquín pepper landraces as they were sampled at different field locations in México.

Landrace	Capsaicin	Dihydrocapsaicin	Total capsaicinoids	Pungency
	µg mL^{-1}	µg mL^{-1}	µg mL^{-1}	SHU
Ags01	118 ± 43cd	132 ± 42cd	250 ± 85cd	3748 ± 1273cd
Ags02	228 ± 78ad	295 ± 170bd	524 ± 247bd	7860 ± 3711bd
Ags03	265 ± 81ad	390 ± 170bd	655 ± 251bd	9832 ± 3760bd
Dur01	165 ± 72bd	219 ± 77bd	375 ± 144cd	5620 ± 2163cd
Hgo01	193 ± 5ad	490 ± 07bc	683 ± 12bd	10 243 ± 176bd
Mich01	186 ± 2ad	479 ± 3bd	665 ± 5bd	9973 ± 71bd
Mich02	228 ± 8ad	192 ± 17cd	420 ± 21bd	6299 ± 319bd
Nay02	459 ± 194ab	920 ± 163a	1379 ± 95a	20 693 ± 1425a
Oax01	108 ± 8cd	93 ± 8cd	201 ± 16cd	3023 ± 237cd
Oax03	69 ± 30cd	92 ± 5cd	162 ± 27cd	2425 ± 399cd
Oax05	134 ± 20cd	180 ± 164cd	313 ± 175cd	4701 ± 2630cd
Oax07	89 ± 12cd	136 ± 61cd	225 ± 60cd	3376 ± 893cd
Oax10	82 ± 25cd	120 ± 18cd	203 ± 20cd	3039 ± 300cd
Pue01	64 ± 6cd	70 ± 13d	135 ± 19d	2023 ± 285d
Pue04	80 ± 2cd	121 ± 4cd	202 ± 5cd	3025 ± 72cd
Qro02	165 ± 84bd	285 ± 94bd	450 ± 178bd	6746 ± 2671bd
Qro04	470 ± 246a	618 ± 310ab	1088 ± 556ab	16 325 ± 8341ab
Qro06	126 ± 35cd	190 ± 88cd	316 ± 122cd	4739 ± 1828cd
Qro08	89 ± 4cd	103 ± 4cd	192 ± 8cd	2877 ± 122cd
Qro11	86 ± 20cd	111 ± 39cd	197 ± 58cd	2959 ± 877cd
Qro01	76 ± 00cd	111± 1cd	187 ± 2cd	2804 ± 24cd
Qro03	60 ± 1cd	170 ± 1cd	230 ± 2cd	3450 ± 24cd
Qro09	53 ± 3cd	87 ± 11cd	140 ± 9cd	2101 ± 133cd
Sin05	69 ± 9cd	105 ± 11cd	174 ± 19cd	2614 ± 289cd
Ver01	279 ± 132ad	190 ± 24cd	469 ± 156bd	7031 ± 2337bd
Ver04	334 ± 228ac	471 ± 361bd	805 ± 589ac	12 079 ± 8834ac
Ver06	238 ± 176ad	415 ± 279bd	653 ± 455bd	9798 ± 6830bd
Ver08	36 ± 00d	108 ± 2cd	144 ± 2cd	2161 ± 28cd
Ver09	133 ± 124cd	233 ± 213bd	367 ± 337cd	5504 ± 5052cd
Ver11	95 ± 75cd	174 ± 115cd	270 ± 190cd	4051 ± 2847cd
Ver12	237 ± 110ad	323 ± 126bd	560 ± 230bd	8408 ± 3445bd
Average	162 ± 134	246 ± 217	408 ± 337	6114 ± 5060
MSD	296	418	670	10 047

Values with different letters in each column are significantly different (Tukey, $p < 0.05$).
MSD: Minimal significant difference; C: capsaicin; D: dihydrocapsaicin; SHU: Scoville heat units.

Postharvest handling is particularly relevant for chili peppers, as capsaicinoid content varies during ripening and decreases during storage, as these results show. Our fruit samples collected from the field were mildly to intermediately pungent (6 114 SHU), like the jalapeño pepper and tabasco sauce which have a moderate hot flavor for the Mexican consumer (Chasing-Chilli, 2020). The field-sampled piquín fruits in the current research are suitable for low-heat dishes. In nine Mexican Cag morphotypes from the central-eastern region of Yucatán, Mexico, the average pungency (Cázares-Sánchez et al., 2005) was 70 374 SHU (3584 μg g^{-1} capsaicin and 1707 μg g^{-1} dihydro-capsaicin content). In a wild piquín landrace from Sonora, Mexico, Montoya-Ballesteros et al. (2010) reported contents of 8220 and 4240 μg g^{-1} capsaicin and dihydrocapsaicin, respectively, which result into a high pungency (160 000 SHU). On their part, González-Zamora et al. (2013) registered 480089 SHU in piquín/chiltepín fruits from northern Mexico, a level even higher than for habanero chili (*C. chinense* Jacq.)

Pungency changes in piquín fruits from plants grown in greenhouse conditions

Under greenhouse conditions, the average fruit capsaicin content was 33% higher than dihydrocapsaicin (1121 *vs*. 844 μg mL^{-1}, respectively) (Table 3). Among landraces, pungency varied from low (Oax10, 4513 SHU) to high levels (Ags01, 55 781 SHU). Pue01 (41 516 SHU) and Qro03 (40 396 SHU) were other highly pungent landraces. According to the pungency scale mentioned above, most of these landraces may be classified as hot peppers, with values from 20 000 to 50 000 SHU. In contrast, landraces Oax07 and Oax10 had less than 5000 SHU. In any case, the greenhouse conditions greatly improved pungency in all landraces, while preserving the pungency diversity. Some landraces were of moderate pungency suitable for vast consumption, while the highly pungent landraces would be preferred by special consumers

Table 3. Capsaicinoid content in fruits of 31 piquín Mexican landraces harvested from plants grown under greenhouse conditions.

Landrace	Capsaicin	Dihydrocapsaicin	Total capsaicinoids	Pungency
	μg mL^{-1}	μg mL^{-1}	μg mL^{-1}	SHU
Ags01	1843 ± 27a	1876 ± 89a	3719 ± 101a	55 781 ± 1511a
Ags02	946 ± 178il	1098 ± 103bd	2044 ± 246be	30 665 ± 3682be
Ags03	1733 ± 57ab	418 ± 51ei	2152 ± 24be	32 278 ± 361be
Dur01	1524 ± 94bc	329 ± 23gi	1852 ± 83de	27 788 ± 1240de
Hgo01	1272 ± 23cg	350 ± 64gi	1622 ± 69de	24 331 ± 1034de
Mich01	1485 ± 78bd	401 ± 126fi	1887 ± 119de	28 300 ± 1781de
Mich02	959 ± 181hl	522 ± 22dh	1481 ± 167de	22 217 ± 2500de
Nay02	1202 ± 31di	754 ± 24ch	1957 ± 55ce	29 352 ± 823ce
Oax01	1206 ± 167di	811 ± 686bg	2017 ± 840be	30 261 ± 1259be
Oax03	1350 ± 133cf	389 ± 149fi	1739 ± 275de	26 083 ± 4128de
Oax05	1043 ± 237fk	1012 ± 319bd	2055 ± 554be	30 821 ± 8312be
Oax07	141 ± 6m	178 ± 11hi	319 ± 17f	4784 ± 254f
Oax10	155 ± 4m	145 ± 38i	301 ± 34f	4513 ± 509f
Pue01	1567 ± 150ac	1200 ± 117bc	2768 ± 267b	41 516 ± 4000b
Pue04	1130 ± 143ej	1017 ± 267bd	2147 ± 410be	32 200 ± 6147be
Qro01	1283 ± 106cg	955 ± 205bf	2239 ± 311be	33 582 ± 4671be
Qro02	1275 ± 18cg	991 ± 72be	2267 ± 89bd	33 999 ± 1340bd
Qro03	1357 ± 116ce	1336 ± 217ab	2693 ± 101bc	40 396 ± 1516bc
Qro04	1175 ± 4di	1036 ± 8bd	2210 ± 11be	33 155 ± 170be
Qro06	1277 ± 6cg	1096 ± 3bd	2373 ± 9bd	35 595 ± 133bd
Qro08	1044 ± 4ek	835 ± 4bg	1880 ± 8de	28 196 ± 114de
Qro11	1047 ± 33ek	1019 ± 152bd	2066 ± 120de	30 992 ± 1801be
Qro09	1144 ± 141ej	1047 ± 14bd	2191 ± 155be	32 875 ± 2321be
Sin05	729 ± 34l	1040 ± 20bd	1770 ± 52de	26 547 ± 784de
Ver01	1082 ± 110ej	1165 ± 72bc	2248 ± 178be	33 715 ± 2663be
Ver04	1206 ± 15di	892 ± 2bg	2098 ± 17be	31 467 ± 251be
Ver06	768 ± 60kl	956 ± 254bf	1724 ± 314de	25 863 ± 4706de
Ver08	1037 ± 3fl	836 ± 1bg	1873 ± 4de	28 093 ± 64de
Ver09	1002 ± 31gl	788 ± 284bg	1790 ± 312de	26 845 ± 4674de
Ver11	918 ± 5il	797 ± 6bg	1715 ± 10de	25 725 ± 142de
Ver12	857 ± 8jl	883 ± 5bg	1740 ± 7de	26 105 ± 97de
Average	1121 ± 370	844 ± 393	1966 ± 633	29 485 ± 9488
MSD	313.5	576.8	785.3	11 779.7

Values with different letters in each column are significantly different (Tukey, $p < 0.05$).
MSD: Minimal significant difference. C: capsaicin; D: dihydrocapsaicin; SHU: Scoville heat units.

or suitable for capsaicinoid extraction. Since environmental and handling conditions were similar for all landraces in the greenhouse, pungency variability recorded among landraces could be attributed to inherited genetic differences.

Highly pungent landraces have a large market as many consumers prefer spicy foods and the characteristic pungency and flavor of the piquín pepper in its various presentations (sauces, pickles, dried, or fresh). The pungency levels found here in the 31 studied landraces agree with the range of 22 190-44 035 SHU reported for nine ecotypes of wild piquín pepper collected in northeast Mexico (Moreno-Ramírez et al., 2018). In the highly pungent habanero pepper, Contreras-Padilla and Yahia (1998) observed that fruits harvested from greenhouse-grown plants outperformed fruits from open-air grown plants by 72% in accumulation of total capsaicinoids (195 380 *vs*. 113 040 mg kg^{-1} dry weight, respectively). Therefore, it can be inferred that *Capsicum* fruits are able to synthesize more capsaicinoids under favorable conditions than outdoors. The significant effect of environmental factors on the synthesis and accumulation of capsaicinoids was also observed by Harvell and Bosland (1997) in a dihaploid population of *C. annuum*, in which the capsaicinoid content varied up to eight-fold between different environmental conditions.

Pungency changes during fruit maturation

Across the maturation stages (green, intermediate, and red) the greenhouse piquín fruits showed significant differences ($p < 0.05$) among maturity stages and among landraces (Table 4). For most landraces, fruit pungency increased as maturity advanced from the green stage (14 813 SHU) to the intermediate stage (24 767 SHU), and kept increasing to the mature red stage (29 485 SHU). Similarly, Díaz et al. (2004) reported that capsaicinoids gradually accumulated through

Table 4. Pungency in piquín pepper fruits of 31 Mexican landraces harvested at three maturity stages when grown under greenhouse conditions.

Landrace	Green	Intermediate	Red
	SHU	SHU	SHU
Ags01	14 894 ± 1061gkC	23 748 ± 3585eiB	55 781 ± 1511a
Ags02	17 161 ± 1175fiC	21 350 ± 912gkB	30 665 ± 3682beA
Ags03	22 822 ± 3455acC	28 896 ± 4490ceB	32 278 ± 361beA
Dur01	18 749 ± 2375dgC	23 000 ± 2007ejB	27 788 ± 1240deA
Hgo01	17 169 ± 1307fiC	28 442 ± 357cfB	24 331 ± 1034deA
Mich01	10 091 ± 1736moC	26 825 ± 1030dgB	28 300 ± 1781deA
Mich02	7693 ± 1054oC	36 646 ± 1015abB	22 217 ± 2500deA
Nay02	22 217 ± 1957bdC	25 356 ± 622dhB	29 352 ± 823ceA
Oax01	20 078 ± 1698cfC	30 732 ± 2508bdB	30 261 ± 1259beA
Oax03	13 314 ± 266imC	33 696 ± 10acB	26 083 ± 4128deA
Oax05	15 923 ± 949gjC	38 867 ± 1806aB	30 821 ± 8312beA
Oax07	13 057 ± 189jnC	22 530 ± 2076fjB	4784 ± 254fA
Oax10	17 816 ± 809ehC	27 144 ± 3836dgB	4513 ± 509fA
Pue01	7608 ± 1025oC	27 976 ± 2633cfB	41 516 ± 4000bA
Pue04	14 212 ± 517hiC	25 734 ± 2154dhB	32 200 ± 6147beA
Qro01	15 850 ± 457gjC	24 710 ± 117dhB	33 582 ± 4671beA
Qro02	14 603 ± 332hkC	27 513 ± 356cgB	33 999 ± 1340bdA
Qro03	20 334 ± 1994cfC	16 055 ± 86klB	40 396 ± 1516bcA
Qro04	13 185 ± 194imC	36 662 ± 605abB	33 155 ± 170beA
Qro06	7073 ± 303oC	24 772 ± 3506dhB	35 595 ± 133bdA
Qro08	21 511 ± 2429ceC	22 218 ± 1446fkB	28 196 ± 114deA
Qro09	26 107 ± 706abC	25 006 ± 157dhB	32 875 ± 2321beA
Qro11	14 450 ± 955hkC	25 070 ± 99dhB	30 992 ± 1801beA
Sin05	11 081 ± 103koC	26 598 ± 397dgB	26 547 ± 784deA
Ver01	9787 ± 391moC	19 819 ± 1157hkB	33 715 ± 2663beA
Ver04	8861 ± 224oC	15 981 ± 894klB	31 467 ± 251beA
Ver06	10 236 ± 670oC	15 990 ± 620klB	25 863 ± 4706deA
Ver08	8817 ± 186oC	20 068 ± 1656hkB	28 093 ± 64deA
Ver09	9083 ± 78noC	18 120 ± 1712ikB	26 845 ± 4674deA
Ver11	26 714 ± 194aC	16 782 ± 668jlB	25 725 ± 142deA
Ver12	8702 ± 501oC	11 450 ± 3618B	26 105 ± 97deA
Average	14 813 ± 5584	24 767 ± 6580	29 485 ± 9487
MSD	4026	6305	11780

Values with different lowercase letters in each column are significantly different ($p < 0.05$). Values with different uppercase letters in each row are significantly different ($p < 0.05$).

fruit development until reaching the highest content at the end of ripening. However, some piquín landraces behaved differently. Figure 2 illustrates the behavior of three contrasting landraces through fruit maturation. Landraces Oax10 and Mich02 increased in pungency from the green stage to the intermediate stage, and thereafter decreased sharply when reaching the red stage. However, landrace Ags01 followed the average trend: the fruit maintained an increasing pungency from the green stage to the red stage when it reached the highest pungency.

It has been reported that capsaicinoid concentration during fruit development starts a few days after flowering, followed by linear increments for the next 30 to 50 d to then decrease until harvest (Iwai et al., 1979). According to Estrada et al. (2000), the final upsurge until the red stage results from increased rate of capsaicin synthesis and decreased degradation rate due to diminished activity of oxidating isoenzymes. That is, capsaicinoid content in chili peppers depends on the balance between simultaneous synthesis and degradation during fruit ripening. The decreasing capsaicinoid content that occurs with fruit maturity in some genotypes has been associated to the activity of peroxidase enzymes (Bernal et al., 1993; Contreras-Padilla and Yahia, 1998).

Fruit size and shape

The piquín plants grown in greenhouse produced larger fruits than those collected in the field or purchased in markets. Greenhouse fruits were 47% longer, 43% wider, and 100% heavier than fruits collected from field-grown plants. Therefore, piquín landraces can produce larger fruits and can potentially yield more fruit when grown under greenhouse protected conditions compared to field-grown plants. Since at this experimental site there were small differences in temperature between the greenhouse and the open-air conditions, we postulate that the larger and more pungent fruits produced in the greenhouse can be attributed to a better watering and fertilization given by the drip irrigation system which provided plants with nutrient solution 9 times a day. Nonetheless, these environmental differences did not produce significant changes on the fruit roundness index (Table 5).

Among field-collected samples, fruit length ranged from 2.9 to 17.3 mm, while the greenhouse fruits ranged from 6 to 37 mm. The longest field-collected fruits were produced by landraces Oax10, Dur01, and Mich02, with 15.9, 17.0, and 17.3 mm, respectively. Landraces Mich02, Nay02, and Oax10 produced the longest fruits in the greenhouse with 21.1, 21.5, and 37 mm, respectively. Most field-collected fruits weighed less than 270 mg, except for Mich02 fruits that registered 540 mg. Similar variations in fruit size were reported by Narez-Jiménez et al. (2014) for wild chili landraces collected in the Mexican state of Tabasco. The results hint that Mich02 landrace may not belong to the Cag species because field and greenhouse fruits markedly exceeded the average size of piquín fruits (Figure 3). Only three landraces (Qro03, Qro10, and Ver08) produced fruits of similar size and weight at field locations and greenhouse conditions.

Figure 2. Pungency levels at three fruit maturation stages (green, intermediate, and red) in three contrasting piquín pepper landraces and in the average of 31 landraces.

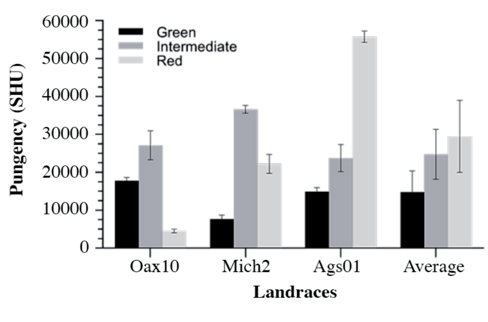
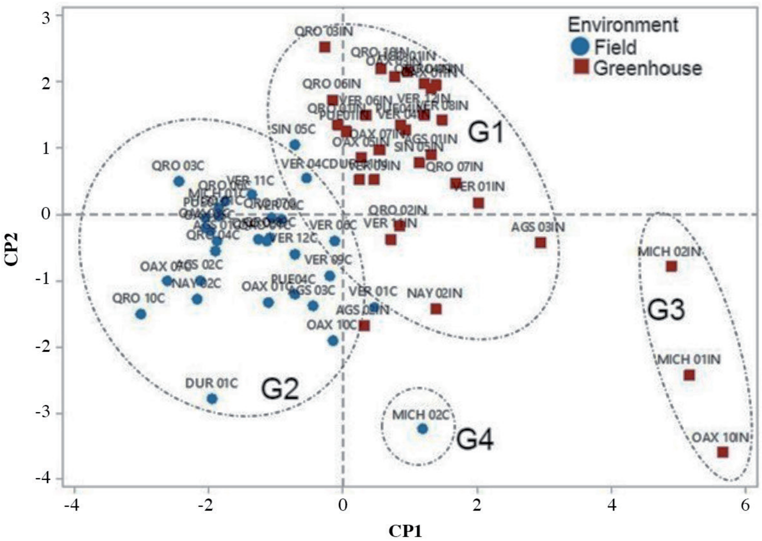


Table 5. Comparison of fruit size traits of 31 piquín pepper Mexican landraces sampled from two contrasting environments: field locations *vs*. harvested in the greenhouse (n = 124).

Environment	Length	Width	Roundness index (length/width)	Dry weight
	mm	mm		g
Greenhouse	14.4 ± 2.5a	6.7 ± 2.0a	2.5 ± 0.6a	0.24 ± 0.1a
Field	9.8 ± 1.4b	4.7 ± 0.6b	2.6 ± 0.8b	0.12 ± 0.0b

Averages ± standard deviations. Means with different letters are significantly different (Tukey, $p < 0.05$).

Figure 3. Grouping of 31 piquín pepper landraces sampled in two contrasting environments, 31 field locations *vs.* greenhouse grown plants, according to the cluster analysis based on fruit size and quality traits.



Fruit quality

Titratable acidity (TA). In most landraces, greenhouse fruits had a higher TA (0.23% to 0.38%) than field-harvested fruits (0.21% to 0.28%). Landraces Sin05 and Ver11 were less affected by the different growing conditions, as TA was similar in both environments. The changes recorded between environments for most landraces might be related to postharvest fruit handling. The greenhouse-collected fruits were analyzed immediately after harvest. In contrast, field-collected fruits had heterogeneous and unknown storage and handling conditions. In all cases, however, TA decreased as the fruit ripens, probably because in *Capsicum* the organic acids are used up for respiration (Durán-Acevedo et al., 2014). On the other hand, pH values varied much less than TA, since field-collected samples ranged from 4.8 to 6.4 while greenhouse-grown fruits varied between 5.1 to 5.6.

Total soluble solids (TSS). Most landraces showed higher TSS values in fruits sampled at field locations than in greenhouse fruits. These differences might depend on the fruit maturity stage: field-collected samples were older and drier, and had a higher concentration of soluble solids than the freshly ripened fruits harvested at the greenhouse. Field-sampled landraces Qro10 and Dur01 had 3.01 and 3.02 °Brix, respectively, which were the highest recorded values. However, in the greenhouse, the highest TSS values barely exceeded 2.0 °Brix by landraces Dur01, Nay02, and Qro01. TSS content increases with fruit maturation due to starch degradation (Nuez-Viñals et al., 1996). In wild piquín landraces from Nuevo León, Mexico, values ranged from 1.3 to 2.8 °Brix (Flores-González et al., 2018), similar to the range found in our study.

Maturity index (MI). Maturity index (TSS/TA ratio) for field-collected fruits was higher than for greenhouse-grown fruits. It indicates the balance between fruit sweetness and acidity, and it is often used as flavor quality criteria for fruits. Although MI by itself does not control fruit flavor, it allows analysis of postharvest fruit evolution. This index reveals the hydrolytic degradation of starch and pectins that increase the sweet taste (Nuez-Viñals et al., 1996), due to an increase in soluble solids and sugars associated with the decrease in acid content during ripening. Thus, the longer postharvest and storage handling should have allowed a higher concentration of soluble sugars to produce a sweeter taste.

Cluster analysis for fruit size and quality

The 31 landraces observed in two environmental conditions were classified into four differentiated groups. Group 1, located between quadrants I and II, included most greenhouse fruits. Group 2, containing most field-collected fruits, was located between quadrants II and III. Group 3 located in quadrant IV, farther from the center and contained only three landraces (Mich01, Mich02, and Oax10) that under greenhouse conditions produced larger fruits than on the open field. Finally, field-collected fruits of landrace Mich02 formed Group 4, because they were unusually larger and wider than other landraces. Thus, landrace Mich02 as collected from the field, may not be a piquín pepper, although it is named and sold as such. These results confirm the strong effect that favorable growth conditions under a greenhouse combined with appropriate postharvest handling have on fruit size and quality; fruits harvested in the greenhouse are larger and heavier than those collected in the field and taste more acidic (higher TA).

CONCLUSIONS

Fresh greenhouse-harvested piquín pepper fruits contain almost five times more capsaicin and dihydrocapsaicin than fruits collected from the field, which were stored and handled in unknown ways. Capsaicin content in field-collected fruits was lower than dihydrocapsaicin content, contrary to recorded capsaicinoids content in recently greenhouse-harvested fruits. After harvest, capsaicin is degraded faster than dihydrocapsaicin. During fruit ripening of greenhouse harvested fruits, 70% of the piquín landraces increased their pungency during the whole maturity stages, so they reached the highest level at the mature red stage. Only in few landraces fruit pungency decreased in their maturation from the intermediate stage to the red stage.

In most piquín landraces, greenhouse harvested fruits were longer, wider, and heavier than fruits sampled from the 31 field locations. For example, the landrace Oax10 doubled its fruit size in the greenhouse compared to its field-grown fruits. The quality traits (pH, acidity, total soluble solids, and maturity index) changed across the maturity stages for both field and greenhouse collected fruits. The landrace grouping based on fruit traits showed four well-differentiated groups, mostly due to the environmental growing conditions. Three landraces (Mich01, and Mich02, and Oax01) differed from all others both in the field collected and in greenhouse harvested fruits, so they might belong to a different species.

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