Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2019, 47(1):119-127. DOI:10.15835/nbha47111241

AcademicPres

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Original Article

Content of Capsaicinoids and Physicochemical Characteristics of Manzano Hot Pepper Grown in Greenhouse

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Abstract

The hotness of the chili fruit (*Capsicum* spp.) is mainly due to the presence of capsaicinoids (capsaicin, nordihydrocapsaicin and dihydrocapsaicin). The aim of the present research was to evaluate the content of capsaicinoids and characteristics of physicochemical quality in fruits of manzano hot pepper grown in the greenhouse. The experimental design used was completely randomized with 3 and 4 replications. The parameters evaluated were total capsaicinoids, vitamin C, total carotenoids (TC), total soluble solids (TSS), titratable acidity, pH, firmness and color of the fruit. Among the hybrids with the highest content of total capsaicinoids and vitamin C, L4XL8 and L5XL7 (27 371 and 21 700 SHU, respectively) stand out as well as L2XL5 with 809.35 mg 100 g^{-1}. On the other hand, L2XL3 stood out for its TC content (1 515.98 µg 100 g^{-1}); L2XL7 and L4Xl7 stood out for the concentration of TSS, maintaining the acidity level without major changes. Additionally, L7XL8 was a material that was characterized to have fruits with greater firmness (2.31 N) and chromaticity of color (intense yellow) of 72.96. Among the evaluated hybrids, there are materials that presented fruits with physical and chemical characteristics of quality, which could be considered important from the commercial point of view or genetic improvement.

Keywords: *Capsicum pubescens* Ruiz y Pavón; color; fruit quality; scoville heat unit; titratable acidity; total carotenoids

Abbreviations: Ci: Capsaicin; Dhc: Dihydrocapsaicin; N: Newton's; Nhc: Norhydricapsaicin; TC: Total carotenoids; TCi: Total capsaicinoids; TSS; total soluble solids; TA: Titratable acidity; VC: Vitamin C.

Introduction

The consumption of products of vegetable origin among the various prehispanic people, according to the Florentino Codex, was basically constituted by corn (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), squash (*Cucurbita* spp.) and chili pepper (*Capsicum* spp.) (Vera-Guzmán *et al.*, 2011; Meckelmann *et al.*, 2015). The genus *Capsicum* is composed of 30 species (Agostini-Costa *et al.*, 2017), among them, the domesticated (*C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens*), which originated from Mesoamerica (Mexico and Central America) to the Andean region (Ecuador, Bolivia, Chile and Peru), that is, zones with contrasting climatic and tropical characteristics (Rodríguez-Burruezo *et al.*, 2010; Yamamoto *et al.*, 2013). In the particular case of *C. pubescens* Ruiz and Pavón, its origin goes back to areas with altitudes from 1500 to 3000 masl and temperate climate characteristics in South America (Bolivia, Ecuador and Peru) (Wahyuni *et al.*, 2013; Bo and Carpizo, 2015), where the rest of the species would hardly prosper (Espinosa-Torres and Ramírez-Abarca, 2016).

In Mexico, due to its coloration and morphological characteristics, the fruit of *C. pubescens* is called manzano hot pepper (Espinosa-Torres *et al.*, 2010), and its cultivation is mainly carried out under temporary or backyard conditions, however, in the last decade, its production has

Received: 12 Apr 2018. Received in revised form: 30 Jul 2018. Accepted: 01 Aug 2018. Published online: 01 Aug 2018.

extended to greenhouse conditions, mainly in the temperate zones of Michoacán, Puebla, State of Mexico, Veracruz and to a lesser extent Chiapas and Oaxaca (Espinosa-Torres *et al*., 2014; Espinosa-Torres and Ramírez-Abarca, 2016), where the use of protected structures allows the incorporation of various technologies such as hydroponics and the control of climatic factors limiting temperature and humidity, which as a whole give the producer an increase in their level of income and productivity of the culture (Pérez and Castro, 1998; Islam *et al*., 2015), however, due to their physiological and morphological characteristics, prevent proper postharvest handling (Espinosa-Torres *et al*., 2010) problems that are associated with dehydration, color changes and mechanical damage to the fruit (Vera-Guzmán *et al*., 2011), as well as a decrease in the expression of characteristics related to its nutritional value (Hwang-Sung *et al*., 2015).

The intake of chili allows the incorporation into the diet of various bioactive compounds such as anthocyanins, ascorbic acid, phenolic compounds, flavonoids, carotenoids (α- and β-carotene), capsaicionoids and vitamins (Sánchez-Sánchez *et al*., 2010; Wahyuni *et al*., 2013). In this sense, a characteristic that distinguishes the fruits of chili, is the synthesis and accumulation of capsaicinoids, alkaloid group responsible for its pungency (itching), compounds that are located mainly in the tissue of the placenta adjacent to the seeds (Vera-Guzmán *et al*., 2017), where 90% is made up of capsaicin, dihydrocapsaicin and nordihydrocapsaicin (Wahyuni *et al*., 2013; Bo and Carpizo, 2015), whose main function is defense against the attack of mammals (Rodríguez-Burruezo *et al*., 2010) and the presence of fungi and bacteria (Segura *et al*., 2013).

There is a great variety of methods of extraction and quantification of capsaicinoids, however, it is common to find among the pungency reports of the various species of *Capsicum*, values that are expressed by the Scoville (Scoville Heat Units) SHU organoleptic test, and their values they range from 0 for non-pungent varieties (bell pepper) and 2 200 000 in chili habanero (*C. chinense* Jacq.) (Yamamoto *et al*., 2013; De Aguiar *et al*., 2016), and in a subjective way it represents a characteristic of nutritional quality which affects its acceptance among consumers. In the case of *C. pubescens* its content varies from 4 032 to 60 000 SHU (Sánchez-Sánchez *et al*., 2010). Therefore, the aim of the present research was to evaluate the content of capsaicinoids and characteristics of physicochemical quality in fruits of manzano hot pepper grown in the greenhouse.

Materials and Methods

Plant material, management and experimental design

The study involved the use of 25 manzano hot pepper hybrids, obtained as a product of five self-fertilization cycles between eight lines (L1, L2, L3, L4, L5, L6, L7, and L8), as well as the commercial variety 'Grajales St.' (control) (Pérez and Castro, 2012). The cultivation was carried out in greenhouse conditions (double zenithal type and 50% shade), located in the Experimental Agricultural Field of the Chapingo Autonomous University, State of Mexico (19° 29' 23" N; 98° 52' 24" W), 2 268 meters above sea level (masl) and average annual temperature of 15.9 °C. The sowing was carried out during the month of November 2011, in trays of polystyrene with 200 cavities and Growing Mix No. 2 as a substrate, to be subsequently transplanted in January 2012. The crop was managed according to a proposed intensive production system by Pérez and Castro (2012), for this, they were placed in white polyethylene bags (40 cm wide and 45 cm high) in which 50% course tezontle was used as substrate in the lower part of the bag and a mixture of 25% Growing Mix No. 2 and 25% fine tezontle was used in the upper portion. The pots were placed at a distance of 50 cm between plants and 1 m between rows. A nebulization and tutoring system was used; For the supply of nutrients, the application of a nutritive solution adapted from Pérez and Castro (1998) was implemented through a drip irrigation system, with an expenditure of 3 L / h distributed during the day in three irrigations. The fruits used for the laboratory analysis were harvested in the month of April 2014 and were characterized by presenting average weight of 120 ± 2 g, intense and uniform yellow color (consumer maturity), where they were visually inspected that they did not present mechanical damage and evidence of damage caused by pests or diseases. The laboratory analyzes were carried out in the Department of Plant Science of the Autonomous University Chapingo. The experimental design was completely randomized with 3 and 4 replicates. The concentration of capsaicionoids consisted of three replicates with 2 fruits per experimental unit. A set of 3 fruits was used as an experimental unit and four replicates for the determination of vitamin C, total carotenoids, total soluble solids, titratable acidity, firmness and fruit color.

Extraction and quantification of capsaicinoids

Six fruits of each genotype were harvested and placed in ziploc plastic bags (27 × 28 cm), transported to the laboratory, where they were lyophilized for 4 days and then homogenized using an Osterizer® blender (Cruz-Pérez *et al*., 2007). The extraction was performed with 1 g of lyophilized sample, adding 10 mL of acetonitrile, the mixture was placed in a water bath for 5 hours at 60 °C, a 3 mL aliquot was taken and filtered through a nylon membrane with pore 45 μm and 25 mm in diameter. The quantification was carried out through the injection of 20 μL of the extract to the Agilent® chromatograph model HP-1100 equipped with UV detector and Supelcosil LC-18 column (25 cm × 4.6 mm, 5 μm), whose mobile phase consisted of acetonitrile: water (45:55), 1.5 mL of flow, 23 ± 3 °C and wavelength of 280 nm. Prior to the calculation of the concentration of total capsaicinoids, the content of individual capsaicinoids was determined by method 995.03 (AOAC, 1995) with the application of the expressions:

$$N = \left[\left(\frac{Pn}{Ps}\right) \times \left(\frac{Cs}{Wt}\right) \times \left(\frac{10}{0.98}\right)\right] \times 9300;$$

$$C = \left[\left(\frac{Pc}{Ps}\right) \times \left(\frac{Cs}{Wt}\right) \times \left(\frac{10}{0.89}\right)\right] \times 16100;$$

$$D = \left[\left(\frac{Pd}{Ps}\right) \times \left(\frac{Cs}{Wt}\right) \times \left(\frac{10}{0.93}\right)\right] \times 16100;$$

where:

N = nordihydrocapsaicin (SHU); C = capsaicin (SHU); D = dihydrocapsaicin (SHU); Pn, Pc, Pd = peak area for nordihydrocapsaicin, capsaicin and dihydrocapsaicin; Ps =

peak area of the corresponding standard; Cs = concentration of the standard solution (mg·mL^{-1}); Wt = weight of the sample (g).). The total capsaicinoids were the result of the sum of individual capsaicinoids (N + C + D), and 1 μg of capsaicinoids g^{-1} = 15 Scoville Heat Unit (SHU) (Collins *et al.*, 1995).

Vitamin C (ascorbic acid)

The determination of the vitamin C content was carried out according to the method of Tillman (AOAC, 1990) known as DFI-2, 6 dichlorophenol-indophenol, for which the maceration of a sample of pulp was carried out with a stabilizing agent as oxalic acid (to maintain proper acidity for the reaction and avoid self-oxidation of ascorbic acid at high pH) and reduction of 2,6-dichlorophenol-indophenol (Tillman's solution). It was estimated from 5 g of finely chopped fruit and homogenized with 50 mL of oxalic acid, taking an aliquot of 10 mL. The concentration was expressed in mg 100 g^{-1} fresh weight by a standard curve of ascorbic acid.

Total carotenoids

The quantification of carotenoids was carried out according to the technique proposed by Lichtenhaler (1987); a sample of 5 g of tissue was homogenized with 80% acetone, the residue was filtered and adjusted to 10 mL, the absorbance reading was obtained at 476, 646 and 663 nm using the Thermo Scientific Genesys™ 10 UV Scanning spectrophotometer. The content of carotenoids was calculated with the application of the following equations: Ca = 12.25 A663 - 2.79 A646; Cb = 21.50 A646 - 5.10 A663 and Cx + c = 1000 A476 - 1.82 Ca - 85.02 Cb; where: Ca = chlorophyll a; Cb = chlorophyll b; Cx + c = total carotenoids; where A = absorbance reading with different wavelength (476, 646 and 663 nm). The results were expressed in μg 100 g^{-1} fresh weight.

Total soluble solids

The total soluble solids were quantified with a PAL-1® portable digital refractometer (ATAGO, USA) which uses a scale of 0-53°, and to make the measurement a drop of fruit juice was placed on the refractometer screen to take the reading. The results were expressed in °Brix.

Titratable acidity

The titratable acidity was determined according to the methodology proposed by the AOAC (AOAC, 1990), with 5 g of pulp that was neutralized with 0.1 N NaOH, using 1% phenolphthalein as indicator. The results are reported in % of citric acid.

Firmness

The determination of the firmness was made in the equatorial zone of the fruit by means of a Compac Gauge digital penetrometer (Mecmesin®, USA) with a cone-shaped strut with a diameter and height of 9 mm, recording the applied force until the penetration of the strut, where the readings were expressed in Newton's (N).

Color

The surface color of the epidermis of the fruit was determined with a Color Tec-PCM® colorimeter (D25-PC2 Cole Palmer, USA), recording the initial values of L, a and b. With these values the hue angle (hue) and the purity of the color (chroma) were calculated with the formulas: hue = arctan (b * a^{-1}); chroma = $(a^2+b^2)^{½}$; and the luminosity (L) obtained directly with the device, which corresponds to the color space L * a * b * (Minolta, 2007).

Statistical analysis

An analysis of variance and Tukey's mean comparison test (P ≤ 0.05) were applied to the set of data obtained from each parameter, in which the statistical analysis program Statistical Analysis System was used (SAS), ver. 9.1 (SAS, 2003).

Results and Discussion

Capsaicinoids

It is well known that the fruits of the species from the *Capsicum* genus have different levels of hotness or pungency, and when compared to each other, between the extremes in relation to this characteristic, is the habanero pepper (*C. chinense* Jacq.) and bell pepper (*C. annuum* L.) (Figueroa *et al.*, 2015), in which its determination is usually an aspect of important quality among consumers (Wahyuni *et al.*, 2013). As shown in Table 1, the manzano hot pepper showed significant variation (P ≤ 0.05) with respect to the content of total capsaicinoids (level of hotness), which highlights the hybrid L4XL8 with 273 706 SHU (Scoville Heat Units), however, L5XL7 presented a statistically similar behavior of 217 001 SHU, and the contribution of each individual capsaicinoid (norhidrocapsaicin, capsaicin and dihydrocapsaicin) was 21.78, 31.63 and 46.38%, respectively, this last coincides with that indicated by Orellana-Escobedo *et al.* (2013) and Meckelmann *et al.* (2015). Additionally, it is observed that 48% of the hybrids (L7XL8, L6XL7, L3XL6, L2XL7, L2XL3, L4XL7, L3XL7, L4XL6, L2XL8, L3XL8, L4xL5 and L2XL5) presented values between 127 162 and 191 459 SHU, which exceeded the commercial variety ('Grajales St.') (126 778 SHU). These results contrast with that observed by Cruz-Pérez *et al.* (2007) who when evaluating genetic material (progenitors and hybrids) of manzano-pepper with different storage periods, found that fruits with 58 days after flowering showed the maximum values of 55 927 and 68 337 SHU, and at day 94, these values decreased to 4 472 and 11 268 SHU. On the other hand, Sánchez-Sánchez *et al.* (2010) indicate varieties of manzano hot pepper (Puebla, Huatusco, Zongolica, Peru and Tacámbaro) with a fluctuation between 4 032 and 36 712 SHU. In other chili species such as *C. chinense*, similar values of 211 247.65 SHU are reported (Orellana-Escobedo *et al.*, 2013), as well as De Aguiar *et al.* (2016) report in 'Naga Jolokia' and 'Murupi' (*C. chinense*) maximum values of 119 016 and 38 910 SHU, respectively, being the first reported value, comparable with the hybrid L1XL7 (119 334) and lower with the obtained in the commercial control. In contrast, Islam *et al.* (2015) when evaluating the variability in the content of capsaicinoids in chili fruits (*Capsicum* spp.) (collected and commercial) grown in northeastern India report values between 317 and 1 152 832 SHU. On the other hand, Collins *et al*. (1995) report values of 216 345 SHU for jalapeño pepper (*C. annuum* L.), which surpasses that reported by Orellana-Escobedo *et al*. (2013) in nuts of this same type of chili, however, are lower than the maximum values found in this study. The high inter- and intraspecific variation, in relation to the synthesis and concentration of capsaicinoids, can be linked according to Wahyuni *et al*. (2013) and Rodríguez-Uribe *et al*. (2014) to the significant effect of the interaction of the fruit with the prevailing environment during the final stages of its growth and development, conditions that are mainly associated with environmental temperature and shading level (Jiménez *et al*., 2013), as well as to the state of maturity (Orellana-Escobedo *et al*., 2013) and to the extraction method used (Sánchez-Sánchez *et al*., 2010).

Vitamin C (ascorbic acid)

One of the outstanding nutritional characteristics of the chili fruit is its high content of ascorbic acid (Teodoro *et al*., 2013), in this study it was observed that the L2XL5 hybrid stands out for having the highest content of this vitamin (809.35 mg 100 g^{-1}), which represents 40% more concentration with respect to the materials L2XL7 and L1XL7 (485.75 mg 100 g^{-1} in both cases) (Table 1). However, it is important to point out that the hybrid L2XL5 presented a similar behavior to that of the majority of the analyzed genotypes (hybrids and commercial variety), whose values ranged from 518.09 to 766.99 mg 100 g^{-1}. In contrast, Cruz-Pérez *et al*. (2007) when evaluating three hybrids ('Puebla' × 'Chiapas', 'Puebla' × 'Huatusco', and 'Puebla' × 'Zongolica') and their progenitors ('Chiapas', 'Puebla', 'Huatusco' and 'Zongolica') with three stages of maturity, found maximum values of 455 mg 100 g^{-1}, corresponding to the progenitor 'Puebla', with a tendency to present a decrease as the maturation process progresses. Espinoza-Torres *et al*. (2010) reported values of 230 mg 100 g^{-1} for manzano hot pepper 'Puebla' stored with different packaging and storage temperatures.

On the other hand, Vera-Guzmán *et al*. (2011) when evaluating a sample of nine regional varieties or chili morphotypes collected in Oaxaca, Mexico; among them

Table 1. Content of capsaicinoids evaluated in manzano hot pepper fruits grown in greenhouse

Hybrids	Nhc	Ci	Dhc	TCi
L4XL8	60 434 a	85 239 a	128 033 a	273 706 a
L5XL7	46 131 ab	69 737 ab	101 133 ab	217 001 ab
L7XL8	44 040 abc	59 911 bcd	87 509 a-d	191 459 bc
L6XL7	28 718 b-f	60 826 bcd	91 432 a-d	180 976 bcd
L3XL6	26 075 c-g	64 235 bc	89 133 a-d	179 442 b-e
L2XL7	36 186 d-g	61 787 bcd	78 061 a-e	176 034 b-e
L2XL3	21 923 d-g	41 806 e-i	93 939 abc	157 667 b-f
L4XL7	29 447 b-e	51 660 cde	75 907 b-f	157 013 b-f
L3XL7	22 888 d-g	46 732 def	75 319 b-f	144 939 c-g
L4XL6	26 063 c-g	46 615 d-g	62 797 b-f	135 475 c-g
L2XL8	22 851 d-g	37 652 e-k	73 920 b-f	134 422 c-g
L3XL8	21 657 d-g	36 610 e-k	74 932 b-f	133 198 c-g
L4XL5	26 743 c-g	36 433 e-k	69 892 b-f	133 069 c-g
L2XL5	18 623 d-g	42 752 e-h	65 787 b-f	127 162 d-h
Grajales St.	18 521 d-g	36 429 e-k	71 829 b-f	126 778 d-h
L5XL8	22 091 d-g	31 209 f-l	68 631 b-f	121 932 d-i
L1XL7	23 816 d-g	40 475 e-j	55 043 b-f	119 334 e-i
L1XL3	12 782 efg	30 426 g-l	61 393 b-f	104 601 f-i
L1XL4	23 791 d-g	34 925 f-l	41 524 def	100 240 f-i
L6XL8	18 860 d-g	30 172 h-l	45 185 cdef	94 217 ghi
L1XL2	21 516 d-g	36 861 e-k	35 417 ef	93 794 ghi
L3XL5	9 962 fg	25 699 i-l	52 124 b-f	87 785 ghi
L3XL4	10 411 fg	23 766 kl	35 822 ef	69 999 hi
L1XL5	18 291 d-g	21 954 kl	27 062 f	67 306 hi
L2XL4	13 704 efg	18 872 l	31 795 ef	64 371 i
L1XL6	9 351 g	24 749 jkl	27 737 g	61 837 i
MDSH	18 986	16 254	50 210	60 499

Note: The concentration of capsaicinoids is expressed in SHU (Scoville Heat Units). Means with the same letter within columns do not show significant statistical differences (Tukey's test, p≤0.05).
HSMD: Honest significant minimum difference.

'Canario' (*C. pubescens*) reported values of 1.9 mg 100 g^{-1} (immature) and 18 mg 100 g^{-1} (mature), highly dependent values on the variety and stage of maturity at harvest. These results surpass those found in other types of pepper such as habanero (*C. chinense* Jacq.) (281.73 mg 100 g^{-1}) (Segura *et al*., 2013; Teodoro *et al*., 2013) and bell pepper (*C. annum* L.) (355.5 mg 100 g^{-1}) (Figueroa *et al*., 2015). As well as with other vegetable species such as husk tomato (*Physalis ixocarpa* Brot, ex Horm) (3.31 mg 100 g^{-1}) (Cruz-Álvarez *et al*., 2012) and carrot (*Daucus carota* L.) (3.5 mg 100 g^{-1}) (Datt *et al*., 2012). In this sense, these results allow to assert that these materials can be useful for commercial use and be an important source of vitamin C and help to be a preventive factor for cancer by inhibiting the synthesis of N-nitroso compounds in the stomach and stimulating the immune system (Cruz-Pérez *et al*., 2007; Datt *et al*., 2012).

Total carotenoids

Color changes (salmon, yellow, orange, red and brown) in the chili fruit are closely related to the accumulation of specific mixtures of carotenoids (capsanthin, α, β-carotene, zeaxanthin, lutein and β-cryptoxanthin) (Wahyuni *et al*., 2013; Hwang-Sung *et al*., 2015) and its concentration varies depending on the cultivar and edaphoclimatic conditions prevailing during its growth and development (Agostini-Costa *et al*., 2017). This compound presented significant variation ($P \leq 0.05$) between hybrids, where L2XL3 stood out for its content of total carotenoids (1 515.98 μg 100 g^{-1}), which represents a variation of 62% with respect to L6X7 (426.56 μg 100 g^{-1}) with the lowest value (Table 1). What is found in this study contrasts with that pointed out by Sun-Hwa *et al*. (2007) for yellow fruits of *C. pubescens* (1 950 μg 100 g^{-1}), *C. baccatum* (1 600 μg 100 g^{-1}) and *C. chinense* Jacq. (2 000 μg 100 g^{-1}), but similar to those obtained by Rodríguez-Uribe *et al*. (2012) in five types of chili (*C. annuum*) with orange-yellow fruits (233 to 1 615 μg 100 g^{-1}). Values of 1 000 to 1 260 μg 100 g^{-1} are reported in habanero chili fruits (*C. chinense* Jacq.) grown in Yucatan, Mexico, with different colorations (orange, red, yellow and brown) by Segura *et al*. (2013), which are similar to those found in L1XL6, L2XL8, L5XL7 and L6XL8 (1 270.87, 1 108.74, 1 273.07 and 1 248.29 μg 100 g^{-1}, respectively.) Additionally, Agostini-Costa *et al*. 2017) when evaluating different varieties of jalapeño (*C. annuum* L.) and habanero peppers cultivated in Brazil, and with red and yellow tones, they report that they did not find variation regarding to the content of total carotenoids with values of 304 ± 92 mg g^{-1}; according to these same authors, this variation is strongly influenced by the stress caused by the high light exposure during its growth and development, where the variety plays an important role (Wahyuni *et al*., 2013).

Total soluble solids

The accumulation of solutes during the maturation process is one of the parameters with greater precision and reliability when used as a harvest index in fruits and vegetables (Sánchez-Sánchez *et al*., 2010), because its determination is rapid, relatively low cost and it correlates directly with flavour (Beckles, 2012). Among the manzano hot pepper genotypes, there was statistical variation regarding the concentration of TSS (Table 2), where the hybrid L2XL7 was higher ($P \leq 0.05$) (7.40 °Brix), without exceeding that observed in L4Xl7 (7.30 °Brix) In addition, the values found were 19.86% higher in relation to the commercial variety (5.93 °Brix). In this sense, Navarro *et al*. (2006) report 6.99 and 4.92 °Brix in fruits with 50 and 100% red hue. On the other hand, Figueroa *et al*. (2015) indicate data with a range of values between 8.1 and 9.5 °Brix in red peppers with different color tones. Additionally, Fox *et al*. (2005) reported values of 6.9 and 7.9 °Brix in fruits with ripeness of pepper consumption 'Robusta' and two harvest dates. The process of fruit maturation involves a series of changes related to respiration, degradation of compound such as chlorophyll and substances involved in obtaining cellular energy (Beckles, 2012), and its variation is widely correlated to its nutritional management (Yamamoto *et al*., 2013), variety and state of maturity (Jiménez *et al*., 2013).

Titratable acidity

The characteristic flavor of each horticultural product must not only be associated as a consequence of the presence of sugars (sucrose, glucose, galactose and fructose) (Giovannoni, 2007), but also to the accumulation of organic acids (citrate and malate) (Beckles, 2012). The acidity data of the fruits expressed as a function of the prevailing organic acid (citric acid) are shown in Table 2, which fluctuated between 0.32 and 0.45% with little variation among genotypes, where L4XL7 was the most outstanding and with the exception of L2XL7 and L2XL3, this hybrid showed a similar behavior to the rest of the materials, including the control. When comparing these values with those reported for this species and other types of chili, these surpass the 0.052% found in fruits of the variety 'Puebla' of manzano hot pepper (Espinoza-Torres *et al*., 2010); but they are similar to those reported by Salinas *et al*. (2010) for four types of Amashito peppers, whose values ranged between 0.30 and 0.40% citric acid, as well as those reported by Fox *et al*. (2005) in red bell pepper fruits harvested on two dates (0.29 and 0.32% citric acid). On the other hand, Figueroa *et al*. (2015) when evaluating six commercial varieties and three fruit colorations: 'Magno' (orange), 'Moonset' (yellow), 'California' (yellow), 'Triple 4' (red), 'Triple Star' (red) and 'Viper' (red), report a maximum value of 0.67% in red fruits. In others species such as tomato (*Solanum lycopersicum* L.) Cheema *et al*. (2014) when performing hexanal applications and in combination of controlled atmospheres, they report values of 0.42 to 0.57%, similar to those found in L4XL7 and L4XL8. The way in which the data of titratable acidity behaved, could also be linked to the pH of the fruits which also did not show significant variation (data not shown), which are widely correlated with the state of maturity during its evaluation (Figueroa *et al*., 2015), which in this work, the fruits evaluated were harvested with a similar state of maturity (consumption maturity). Authors such as Espinoza-Torres *et al*. (2010) and Beckles (2012) indicate may be linked to a decrease in the concentration of organic acids in ionized form and phenolic compounds in plant tissue.

Table 2. Concentration of vitamin C, total carotenoids, total soluble solids and titratable acidity evaluated in manzano hot pepper fruits grown in greenhouse

Hybrids	VC (mg 100 g-1)	TC (µg 100 g-1)	TSS (°Brix)	TA (% citric acid)
L4XL8	679.90 ab	537.03 bc	6.52 a-f	0.44 ab
L5XL7	776.99 ab	1273.07 abc	6.95 a-d	0.39 abc
L7XL8	744.63 ab	1428.43 ab	6.65 a-f	0.40 abc
L6XL7	615.18 ab	426.56 c	6.55 a-f	0.39 abc
L3XL6	615.18 ab	1355.07 abc	6.43 a-f	0.39 abc
L2XL7	485.73 b	565.62 abc	7.40 a	0.32 bc
L2XL3	679.90 ab	1515.98 a	6.62 a-f	0.32 bc
L4XL7	679.90 ab	680.87 abc	7.30 ab	0.45 a
L3XL7	744.63 ab	700.53 abc	6.88 a-e	0.34 abc
L4XL6	776.99 ab	508.08 bc	5.80 f	0.35 abc
L2XL8	582.82 ab	1108.74 abc	6.15 c-f	0.33 abc
L3XL8	744.63 ab	885.39 abc	5.73 f	0.38 abc
L4XL5	744.63 ab	714.86 abc	6.32 c-f	0.35 abc
L2XL5	809.35 a	860.39 abc	6.52 a-f	0.32 bc
Grajales St.	647.54 ab	638.07 abc	5.93 ef	0.35 abc
L5XL8	615.18 ab	853.20 abc	6.00 def	0.41 abc
L1XL7	485.73 b	679.97 abc	7.10 abc	0.39 abc
L1XL3	518.09 ab	648.29 abc	6.10 def	0.41 abc
L1XL4	550.45 ab	811.92 abc	6.95 a-d	0.36 abc
L6XL8	582.82 ab	1248.29 abc	6.00 def	0.33 abc
L1XL2	582.82 ab	472.09 bc	6.33 b-f	0.34 abc
L3XL5	582.82 ab	987.78 abc	6.05 def	0.35 abc
L3XL4	744.63 ab	862.56 abc	7.08 abc	0.34 abc
L1XL5	647.54 ab	565.45 abc	6.30 c-f	0.38 abc
L2XL4	647.54 ab	672.78 abc	6.32 c-f	0.37 abc
L1XL6	615.18 ab	1270.87 abc	6.37 b-f	0.41 abc
HSMD	298.01	298.01	0.98	0.117

Note: Means with the same letter within columns do not show significant statistical differences (Tukey's test, p≤0.05)..
HSMD: Honest significant minimum difference.

Firmness

The quantitative expression of the structural characteristics of the cell wall between harvested products constitutes one of the attributes of quality most appreciated by the consumer (Giovannoni, 2007), which is why, among the various plant breeding programs, this parameter it is one of the main selection characters (Figueroa *et al*., 2015). In this study, the evaluated hybrids showed values of significant firmness (P≤0.05) (Table 4), with L7XL8 showing fruits with an average firmness value of 2.31 N, nevertheless, their behavior was similar to 84.61% (22 materials) of the genotypes evaluated, including the commercial variety (2.08 N). These results show a clear contrast with what was found by Figueroa *et al*. (2015) in bell peppers, which, when harvested with different coloration, their firmness values fluctuated between 12.60 and 24.63 N.

Additionally, they also surpass those reported in manzano hot pepper by Espinoza-Torres *et al*. (2010) who, when evaluating the effect of packaging with different storage conditions, found values between 17.65 and 33.34 N. With respect to this behavior, it is of prime interest to indicate that this quality parameter is directly associated with the degree of maturity of the fruit at the time of harvest (Hwang-Sung *et al*., 2015), additionally, due to its morphological and physiological characteristics, once separated from the mother plant, it loses turgor as a result of the transpiration process (Jiménez *et al*., 2013).

Color

The manzano hot pepper fruit is consumed when the coloration of its epidermis has changed to a yellowish hue without the presence of green stripes (Meckelmann *et al*., 2015), and it is the first aspect evaluated by the consumer, so this quality parameter is of vital importance in postharvest (Espinosa-Torres and Ramírez-Abarca, 2016; Tortoe *et al*., 2016). Among the color components evaluated, no statistical variation was found with respect to brightness (L) and hue (hue) (Table 4), however, the chromaticity that

Table 3. Firmness and color (brightness, purity and color tone) evaluated in manzano hot pepper fruits grown in greenhouse

Hybrids	F	Color		
		L	Chroma	Hue (°)
L4XL8	1.96 bc	38.72 a	44.97 b	71.89 a
L5XL7	2.04 abc	39.30 a	47.50 ab	66.44 a
L7XL8	2.31 a	37.50 a	72.96 a	77.38 a
L6XL7	2.08 abc	44.17 a	46.49 b	75.46 a
L3XL6	2.09 abc	42.57 a	53.09 ab	72.24 a
L2XL7	2.09 abc	42.74 a	59.04 ab	66.32 a
L2XL3	2.17 abc	40.41 a	42.49 b	59.77 a
L4XL7	2.03 abc	43.64 a	48.77 ab	73.01 a
L3XL7	2.23 ab	42.32 a	57.74 ab	70.31 a
L4XL6	2.11 abc	36.57 a	45.70 b	69.75 a
L2XL8	2.09 abc	37.22 a	47.19 ab	44.61 a
L3XL8	2.10 abc	38.26 a	56.19 ab	77.82 a
L4XL5	2.15 abc	38.36 a	45.27 b	67.88 a
L2XL5	2.07 abc	40.74 a	47.90 ab	79.70 a
Grajales St.	2.08 abc	38.22 a	48.06 ab	77.35 a
L5XL8	2.06 abc	41.82 a	50.89 ab	73.66 a
L1XL7	2.07 abc	41.66 a	54.35 ab	73.18 a
L1XL3	2.06 abc	39.35 a	45.16 b	68.31 a
L1XL4	1.99 bc	37.69 a	37.96 b	74.31 a
L6XL8	2.01 abc	38.46 a	45.69 b	81.01 a
L1XL2	2.04 abc	35.50 a	39.58 b	66.53 a
L3XL5	2.11 abc	38.71 a	43.92 b	65.70 a
L3XL4	2.07 abc	40.01 a	42.82 b	61.10 a
L1XL5	1.92 c	39.69 a	44.65 b	66.09 a
L2XL4	2.08 abc	36.56 a	39.01 b	73.61 a
L1XL6	1.88 c	34.66	44.47 b	53.92 a
HSMD	0.307	10.318	26.404	38.464

Note: Means with the same letter within columns do not show significant statistical differences (Tukey's test, p≤0.05)..
HSMD: Honest significant minimum difference.

refers to the purity of the characteristic color (Tortoe *et al*., 2016), statistically highlights (P≤0.05) the L7XL8 hybrid with 72.96, however, it is similar to that shown by L5XL7, L3XL6, L2XL7, L4XL7, L3XL7, L2XL8, L3XL8, L2XL5, ′Grajales St.′, L5XL8 and L1XL7 whose values fluctuated between 47.19 and 59.04 and correspond to fruits with intense yellow color and representing 42.30% of the evaluated materials. In contrast, Moreno-Pérez *et al*. (2006) when evaluating mature fruits of 162 collections (plants) of native chili guajillo (*C. annuum* L.), coming from the states of Jalisco, Zacatecas and Durango, Mexico, indicate maximum values of 34.30.

On the other hand, Figueroa *et al*. (2015) report similar data that ranged between 34.17 and 48.96, in fruits of bell pepper varieties with different color, where varieties with orange ('Magno') and yellow ('California' and 'Moonset') tones presented the higher values of chromaticity with 48.96, 47.96 and 45.94, respectively. In this sense, the chili fruit is characterized by a non-climacteric maturation pattern, which is why many processes are not associated with the synthesis and action of ethylene (Bo and Carpizo, 2015), but it is strongly influenced by postharvest handling conditions (Espinoza-Torres *et al*., 2010), resulting in gradual degradation and the accumulation of secondary compounds (carotenoids) in the pericarp chromoplasts (Vera-Guzmán *et al*., 2011).

Conclusions

Differences were found in the content of capsaicinoids (dihydrocapsaicin, capsaicin and nordihydrocapsaicin) and 52% of the evaluated hybrids exceeded the control. On the other hand, L2XL5 and L2XL3 had the highest content of vitamin C and total carotenoids, respectively; where in the same way L2XL7 and L4Xl7 stood out for their concentration of total soluble solids. Additionally, L7XL8 was the one of greater firmness and purity of color. Manzano hot pepper hybrids presented fruits with outstanding physicochemical characteristics, which could be important from the nutritional point of view, and susceptible to commercial use or genetic improvement.

Acknowledgements

The authors thank the Autonomous University of Chapingo (UACh) for the facilities granted and the National Council for Science and Technology of Mexico (CONACYT) for the financial support for carrying out this research.

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