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RESEARCH

PHYTOCHEMICAL EVALUATION OF WILD AND CULTIVATED PEPPER (*Capsicum annuum* L. and *C. pubescens* Ruiz & Pav.) FROM OAXACA, MEXICO

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Reports of the last decade show that some types of food and spices included in the human diet, such as pepper (*Capsicum annuum* L.) can have a positive effect on human health. The Mexican pepper germplasm is poorly documented with regard to variety and the amount of phytochemical compounds that it contains. In the present study, the variation of phytochemical compounds was evaluated in nine fruit variants (morphotypes) of wild and cultivated pepper grown in Oaxaca. ANOVA detected significant differences among pepper morphotypes and ripeness stages of fruits; vitamin C, total phenols, flavonoids, β-carotene, coordinated chromatic of color, and capsaicinoids. The highest values of vitamin C were found in 'Tabaquero', 'Güero' and 'Costeño' morphotypes (151.6 to 183.2 mg 100 g^{-1}). With regard to total phenols and flavonoids, 'Piquín' and 'Solterito' had the highest levels. Coordinates of color a* and b*, and chroma presented a positive correlation with phenol and flavonoid contents. The evaluated morphotypes differed in capsaicin and dihydrocapsaicin; *C. annuum* had higher capsaicin content (4.9 to 142 μg mL^{-1}) than dihydrocapsaicin (1.5 to 65.5 μg mL^{-1}) and *C. pubescens* Ruiz & Pav. showed the opposite pattern.

Key words: Capsaicionids, *Capsicum annuum*, carotenoids, phenols, flavonoids, vitamin C.

Mexico is the center of origin and diversity of *Capsicum annuum* L., it is mainly found in tropical and subtropical regions which have a high genetic diversity within and among wild botanical varieties; for example in *C. annuum* var. *aviculare*, *C. annuum* L. var. *annuum* and *C. annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill, which also exchange genes with landraces grown in backyards (Eshbaugh, 1980; Hernández-Verdugo *et al*., 2001; Votava *et al*., 2002). *Capsicum pubescens* Ruiz & Pav. and *C. chinense* Jacq. species were added to this historical genetic legacy of peppers that have been part of the Mexican diet for at least a century (Smith and Heiser, 1957; Muñoz and Pinto, 1966; Vela, 2009). According to the findings of Perry and Flannery (2007) at the Guilá Naquitz caves in Oaxaca, this State is one of the centers of origin of *C. annuum*, where archaeobotanical traces date back from 600 to 1521 B.C.

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Every year, in Mexico more than 140 000 ha of fresh pepper are planted. In particular, the state of Oaxaca planted an area which accounts for almost 2200 ha, with an average yield of 5.1 t ha^{-1} (SIAP, 2009). López-López (2007b), in a collection of 116 individual and population-based samples, determined by at least 22 different morphotypes or landraces of regional peppers that were differentiated by fruit shapes, local names, and a high morphological variability of plant traits. In the last decade of pepper documented history in Oaxaca, 'Chile de Agua' (*C. annuum*) landrace has been the main focus of researchers, because of its popularity in the Central Valleys of Oaxaca (López-López, 2007a; Pablo *et al*., 2009; Vásquez *et al*., 2009), followed by 'Paradito' landrace, which has also been widely documented (López-López, 2007b). The genebank of the Regional University Center of the Universidad Autonoma Chapingo has a collection of 304 accessions from Oaxaca (Córdova and Molina, 2006). Nevertheless, little is known about the composition of the fruits; neither the accession, nor the landraces of those which are cultivated and gathered in their native area of Oaxaca.

Carotenoid rich food consumption is directly related to a lower risk of cardiovascular disease and moreover some types of cancer (Pérez-Gálvez *et al.,* 2003). Pepper fruit contains a broad variety of carotenoids, flavonoids, phenols, ascorbic acid, capsaicin, and other components, which determine the great variability of the fruit's smell, flavor, taste and consequently consumer preference. However, the fruit composition changes according to the ripeness stage (Navarro *et al*., 2006; Conforti *et al*., 2007; Deepa *et al*., 2007), and the environmental conditions in which the fruit was grown and in the case of cultivated varieties, the crop management (Medina-Lara *et al*., 2008; Monforte-González *et al*., 2010). Therefore, the purpose of this study was to evaluate the variation of phytochemical composition and the color of nine morphotypes of wild and cultivated peppers from Oaxaca, Mexico.

METHODS AND MATERIALS

Plant material and samples preparation

A sample of nine regional landraces or morphotypes of pepper: 'Chile de Agua', 'Güero', 'Nanchita', 'Piquín', 'Solterito', 'Tusta', 'Costeño', 'Canario', and 'Tabaquero', were collected from backyards and local markets in Valles Centrales, Sierra Sur, and Istmo regions of Oaxaca State, Mexico (Table 1). Morphotypes were determined according to fruit traits and classification proposed by López-López (2007a; 2007b) and Aguilar *et al*. (2010).

Each sample was composed of 250 to 750 g of immature pods completely developed, but not colored (commercial or consumption maturity), defined in local terms as light green for 'Chile de Agua' and 'Güero', greenish yellow for 'Tusta' and 'Güero', dark green for 'Nanchita', 'Piquín', 'Solterito', Tabaquero', and 'Canario'. The definition of unripe (immature, green) and ripe (mature red or yellow) was based on the criteria described by Howard *et al*. (2000) and Conforti *et al*. (2007). A sample corresponded to a lot of immature pods harvested from one plant found in the backyard or a pods lot purchased from just one seller, thereby avoiding a mixture of lots. All samples were matured (pods completely red or yellow) at room temperature during no more than 8 d without losing less than 80% of the pods humidity, according to the results of Krajayklang *et al*. (2000). In the maturation process, not all of the pods of the samples of 'Guero' and 'Tusta' reached the maturity stage (red), and just one part of the pods of 'Tabaquero', 'Solterito', 'Piquin', 'Nanchita' and 'Chile de Agua' reached maturity. On the basis of a limited sample size, the laboratory analyses were focused on the most common stages of consumption for each morphotype. For example, the consumption of immature pods (unripe) of 'Chile de Agua', 'Güero', 'Nanchita', 'Solterito', 'Tusta', and the mature (ripe) and/or immature in 'Tabaquero', 'Piquín', 'Costeño' and 'Canario' (Aguilar *et al*., 2010). The final pod lots analyzed were considered as randomized samples of the landrace diversity of pepper grown in Oaxaca, Mexico.

Color, vitamin C, total phenols and flavonoids

Color in fresh fruits was measured in the mid-part of a random sample of nine pods by a portable Mini Scan photo-colorimeter (model MS/B-200S; Hunter Lab®, Reston, Virginia, USA) using the CIE Lab coordinates L*, a* and b*; whereby L* values indicate brightness or luminosity (0, white to 100, black); a* is defined as the variation from green (-) to red (+); while b* is defined as the variation from blue (-) to yellow (+). With L*, a* and b* coordinates were calculated the chroma $(C^*) = [(a^*)^2 + (b^*)^2]^{1/2}$ and hue angle $(h^o) = \tan^{-1}(b^*/a^*)$ values, according to McGuire (1992).

Vitamin C content in fresh fruits was determined by Durust *et al*. (1997) method. Samples were ground with oxalic acid at 0.4% in a ratio of 1:10 w/v and put in a dark room for 20 min before its centrifugation at 660 rpm. Later, 1 mL of the supernatant was mixed with sodium acetate buffer solution and a 2,6-dichlorophenol indophenol solution. Then, absorbance of the solution was measured by spectrophotometer at a wavelength of 520 nm, and the vitamin C was calculated on the basis of an adjusted calibration curve of L-ascorbic acid standard (99% purity; Reg. 84272 Sigma, St. Louis, Missouri, USA). The estimated concentration of vitamin C was reported as ascorbic acid mg 100 g^{-1} fresh weight.

Total phenolic contents were determined by the Folin-Ciocalteu method proposed by Lin and Tang (2007). A

Table 1. List of plant material used in the phytochemical analysis of fruits.

Sample number	Local name	Location of sample collection in Oaxaca, Mexico	Altitude (m)	Latitude	Longitude
C-01	Solterito or Paradito[1]	Villa de Zaachila	1520	16°56' N	96°45' W
C-02	Nanchita[1]	Villa de Zaachila	1520	16°56' N	96°45' W
C-03	Piquín[1]	Villa de Zaachila	1520	16°56' N	96°45' W
C-04	Chile de Agua[1]	Villa de Zaachila	1520	16°56' N	96°45' W
C-05	Tusta[1]	Miahuatlán de Porfirio Díaz	1600	16°19' N	96°35' W
C-06	Tusta[1]	Miahuatlán de Porfirio Díaz	1600	16°19' N	96°35' W
C-08	Costeño[1]	San Pedro Amuzgos	520	16°39' N	98°05' W
C-09	Costeño[1]	San Isidro Amatitlán, Santa María Zacatepec	320	16°45' N	97°59' W
C-10	Costeño[1]	San Antonio Zaragoza, Santa María Zacatepec	310	16°45' N	97°59' W
C-11	Costeño[1]	Guadalupe, Santa María Zacatepec	1412	16°45' N	97°59' W
C-12	Canario[2]	San Juan Achiutla	2000	17°18' N	97°29' W
C-13	Canario[2]	San Francisco Chindua	2120	17°25' N	97°19' W
C-14	Tabaquero[1]	Linda Vista Montenegro, Santiago Jocotepec	100	17°35' N	95°53' W
C-15	Güero[1]	Miahuatlán de Porfirio Díaz	1600	16°19' N	96°35' W

[1]*Capsicum annuum*; [2]*Capsicum pubescens*.

fresh fruit sample without seeds was sliced and stored at -20 °C for 16 h, before grinding it in a stainless steel blender for 30 s. The extraction used deionized water in a ratio of 1:10, in an ice bath with a stirring mechanism for 15 min. The homogenate was centrifuged at 3640 rpm. In order to measure the phenolic content, 0.1 mL of the supernatant it was mixed with 2.8 mL of deionized water, 2.0 mL of 2% sodium carbonate (Na_2CO_3), and 0.1 mL of Folin-Ciocalteau reagents. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 750 nm on a UV-visible spectrophotometer (UV-1601; Shimadzu®, Kyoto, Japan) and compared with the absorbance of the control of deionized water. Gallic acid (3,4,5-trihydroxybenzoic acid with 97.5% purity; Reg. 2050271 Sigma, St. Louis, Missouri, USA) was chosen as a standard. The calculation of total phenol content was based on the calibration curve of the gallic acid standard and the data was expressed as milligram gallic acid equivalents (GAE) 100 g^{-1} fresh weight.

The total flavonoid content was determined according to the aluminum chloride colorimetric method (Lin and Tang, 2007). From the homogenate, 0.5 mL of supernatant was mixed with 1.5 mL of 95% alcohol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCL_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of deionized water reagents. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm on a UV-visible spectrophotometer (model UV-1601; Shimadzu®, Kyoto, Japan) and compared with the absorbance of deionized water as the control. Flavonoid contents were calculated on the basis of the calibration curve of quercetin standard (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one with 98% purity; Reg. 317313 Sigma, St. Louis, Missouri, USA). Data was expressed as milligrams quercetin equivalents (QE) 100 g^{-1} fresh weight.

β-Carotene and capsaicinoids

β-Carotene was measured according to the method suggested by Davis *et al*. (2003). The β-carotene analysis was carried out only on the samples of ripened pods that had reached maturity before 8 d at room temperature; 'Chile de Agua', 'Canario', 'Costeño', 'Piquin', and 'Tabaquero'. Once the pods had matured, the samples were ground using an extraction mix compound of ethanol, acetone and hexane in a ratio of 1:1:2 v/v. The ground sample was left in an ice bath with a stirring mechanism; distilled water was added and it was left to rest at room temperature, but the sample was protected from the light. An aliquot of the upper phase was taken to prepare a hexane-based dilution. The solution absorbance was measured with a UV/vis spectrophotometer at 446 nm. The quantity of β-carotene in the sample was calculated using its data absorbance and the absorbance reading from the calibration curve of β-carotene standard (β,β-carotene with 97.0% purity, Reg. 18174416; Fluka, Buchs SG, Switzerland). The β-carotene concentration was expressed as mg 100 g^{-1} fresh weight.

Capsaicin and dihydrocapsaicin determination was made by using gas chromatography (GC) (Abraham-Juárez *et al*., 2008). Whole fruits were dried in an oven with an air circulation (2 mL min^{-1} flow rate) of 58 to 60 °C for 2 to 3 d or until a constant weight was reached. The numbers of pepper used for each sample varied from 3 > 20 depending on the peppers and how many were required to produce at least 10 g (air-dried tissue) of sample. The fruits were then ground and placed inside amber plastic flasks to be stored at 20 °C until the analysis was made (Cázares-Sánchez *et al*., 2005). The capsaicinoids were extracted from 20 mg of the ground sample in 1 mL of 95% ethanol and heated at 70 °C for 4 h. The suspended material was centrifuged at 2500 rpm for 15 min, and the supernatant was transferred to a vial. The sample was conserved at -20 °C until further analysis.

Capsaicin and dihydrocapsaicin analysis were performed using a gas chromatograph (model Autosystem XL; Perkin Elmer®, Norwalk, Connecticut, USA) with a capillary column (HP-5MS crosslinked 5% phenyl, 95% dimethylpolysiloxane; ID 0.25 mm, length 30 m, and film thickness 0.25 μm, Agilent, USA). Two-microliter samples of the extracts were injected directly into the GC and then the data was recorded. The column temperature program was as follows: 220 °C for 0 min, 3 °C min^{-1} to 270 °C for 20 min. The carrier gas was helium with a flow rate of 0.80 mL min^{-1}. Injector temperature was held at 260 ºC during the analysis and the detector's temperature (FID) of 300 °C. Capsaicin and dihydrocapsaicin contents were estimated on the basis of the external standard method which was defined with capsaicin (8-methyl-N-vanillyl-*trans*-6-nonenamide of capsicum with 95% purity; Reg. 2816484 Sigma, St. Louis, Missouri, USA) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide of capsicum with 90% purity; Reg. 2815150 Sigma, St. Louis, Missouri, USA) calibration curves. All analyses were made with three replicates.

Statistical analysis

Before making a statistical analysis all of the data that was obtained, it was transformed using $\sqrt{X}$ expression except for the chromatic coordinate a* values and the hue angle, which were transformed by $\sqrt{X+20}$ due to the presence of negative values, and the chroma (C*) percentages were transformed by arcsin $\sqrt{(X/100)}$. For the statistical analysis, the chromatic coordinates, hue angle and chroma were considered as independent transformed variables. Then, an ANOVA was performed on each variable using a lineal model of completely random design (unbalanced number of observations/replicates per sample evaluated) where landraces or morphotypes were considered as a random effect and the ripeness stage as a nested effect within the morphotypes (Steel and Torrie, 1985). A Tukey's multiple comparison test ($p \leq 0.05$)

was carried out when differences were found between the morphotypes and the ripeness stages. In order to determine the relationship between chemical compounds and color coordinates, a Pearson's correlation analysis was made (Student test, $P \leq 0.05$). All data analysis was done using SAS software (SAS Institute, 1999).

RESULTS AND DISCUSSION

Mean squares resulting from ANOVA for all phytochemical variables, color coordinates, and parameters showed significant differences among morphotypes and ripeness stages, except for vitamin C in ripeness stages. A high variability of carotenoids, flavonoids and vitamin C content was found, with variation coefficients of 25.2 to 37.8% (Table 2). The estimated variability suggests that the samples evaluated showed very varied phenotypic expressions where the environmental effects of the place where plants grown and genotypic effects of the morphotypes are confused due to the fact that each sample was not related and was collected in different backyards or purchased with different market sellers.

Table 2. Significance of the mean squares resulting from the variance analysis of phytochemical compounds and color chromatic coordinates in nine pepper morphotypes.

Variables	Mean squares of morphotypes	Mean squares of ripeness stages	CV (%)
Vitamin C	80.87**	12.38^{NS}	26.4
Phenols	30.91**	92.44**	15.4
Flavonoids	23.67**	26.42**	25.2
β-carotene	69.39**		37.8
L*	4.13**	1.43**	10.1
a*	1.51**	38.69**	6.7
b*	4.11**	1.89**	9.8
Hue angle (h°)	1.17**	10.64**	2.4
Chroma (C*)	539.77**	749.83**	14.6
Capsaicin	62.10**		23.2
Dihydrocapsaicin	43.20**		25.3

^{NS} = non significant at p > 0.05; ** = significant at p ≤ 0.01; CV = variation coefficient.

Phytochemical composition and color

Mean values of vitamin C, total phenols, flavonoids, β-carotene, color coordinates and parameters in the nine pepper morphotypes are shown in Table 3. Vitamin C (ascorbic acid concentration) varied greatly from sample to sample and within pepper types, with values ranging from 1.9 to 18.0 in 'Canario' (*C. pubescens*) to 183.2 mg 100 g^{-1} in 'Tabaquero' (*C. annuum*). The ascorbic acid variation presented three general patterns: 'Costeño', 'Guero' and 'Tabaquero' had the highest values, while 'Chile de Agua', 'Nanchita' and 'Solterito' had medium values, and 'Tusta', 'Piquín' and 'Canario' had the lowest values, showing part of the differences in fruit composition among landraces grown in Oaxaca, Mexico. As other studies have shown, the highest or the lowest values of vitamin C in *C. annum* are dependent on the varieties and the maturity stage of the fruits (Khadi *et al*., 1987; Howard *et al*., 2000). The vitamin C content found in this research was within reported ranges in other studies. For example, in the *C. annuum* pepper grown in Turkey, a variation that ranged from 15.2 to 64.9 mg 100 g^{-1} fresh fruit was reported (Topuz and Ozdemir, 2007). And another study conducted in India with the same species showed a variation that ranged from 48.23

Table 3. Comparison of averages within and among pepper morphotypes by ripeness stage, in terms of phytochemical compounds and color chromatic coordinates.

Morphotypes and ripeness stage	Vitamin C	Total phenols	Flavo-noids	Carote-noids	Hue angle	Chroma (C*)	Chromatic coordinates L*	a*	b*
	mg 100 g^{-1}								
Tusta[1]									
Unripe	41.5a[3]	186.4bc	17.9bc		-84.3b	38.6ab	72.1ab	-3.7c	38.4abc
Tabaquero[1]									
Ripe	LS	69.7d	9.5bc	64.4ab	44.4a	56.0a	32.3d	40.0a	39.2abc
Unripe	183.2a	402.2a	26.4bc	ND	-74.0b	42.0ab	39.9cd	-11.5c	40.4abc
Solterito[1]									
Ripe	LS	LS	LS		37.3a	38.7ab	32.1d	30.8ab	23.5c
Unripe	86.7a	243.9abc	63.4ab		-69.5b	8.4b	21.8d	-2.9c	7.8c
Piquín[1]									
Ripe	LS	309.5ab	50.1abc	132.9a	45.0a	54.4a	32.6d	38.5a	38.3bc
Unripe	29.1a	215.6bc	97.4a	ND	-80.8b	41.0ab	49.4bcd	-6.4c	40.3abc
Nanchita[1]									
Ripe	LS	LS	LS		38.8a	43.0ab	31.7c	33.5ab	27.0bc
Unripe	90.4a	198.3bc	34.0bc		-72.3b	45.5ab	51.7abcd	-13.8c	43.4abc
Güero[1]									
Unripe	151.6a	233.0abc	33.3bc		-86.4b	56.2a	74.6a	-3.4c	56.0ab
Costeño[1]									
Ripe	175.4a	350.4a	60.8abc	29.3bc	54.2a	68.6a	47.5cd	37.6a	55.8ab
Unripe	119.2a	175.5bc	14.9bc	ND	-75.4b	49.0a	45.7cd	-12.1c	47.4abc
Canario[2]									
Ripe	18.0a	112.7c	6.4c	3.4c	68.4a	60.9a	52.8abc	22.4b	56.5a
Unripe	1.9a	113.8c	13.1bc	ND	-74.8b	35.4ab	42.1cd	-9.2c	34.1bc
Chile de Agua[1]									
Ripe	LS	LS	LS	15.2c	49.2a	58.3a	37.3cd	38.1a	44.1abc
Unripe	90.1a	180.4bc	44.4ab	ND	-78.8b	56.8a	52.0abcd	-11.1c	55.7abc

LS; limited sample; ND; non detected.
[1]*Capsicum annuum*; [2]*Capsicum pubescens*; [3]Means followed by the same letters are not significantly different (Tukey, p ≤ 0.05).

to 192.63 mg 100 g^{-1} as reported by Deepa *et al*. (2006). Nevertheless, differences were found in vitamin C for *C. pubescens* within this study and the values reported by Cruz-Pérez *et al*. (2007), where vitamin C concentration rates varied from 238.35 to 455.4 mg 100 g^{-1} fresh weight.

Among morphotypes, the total phenol and flavonoid values averaged from 113.2 to 262.9 and 9.7 to 73.7 mg 100 g^{-1} fresh weight, respectively. 'Canario' (*C. pubescens*) presented the lowest values, in phenols as well as in flavonoids, which are the opposite to the values found in 'Piquin', 'Solterito' and 'Costeño', of *C. annuum*. Phenolic values found in this study are within the ranges reported by Marinova *et al*. (2005) and Lin and Tang (2007) for unripe and ripe *C. annuum* fruits (173.2 to 246.7 mg 100 g^{-1} fresh weight). On the contrary, with respect to flavonoids, their values (4.1 to 27.4 mg 100 g^{-1} fresh weight) were lower than the values found in this study for *C. annuum* morphotypes such as 'Chile de Agua', 'Costeño', 'Güero', 'Nanchita', 'Piquin' and 'Solterito' (33.2 to 73.7 mg 100 g^{-1}) (Table 3).

The results show an important difference between *C. pubescens* ('Canario') and *C. annuum* in total phenols and flavonoids but also within *C. annuum* morphotype ones. Nevertheless, the concentrations of flavonoids and phenols depend on cultivation, ripeness, storage and soil salinity, among other factors (Zhang and Hamauzu, 2003; Marinova *et al*., 2005; Navarro *et al*., 2006). In this research, the samples evaluated did not increase the phenol and flavonoid contents from unripe (green) to ripe (red or yellow) stage as was determined by Deepa *et al*. (2007) in *C. annuum,* probably due to there being no control over the maturation process, but the results were similar to those evaluated by Howard *et al*. (2000) in *C. annuum*.

All pigments responsible for the color of pepper fruits have characteristics of chromophores as a result of its conjugated double bonds system in its molecules. The color of the fruits at their ripe stage varied from yellow variants most likely produced by zeaxanthin, β-cryptoxanthin, and β-carotene to red induced by capsanthins and capsorubin depending on the length of the conjugated double bonds system and the presence of different functional groups (Hornero-Méndez and Minguez-Mosquera, 2001). This research measured β-carotene in ripe fruits; the highest values were calculated in 'Costeño', 'Tabaquero', and 'Piquin' (29.3 to 132.9 mg 100 g^{-1} fresh weight) that matured to a red color, and also the results did not show substantial differences between 'Chile de agua' and 'Canario' (yellow) morphotypes (Table 3). Howard *et al*. (2000) found that the highest values of β-carotene in *C. annuum* and *C. frutescens* were determined by its maturity stage (33.7 to 118.7 mg 100 g^{-1} fresh weight) but slightly less than the 'Piquin' type (132.9 mg 100 g^{-1} fresh weight). The data found on β-carotene content provides information on the variation in pigments in pepper landraces grown and consumed in Oaxaca.

Visual color was measured by coordinates CIE Lab L*, a* and b*, and estimators Hue and chroma. The values of these coordinates varied greatly from sample to sample depending on the pepper type. 'Güero' morphotype presented the highest values of L* and b*, as well as a negative value of a*, which is congruent since only unripe fruit (green-yellowish) were analyzed. Cultivated morphotype 'Tusta' presented a similar pattern to the 'Güero' type. These landraces have similar colors except that 'Tusta' has a rough epidermis while 'Güero's' skin is smooth (Table 3).

Regarding ripeness stages within each morphotype of pepper, the content of vitamin C did not present significant differences among and within morphotypes, which seems to indicate a low variability among samples. The results showed that 'Tabaquero' type has a higher phenolic content in the unripe stage (green) than in the ripe stage (red); 'Costeño' landrace showed the opposite pattern, having more phenols in the ripe stage (red and yellow) than in the unripe stage (green). 'Canario' (*C. pubescens*) had the same quantity of phenols in the ripe stage (yellow) than in the unripe stage (green). Flavonoids varied among pepper types, but did not vary according to the different stages of ripeness (Table 3). These results suggest that there are differences in phenols and flavonoids among fruit types, with mixed pattern variations according to ripeness stages and pepper types.

With respect to chromatic coordinates, unripe fruits had negative values in a*, according to the different color intensity of green. 'Tabaquero', 'Nanchita', 'Costeño' and 'Chile de Agua' were greener fruits. On the other hand, redder or yellower ripe fruits (a* positive) were found in 'Tabaquero', 'Solterito', 'Piquín', 'Nanchita', 'Costeño' and 'Chile de Agua' (Table 3). Hue angle was useful to differentiate the unripened and ripe pods by its negative and positive values, respectively. In this work hue angle, chroma and CIE coordinated were useful to explain the differences in the skin color of the fruit among and within samples, and they proportioned complementary information such as was discussed by Kim *et al*. (2008) in *C. annuum*.

Table 4 shows the correlations between vitamin C, phenols, flavonoids, β-carotene and color coordinates and parameters. For instance, b* coordinate and C* parameter showed significant correlations ($p < 0.05$) with every phytochemical compound, regardless of the ripeness stage. L*, b*, C*, and hue angle had a negative correlation with β-carotene, meaning that the higher carotenoid content is related with low brightness and variations in the visual expression of the mature stage of the fruits. Coordinate a* showed a positive and significant correlation (r = 0.42) with flavonoids present in the fruit; that is, the fruit with a tendency towards red presented the higher flavonoid concentration. These relationships confirm part of the results shown in Table 3; flavonoid content increases, in certain pepper types, at ripe more than unripe stages and sometimes phenols follow the same pattern.

Table 4. Pearson correlations (r) among vitamin C, total phenols, flavonoids, and β-carotene, and color chromatic coordinates in wild and cultivated peppers of Oaxaca.

Phytochemical compounds	Hue angle (hº)	Chroma (C*)	Chromatic coordinates L*	a*	b*
Vitamin C	0.07^{NS}	0.38*	-0.09^{NS}	0.22^{NS}	0.30*
Phenols	0.23^{NS}	0.36*	$< 0.01^{NS}$	0.28*	0.27*
Flavonoids	0.34*	0.41**	-0.08^{NS}	0.42**	0.28*
β-carotene	-0.38*	-0.38*	-0.66**	0.20^{NS}	-0.41**

^{NS}: non significant at $p > 0.05$; *: significant at $p < 0.05$; **: significant at $p < 0.01$ (Student test).

Capsaicinoids

Pungency or the hot taste of pepper fruits is attributed mainly to capsaicinoid concentration, which adds flavor to food when used as spices. These compounds are recognized for their therapeutic effects on gastric ulcers and rheumatoid arthritis (Matucci-Cerinic *et al*., 1990; Sathyanarayana, 2006). Capsaicinoids identified in *Capsicum* fruits are vanillylamides of branched fatty acids, with 9 to 11 carbons, of which capsaicin (vanillylamide of 8-methylnontrans-6-enoic acid) and dihydrocapsaicin (vanillylamide of 8 mehylnonanoic acid) are the most abundant capsaicinoids (Topuz and Ozdemir, 2007). In this study, capsaicin concentration (CAP) was higher than dihydrocapsaicin (DH) in all morphotypes, with the exception of 'Canario' (*C. pubescens*), and it suggests a difference between *C. annumm* and *C. pubescens* (Table 5). However, the results show significant differences within *C. annuum* types; for example, type 'Chile de Agua' and 'Tabaquero' showed values of 4.9 and 6.7 µg mL^{-1} contrasting with 'Piquin' and 'Solterito' that presented value of 116.2 to 142.0 µg mL^{-1} in capsaicin content. The same pattern was observed in dihydrocapsaicin.

The estimated pattern in CAP:DH ratio to *C. annuum* morphotypes was similar (except for the values) to the pattern determined by Cázares-Sánchez *et al*. (2005) and Morán-Bañuelos *et al*. (2008) in the same species, although in this work there were 1.9 to 6.5 units of capsaicin per every unit of dihydrocapsaicin. 'Piquin' and 'Solterito' showed the significantly highest capsaicin and dihydrocapsaicin values among the morphotypes and they can be considered the hottest peppers, according to consumer opinion; followed by 'Tusta', 'Canario' and 'Nanchita' (Table 5). Results indicate that pepper morphotypes may differ in their content of capsaicinoids and dihydrocapsaicinoids, which are responsible for the typical hot taste, sometimes preferred by consumers. That is why these peppers are still preserved in local communities, arable lands and adjacent areas where wild peppers like 'Piquin', 'Tabaquero', 'Nanchita' and 'Solterito' grow without any human intervention.

Table 5. Averages and standard deviation of capsaicin and dihydrocapsaicin in ripe fruits of nine pepper morphotypes.

Morphotypes	Capsaicin (CAP)	Dihydrocapsaicin (DH)	CAP:DH
	µg mL^{-1}		
Chile de Agua[1]	4.9 d[4] (± 1.5)	1.6e (± 0.7)	3.1:1.0
Canario[2]	15.2cd (± 6.0)	33.0b (± 9.9)	1.0:2.2
Costeño[1]	14.6cd (± 12.6)	4.0de (± 3.4)	3.6:1.0
Güero[1, 3]	44.5b (± 9.2)	6.8de (± 0.9)	6.5:1.0
Nanchita[1]	27.4bc (± 7.7)	13.2cd (± 4.7)	2.1:1.0
Piquín[1]	116.2a (± 24.5)	62.4a (± 18.3)	1.9:1.0
Solterito[1]	142.0a (± 2.9)	65.5a (± 3.7)	2.2:1.0
Tabaquero[1]	6.7d (± 1.8)	1.5e (± 0.3)	4.5:1.0
Tusta[1, 3]	51.4b (± 24.5)	33.5bc (± 15.1)	1.5:1.0

[1]*Capsicum annuum*; [2]*Capsicum pubescens*; [3]It was only measured in unripe stage.
[4]Means followed by the same letters are not significantly different (Tukey, $p < 0.05$); ± standard deviation.

CONCLUSIONS

Different fruit composition of the nine pepper morphotypes indicates that apart from the evident morphological differences in terms of fruit shape and appearance, they also differ in their content of vitamin C, phenols, flavonoids, β-carotene, chromatic coordinates coloring CIE, hue angle, capsaicin and dihydrocapsaicin, depending on the ripeness stage. The highest values of vitamin C are found in 'Tabaquero', 'Güero' and 'Costeño' morphotypes and the highest values of phenols and flavonoids were found in 'Piquin' and 'Solterito' morphotypes. β-Carotene ranged from 3.4 to 132.9 mg 100 g^{-1} in ripe fresh fruit samples. Coordinates a* and b* and chroma had a positive correlation with the content of phenols and flavonoids; whereas β-carotene showed a negative correlation with L*, b*, hue angle and chroma. Pepper morphotypes analyzed in this study presented significant differences in capsaicinoid and dihydrocapsaicinoid content. *Capsicum annuum* was higher in capsaicin than in dihydrocapsaicin; while *C. pubescens* showed the opposite pattern.

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Evaluación fitoquímica en chile (*Capsicum annuum* L. and *C. pubescens* Ruiz & Pav.) silvestre y cultivado en Oaxaca, México. En la última década, se reportó que el consumo de ciertos alimentos y especias, como el chile (*Capsicum annuum* L.), pueden tener un efecto positivo en la salud. Particularmente, los acervos genéticos mexicanos de chile están poco documentados en relación a la diversidad desde la perspectiva fitoquímica. En este trabajo se evaluó la variación de compuestos fitoquímicos en nueve morfotipos de chile silvestres y cultivados de Oaxaca. El ANDEVA detectó diferencias significativas entre morfotipos y estados de madurez en vitamina C, fenoles, flavonoides, β-caroteno, color, y capsaicinoides. Los valores más altos de vitamina C se determinaron en 'Tabaquero', 'Güero' y 'Costeño' (151.6 a 183.2 mg 100 g^{-1}). En fenoles y flavonoides sobresalieron los tipos 'Piquín' y 'Solterito'. Las coordenadas cromáticas a* y b*, y los tonos (C*) se correlacionaron positivamente con el contenido de fenoles totales y flavonoides.

Los morfotipos evaluados difieren en el contenido de capsaicina y dihidrocapsaicina, en *C. annuum* fue mayor la cantidad de capsaicina (4.9 a 142 μg mL^{-1}) que de dihidrocapsaicina (1.5 a 65.5 μg mL^{-1}) y en *C. pubescens* Ruiz & Pav. el patrón fue inverso.

Palabras clave: capsaicionoides, *Capsicum annuum*, carotenoides, fenoles, flavonoides, vitamina C.

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