Health-promoting Carotenoids and Phenolics in 31 *Capsicum* Accessions

Ivette Guzman, Krystal Vargas, Francisco Chacon, Calen McKenzie, and Paul W. Bosland

Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003

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Abstract. This study investigated the diversity of carotenoids and phenolics in germplasm from three *Capsicum* (chile pepper) species, *Capsicum annuum*, *Capsicum baccatum*, and *Capsicum chinense*. Lutein, a yellow-pigmented carotenoid, and phenolics, a group of secondary metabolites, are reported to have health-promoting properties. The germplasm studied matured to a yellow color. The hypothesis was that all yellow fruits would contain either the carotenoid lutein, a yellow pigment, or a large amount of phenolics, a group of secondary metabolites that may be yellow among other colors. Thirty-one *Capsicum* accessions were grown in the field over a period of two seasons. On a dry weight (DW) basis, lutein ranged from 0.14 to 94.2 $\mu\text{g}\cdot\text{g}^{-1}$, and total phenolics ranged from 5.79 to 15.01 $\text{mg}\cdot\text{g}^{-1}$. No lutein was detected in one accession and β -carotene, another health-promoting compound, was lacking in four accessions. Accessions were grouped into four groups according to a principal component analysis plot. Results from this study indicate that in only nine accessions, lutein represented at least 50% of the total carotenoid amounts in each accession. These accessions are desirable not only as a source of dietary lutein, a natural yellow pigment, but also as genetic material that can be used to breed for higher lutein *Capsicum*. Therefore, yellow color is not a good indicator of lutein content and phytochemical analysis is required to determine the content of health-promoting compounds.

In *Capsicum*, 39 species have been identified including five domesticated species, *C. annuum*, *C. baccatum*, *C. chinense*, *Capsicum frutescens*, and *Capsicum pubescens* (Carrizo García et al., 2016). Chile peppers are an important culinary spice. Historically, the cultural value of chile peppers includes medicinal and culinary uses (Cichewicz and Thorpe, 1996; Guzman et al., 2011); however, due to the rich color palette of the fruits, they are potential sources of natural colorants with dietary health-promoting properties and thus have been gaining more attention as a source of pigments for foods and textiles due to their biological activity (Arimboor et al., 2015; Ksibi et al., 2015).

As chile pepper fruits mature, they become yellow, orange, red, or brown (Guzman

et al., 2011; Kim et al., 2016a). One of the high-value qualities that *Capsicum* is bred for is color which is an accumulation of pigments determined by genetics and the environment (Guzman et al., 2010; Kim et al., 2016a; Stommel and Griesbach, 2008). Fruit pigmentation involves several groups of compounds including lipid-soluble carotenoids and water-soluble phenolics (Guzman et al., 2011; Rodriguez-Amaya, 2019).

The importance of natural pigments is highlighted by the possible negative linkage between synthetic pigments and human behavior issues (Kanarek, 2011; McCann et al., 2007). *Capsicum* fruits produce a red-colored oleoresin that is a good source of red pigments used in human food, animal food, and cosmetics (Arimboor et al., 2015); however, yellow *Capsicum* pigments are also valuable pigments in that they play important roles in human health (Bernstein et al., 2016). *Capsicum* may contain up to 10.76 $\text{mg}\cdot\text{g}^{-1}$ DW of total carotenoids (Guzman et al., 2010; Kim et al., 2016a). Carotenoids are shades of yellow, orange, and red, and include nutritionally important compounds like β -carotene, which is a vitamin A source. Lutein is among the few plant phytochemicals that can be absorbed by the human digestive tract, as well as passed on from a nursing mother's breast milk to her infant (Lipkie et al., 2015). Once lutein enters the human blood stream, it reaches the retina, where it becomes the macular pigment, protecting the eyes from oxidative blue light and reducing cataract formation (Bernstein et al., 2016).

In addition to carotenoids, *Capsicum* fruits contain phenolic compounds that also

contribute to color and health-promoting antioxidant properties (Materska and Perucka, 2005; Taofiq et al., 2017). Plant phenolic compounds are a group of water-soluble metabolites that help plants adapt to abiotic and biotic stresses (Taofiq et al., 2017). Included in the diverse phenolic group are multiple categories of plant compounds consisting of simple and complex phenolic compounds (phenolic acids, coumarins, flavonoids, stilbenes, and tannins) (Minatel et al., 2017; Waterhouse, 2002). Due to their unique structures, they serve as strong antioxidants, and have been shown to have antidiabetic properties (Kim et al., 2016b). Their health-promoting and chronic disease prevention properties make them ideal for nutraceutical and functional foods (Brown et al., 2014; Guzman et al., 2015; Yousef et al., 2014a, 2014b, 2016). Total phenolics for *Capsicum* range from 0.2 to 11.03 $\text{mg}\cdot\text{g}^{-1}$ gallic acid equivalents in dry tissue (Carvalho et al., 2015; de Aguiar et al., 2016; Joshi et al., 2017). Major phenolics found in *Capsicum* fruits were identified as derivatives of quercetin, ferulic acid, sinapic acid, apigenin, and luteolin (Materska and Perucka, 2005).

Due to genetic variability, there is a considerable difference in carotenoid and phenolic amounts across chile pepper cultivars (Carvalho et al., 2015; Guzman et al., 2010; Joshi et al., 2017; Materska and Perucka, 2005). In a previous study, the β -carotene concentrations in orange pepper fruits ranged from 0 to 1.24 $\text{mg}\cdot\text{g}^{-1}$ DW according to genotype (Guzman et al., 2010). The important implication of that research is that not all orange fruits contain β -carotene, a precursor to vitamin A. Consequently, the need exists for more chemical analysis profiling colored chile peppers with the potential to have high amounts of health-promoting nutraceuticals like lutein and phenolics. The hypothesis of this research was that health-promoting carotenoids and phenolic compounds are in equal amounts in yellow *C. annuum*, *C. baccatum*, and *C. chinense* fruits. The goal was to identify *Capsicum* genotypes that can be used by food scientists or by breeders attempting to increase nutritional value in *Capsicum* germplasm.

Materials and Methods

Plant material. Thirty-one *Capsicum* accessions from *C. annuum*, *C. baccatum*, and *C. chinense* with yellow maturing fruit, and one yellow *Solanum melongena* (eggplant) (Table 1) were sown in a planting medium (Sunshine Redi-Earth Plug & Seedling Mix; Sun Gro Horticulture, Bellevue, WA) at the New Mexico State University (NMSU) Chile Pepper Institute greenhouse maintained at 27 °C day/18 °C night $\pm$ 3 °C (Las Cruces, NM) using previously published conditions (Bosland and Walker, 2014). Accessions were selected if their fruit color phenotype was yellow, although color intensity, hue, and chroma were not measured. After 8 weeks, seedlings were transplanted in Glendale loam soil at the NMSU Leyendecker

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I.G. is the corresponding author. E-mail: ivguzman@nmsu.edu.

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Table 1. List of 31 *Capsicum* accessions with carotenoid and total phenolic (as gallic acid equivalents) means from 2 years, fruits from five plants each year, in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW) and $\text{mg}\cdot\text{g}^{-1}$ (DW) $\pm$ SD.

Genus species	Sample no. and accession identifier	$\mu\text{g}\cdot\text{g}^{-1}$ DW $\pm$ SD			$\text{mg}\cdot\text{g}^{-1}$ DW $\pm$ SD	
		β -carotene	Lutein	Total carotenoids	Total phenolics	% lutein
<i>Capsicum annuum</i>	1 12C-162	1.52 $\pm$ 0.27	3.94 $\pm$ 0.59	11.87 $\pm$ 0.73	12.77 $\pm$ 1.17	33.23
	2 15C-388	0.14 $\pm$ 0.01	18.86 $\pm$ 0.86	37.19 $\pm$ 1.43	12.40 $\pm$ 0.65	50.70
	3 16C-353	0.08 $\pm$ 0.03	0.14 $\pm$ 0.03	17.47 $\pm$ 0.97	9.69 $\pm$ 0.36	0.78
	4 16C-358	0.07 $\pm$ 0.03	14.72 $\pm$ 1.81	29.79 $\pm$ 2.95	15.01 $\pm$ 1.16	49.40
	5 16C-359	0.10 $\pm$ 0.04	17.96 $\pm$ 1.26	37.28 $\pm$ 5.22	12.35 $\pm$ 1.00	48.17
	6 16c-460	0.44 $\pm$ 0.07	11.35 $\pm$ 0.24	27.63 $\pm$ 6.94	14.10 $\pm$ 1.18	41.10
	7 16c-469	0.34 $\pm$ 0.08	8.03 $\pm$ 1.71	14.17 $\pm$ 4.14	11.34 $\pm$ 0.41	56.69
	8 16c-470	1.26 $\pm$ 0.08	10.40 $\pm$ 1.27	25.01 $\pm$ 2.05	12.61 $\pm$ 1.42	41.59
	9 16c-472	1.08 $\pm$ 0.66	12.87 $\pm$ 1.86	27.80 $\pm$ 4.26	12.67 $\pm$ 1.14	46.32
	10 16c-479	18.72 $\pm$ 1.15	84.98 $\pm$ 8.83	499.04 $\pm$ 48.28	14.32 $\pm$ 0.90	17.03
	11 Golden Cayenne	ND ²	3.82 $\pm$ 0.14	6.47 $\pm$ 0.31	12.78 $\pm$ 1.82	59.04
	12 Mama Mia Giallo	38.75 $\pm$ 8.53	54.23 $\pm$ 10.78	485.69 $\pm$ 11.00	13.14 $\pm$ 0.95	11.17
	13 NuMex Lemon Spice	0.12 $\pm$ 0.03	15.12 $\pm$ 0.57	30.13 $\pm$ 9.26	8.58 $\pm$ 0.37	50.18
	14 NuMex Pumpkin Spice	8.46 $\pm$ 1.89	52.06 $\pm$ 4.16	461.12 $\pm$ 42.63	9.78 $\pm$ 0.46	11.29
	15 NuMex Sunglo	0.16 $\pm$ 0.06	12.14 $\pm$ 1.31	27.42 $\pm$ 9.55	10.15 $\pm$ 0.83	44.27
	16 NuMex Sunrise	0.12 $\pm$ 0.07	9.13 $\pm$ 0.46	14.17 $\pm$ 1.56	11.21 $\pm$ 0.67	64.41
	17 Yellow Chiltepin	14.15 $\pm$ 1.18	9.12 $\pm$ 1.13	149.48 $\pm$ 1.75	5.79 $\pm$ 0.29	6.10
	18 Yellow Hot	1.46 $\pm$ 0.0	6.42 $\pm$ 1.00	12.39 $\pm$ 1.20	14.11 $\pm$ 1.70	51.82
<i>Capsicum chinense</i>	19 Brazilian Yellow	ND	0.74 $\pm$ 0.10	13.08 $\pm$ 1.28	12.69 $\pm$ 1.69	5.68
	20 Burkina Yellow	60.91 $\pm$ 4.71	55.88 $\pm$ 12.50	604.78 $\pm$ 94.88	12.62 $\pm$ 0.72	9.24
	21 Fatali	39.30 $\pm$ 16.1	49.93 $\pm$ 6.58	382.92 $\pm$ 107.39	13.50 $\pm$ 1.55	13.04
	22 Jamaican Hot Yellow	26.45 $\pm$ 6.07	94.20 $\pm$ 9.14	437.97 $\pm$ 33.25	12.15 $\pm$ 0.83	21.51
	23 Peruvian Yellow	ND	2.63 $\pm$ 0.11	4.70 $\pm$ 0.87	8.69 $\pm$ 0.67	56.03
	24 Scotch Bonnet	49.55 $\pm$ 6.35	58.10 $\pm$ 4.61	476.72 $\pm$ 52.90	9.83 $\pm$ 0.39	12.19
	25 Trinidad Perfume	6.42 $\pm$ 1.44	16.98 $\pm$ 1.11	184.21 $\pm$ 31.90	8.77 $\pm$ 0.56	9.22
	26 Wild Chinense	0.21 $\pm$ 0.04	13.41 $\pm$ 0.60	20.23 $\pm$ 0.93	13.35 $\pm$ 0.63	66.30
	27 Yellow Berk	70.01 $\pm$ 15.4	29.45 $\pm$ 3.89	539.22 $\pm$ 98.59	10.29 $\pm$ 0.42	5.46
	28 Yellow Habanero	70.62 $\pm$ 6.27	58.79 $\pm$ 8.75	767.20 $\pm$ 48.46	11.48 $\pm$ 0.91	7.66
<i>Capsicum baccatum</i>	29 Aji Limon	ND	8.14 $\pm$ 0.87	12.31 $\pm$ 2.44	6.25 $\pm$ 6.18	66.12
	30 Aji Norte	10.91 $\pm$ 1.69	26.11 $\pm$ 4.86	230.73 $\pm$ 63.92	7.56 $\pm$ 0.60	11.32
	31 Aji Pucomucho	28.67 $\pm$ 1.76	55.60 $\pm$ 5.32	437.68 $\pm$ 25.41	13.63 $\pm$ 0.22	12.70
<i>Solanum melongena</i>	32 Thai Yellow Egg	0.62 $\pm$ 0.00	ND	19.38 $\pm$ 1.20	17.96 $\pm$ 0.79	0.00

²ND, not detected by high-performance liquid chromatography method.

Plant Science Center and Fabian Garcia Science Center in Las Cruces, NM. Plants were transplanted in 1-m-wide rows, 30 cm apart, and rows were furrow irrigated. For both years, ripe yellow fruits (ready-to-eat) were harvested in August from healthy, established plants and transported to the laboratory for processing. Five fruits from each replicate were harvested in both years and three plants represented three replicates. Fruits were deseeded, cut, and weighed into pieces under yellow or dim light to prevent oxidation caused by blue light wavelengths. The pericarp tissue was flash frozen in liquid nitrogen, lyophilized, and ground to a fine powder using a coffee bean grinder.

Carotenoid analysis. Under yellow lights, 1 g of finely ground lyophilized tissue was extracted using a method modified from Guzman et al. (2010). Initially, 15 mL petroleum ether:acetone (1:1 v:v) (Sigma-Aldrich Corp., St. Louis, MO) was added followed by sonication for 20 min in a sonicating bath. The sample was centrifuged at 2900 rpm for 10 min at 10 °C. The same procedure was repeated two additional times with 15 mL hexane (Sigma-Aldrich Corp.) as the extraction solvent. All supernatants were combined in an amber glass vial and dried down under a stream of nitrogen gas. The residue was then saponified to de-esterify carotenoids using a previously published method (Richins et al., 2014). After saponification, dried extracts were resuspended in 500 μL of spectrophotometry-grade isopropanol (Sigma-Aldrich Corp.). The

samples were centrifuged to remove any debris and the supernatant was transferred to a high-performance liquid chromatography (HPLC) amber glass vial.

The samples were analyzed using a Waters HPLC system (Waters Corporation, Milford, MA) equipped with a Waters 600 controller and an In-Line Degasser AF, and a 4.6 $\times$ 250-mm, 5- μm , YMC C30 Carotenoid Column (YMC America, Allentown, PA). The solvent system, adapted from Richins et al. (2014), was a gradient method of two solvents, solvent A, methanol:tert-butyl methyl ether (MTBE):water (81:15:4), and solvent B, methanol:MTBE:water (8:88:4). The gradient included two linear phases (0% to 67% B from 0 to 30 min, then 67% to 90% B from 30–40 min). Flow rate was set at 3.0 mL/min, column temperature was 25 °C, and injection volume was 20 μL . A Waters 996 Photodiode Array Detector (PDA) collected spectral absorbance data from 230 nm to 700 nm and chromatographic peaks for each sample were detected at 450 nm using Waters Empower 2 software (Waters Corporation). Peaks were quantified using retention times and visible light spectra of lutein and β -carotene standards (Sigma-Aldrich Corp.). Serial dilutions of lutein and β -carotene were prepared with 50:50 hexane and isopropanol. Unknown peaks were identified as a carotenoid if its spectrum between 230 nm and 700 nm resembled that of a carotenoid per Britton et al. (2009). If the peak's spectrum resembled a carotenoid spectrum, the peak

was labeled a carotenoid, and total carotenoids were quantified using the β -carotene standard curve (Guzman et al., 2010).

Phenolics analysis. Total phenolics were extracted from the lyophilized powder and the method was adapted from Singleton et al. (1999). Approximately 0.5 g of each sample was extracted with 8 mL of the 2% acetic acid in 80% aqueous methanol. The sample was sonicated for 10 min at 45 °C. After sonication, the sample was centrifuged at 2900 rpm for 10 min at 4 °C. The supernatant was transferred to a 25-mL volumetric flask. The same pellet was re-extracted with 8 mL of solvent two more times, for a total of three pellet extractions per sample. The final extraction volume in the 25-mL volumetric flask was made up to 25 mL using the extraction solvent. The extract was stored at –20 °C until further use. The Folin-Ciocalteu assay was used in a 96-well plate (Singleton et al., 1999). Extracts were read against the phenolic gallic acid standard (Sigma-Aldrich) using a Molecular Devices Spectra-Max M2 spectrophotometer (Molecular Devices, LLC, San Jose, CA) set at 765 nm.

Statistical analysis. Means for each sample and analytical standard calibration curves were prepared using Microsoft Excel (Microsoft Corporation, Redmond, WA). Concentrations were plotted against peak areas to obtain a linear regression equation with an R-value higher than 0.95 per Richins et al. (2014). Concentrations for each sample were calculated based on the standard curves.

Fig. 1. Images of *Capsicum* spp. fruits and an eggplant, *Solanum melongena*, that ripen to a final yellow color: (A) 'NuMex Sunrise', a *Capsicum annuum*, (B) 'Aji Puchomucho', a *Capsicum baccatum*, (C) 'Thai Yellow Egg', *S. melongena*, (D) 'Fatali', *Capsicum chinense*, (E) 'Wild Chiltepin', *C. annuum*, and (F) a basket of genetically distinct ripe yellow *Capsicum* fruits with diverse pod types.

Accession means and standard deviations were calculated using Microsoft Excel. Hierarchical clustering and principal component analysis (PCA) of the accessions based on all the metabolite means were generated using JMP Statistical (JMP 14.3.0, Medmenham, Marlow, Buckinghamshire, UK).

Results and Discussion

All 31 *Capsicum* accessions from three species (*C. annuum*, *C. baccatum*, and *C. chinense*) and the eggplant differed in phenotypic pod shape and all were yellow in their final ripening stage (Fig. 1). In spite of their shared color, their nutraceutical pigment content differed considerably when measured using HPLC, and this observation is in agreement with previous results published on *Capsicum* (Guzman et al., 2010). Figure 2 reveals the variation in HPLC carotenoid chromatograms of two *Capsicum* accessions. 'Thai

Fig. 2. High-performance liquid chromatography chromatograms of (A) two carotenoid standards, lutein and β -carotene with 11 and 25 min as retention times, respectively, (B) 'Wild Chinense', *Capsicum chinense*, with 66% of total carotenoids as lutein, and (C) 'Yellow Chiltepin', *Capsicum annuum*, with only 6.10% of total carotenoids as lutein.

Fig. 3. Lutein content ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) by high-performance liquid chromatography in each *Capsicum* accession. Data represent the mean from two independent experiments $\pm$ SD from 2 years with at least three plants per year.

Yellow Egg' (accession 32) contained $19.38 \mu\text{g}\cdot\text{g}^{-1}$ of total carotenoids on a DW basis (Table 1); however, the *Capsicum* accessions ranged from 4.70 to $767.20 \mu\text{g}\cdot\text{g}^{-1}$ DW in total carotenoid concentration. 'Jamaican Hot Yellow', a *C. chinense* accession (accession 22), contained the lowest concentration

of total carotenoids, whereas another *C. chinense* accession, 'Yellow Habanero' (accession 28), contained the highest concentration of total carotenoids across all accessions (Table 1). Lutein concentrations in *Capsicum* ranged from 0.14 to $94.2 \mu\text{g}\cdot\text{g}^{-1}$ DW in 16C353 (accession 3) and 'Jamaican Hot

Yellow' (accession 22), respectively. The concentrations of β -carotene also varied from below detection for several *Capsicum* accessions (accessions 11, 19, 23, and 29) to $70.62 \mu\text{g}\cdot\text{g}^{-1}$ DW in 'Yellow Habanero' (accession 28), respectively.

Lutein, a yellow carotenoid, was variable among all ripe yellow accessions, therefore a mixture of other carotenoids and phenolics contributed to the overall color. Moreover, there was high variability between the concentrations of β -carotene relative to lutein per accession (Table 1). Only four *Capsicum* accessions and the eggplant contained higher concentrations of β -carotene than lutein of total carotenoids. These included 'Yellow Chiltepin', 'Burkina Yellow', 'Yellow Berk', and 'Yellow Habanero'. The other 27 accessions contained higher concentrations of lutein than β -carotene (Table 1).

Figure 3 illustrates concentrations of lutein per accession and the percentage of total carotenoids expressed as lutein. For nine accessions, namely 15C388 (accession 2), 16C469 (accession 7), 'Golden Cayenne' (accession 11), 'NuMex Lemon Spice' (accession 13), 'NuMex Sunrise' (accession 16), 'Yellow Hot' (accession 18), 'Peruvian Yellow' (accession 23), 'Wild Chinense' (accession 26),

Fig. 4. Clustering, multivariate principal component analysis (PCA) plot, of sample observations for (A) total phenolics as gallic acid equivalents, total carotenoids, lutein, and β -carotene, and (B) total carotenoids, lutein, and β -carotene.

and 'Aji Limon' (accession 29), lutein comprised 50% or more of their total carotenoid content. 'Wild Chinense' (accession 26) contained the highest percent of lutein, 66.30% of total carotenoids.

Total phenolics measured in the 31 *Capsicum* accessions ranged from 5.79 to 15.01 mg.g⁻¹ (Table 1). 'Yellow Chiltepin' (accession 17) contained the lowest concentration of total phenolics, whereas 16C358 (accession 4) contained the highest concentration. By comparison, 'Thai Yellow Egg' (accession 32) contained the highest total phenolic concentration of all samples at 17.96 mg.g⁻¹, higher than that previously reported (Carvalho et al., 2015; de Aguiar et al., 2016; Joshi et al., 2017).

The hierarchical clustering and PCA plots indicated that there were four groups of accessions that shared similarities in total phenolics, total carotenoids, lutein, and β -carotene concentrations (Fig. 4A). Four groups also resulted based on similarities of total carotenoid concentrations, lutein and β -carotene concentration (Fig. 4B). Moreover, the accessions did not cluster based on species. In addition, as seen in the PCA results, yellow color is not a good indicator of lutein content given that the PCA summary plots indicated four unique groups and all quantified pigment variables had a positive influence on principal component one. Both PCA and cluster analysis showed that chemical profiles of the 31 accessions are independent of plant species.

The importance of understanding pigments in *Capsicum* fruits is useful when determining the nutraceutical value of an accession. Total carotenoids, lutein, β -carotene, and total phenolics are involved in overall color phenotype. Even though they are all beneficial to human health, their human health properties are different from one another. Lutein is not only a carotenoid with strong antioxidant properties but it can enter the bloodstream and is crucial as the human macular pigment that protects human eyes from age-related macular degeneration (Bernstein et al., 2016). The results of the 31 accessions proves that not all pigments with health benefits are accumulated in the same concentrations in *Capsicum* fruits that are all the same color.

Conclusion

In a previous study profiling *Capsicum* accessions, total carotenoid concentrations were as high as 3733.00 $\mu\text{g.g}^{-1}$ DW and the lutein concentrations ranged from not detected to 70.18 $\mu\text{g.g}^{-1}$ DW (Hervert-Hernandez et al., 2010). In this study, the accessions from the three species (*C. annuum*, *C. baccatum*, and *C. chinense*) that contained the highest total carotenoid concentration were the accessions with the highest lutein amount. However, the novelty of this study is that the percent lutein of total carotenoid concentrations varied from one accession to the other. Accessions of interest to breeders and growers could be those containing high percentages of lutein per

total carotenoids. These accessions have genetics that drive the carotenoid pathway to producing mostly lutein instead of other carotenoids. In conclusion, the analysis of 31 *Capsicum* accessions indicates that *Capsicum* has the potential to be a valuable source of lutein for dietary uses.

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