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# **Bioactive compounds in 28 native tomato accessions from southeast Mexico**

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# **Summary**

The diverse populations of native tomato (Solanum lycopersicum L.) in Mexico have great potential for breeding and nutraceutical benefits, but information on their secondary metabolites is scarce. Here we quantified bioactive compounds with nutraceutical potential in 28 native tomato accessions across southeastern Mexico. Plants were grown from seeds, transplanted to fields in a completely randomized block design with three replications of each accession and assessed for antioxidant activity, phenolic compounds, flavonoids, β-carotene, lycopene, and other carotenoids. The results revealed significant differences in these variables among accessions. accessions Y129 (Bcarotene and lycopene), Y115, T107, and C108 (antioxidant activity), Y123 (flavonoids), and Y119 (carotenoids) had especially substantial nutraceutical value. These accessions could be incorporated into a breeding program to develop new tomato varieties with enhanced nutraceutical quality to improve health, especially in rural areas where these accessions are now grown and consumed, and are important sources of genetic diversity worth conserving.

**Keywords:** antioxidants, chemoprotection, landraces, new varieties, *Solanum lycopersicum* 

#### Introduction

Mexico is considered the center of tomato (Solanum lycopersicum L.) domestication. As expected, a great diversity of cultivated, wild, or partially domesticated native populations can be found in different agricultural regions of the country (BONILLA-BARRIENTO et al., 2014). A wide diversity of fruit size, shape, and color showcases the considerable genetic variability among tomato germplasms. Despite the importance of characterizing this genetic variation, around 80% of the global collections of these genetic resources have not been characterized (PÉREZ-DÍAZ, 2020; MARÍN-MONTES et al., 2016), including in central and southeastern Mexico, where tomatoes are cultivated in traditional agroecosystems in small plots and home gardens, contributing to the in situ conservation of agrobiodiversity. These traditional varieties are highly valued in the region for their organoleptic quality (MALDONADO-PERALTA et al., 2016), but their morphological, phenotypic, and nutraceutical properties have not been characterized. Since these varieties are only consumed locally or regionally, they are at risk of disappearing, leading to the loss of species richness and important germplasm resources (PÉREZ-DÍAZ, 2020).

On the nutraceutical side, tomatoes are thought to help reduce certain chronic degenerative diseases due to their high content of potent antioxidants, including polyphenols, flavonoids, carotenoids, anthocyanins,  $\beta$ -carotene, lycopene, and vitamin C (LAHOZ et al., 2016; RAMÍREZ-FLORES et al., 2020). For instance, lycopene helps to reduce the risk of Alzheimer's mortality in adults and is effective in treating Parkinson's disease and other neurological disorders by protecting cells against oxidative stress (HA et al., 2021).

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In this context, the phenotypic, agronomic, and molecular characterization of native tomatoes from various locations in Mexico have been studied, and although the following works have been carried out (PERALTA and SPOONER, 2005; ÁLVAREZ-HERNÁNDEZ et al., 2009; CSAMBALIK et al., 2016; FIGUEROA-CARES et al., 2018; SZYMAŃSKI et al., 2020; RAMÍREZ-OJEDA et al., 2022; SUMALAN et al., 2022) information on functional or nutraceutical potential is still limited. Therefore, addressing the conservation issues outlined in Mexico's National Agenda for Research, Innovation, and Technological Transfer, we quantified bioactive compounds with nutraceutical potential in 28 native tomato accessions from different regions of southeastern Mexico concerning. The information generated will inform strategies to rescue these accessions.

### Materials and methods

## Seed collection

Seeds were obtained from May to June 2018 directly from producers or at local markets or seed fairs (Tab. 1, Fig. 1). The seeds came from 28 native tomato (*Solanum lycopersicum* L.) accessions: four from the state of Chiapas (tropical, warm, humid, and rainy with mean annual temperature (MAT) of 27 °C), two from Tabasco (warm and humid with abundant summer rains and MAT of 26.4 °C), one from Campeche (warm and subhumid, with summer rains and annual temperature of 26–27 °C), and 21 from Yucatan (hot and humid "Awo" type climate, warm and subhumid with summer rains, average temperature of 26.61 °C). The accessions were identified using the names given by the producers.

# Establishment and cultivation management in the field

All accessions were grown in an experimental area within the municipality of Conkal, Yucatán (21° 04'46" N, 89° 29'52" W, 10 m above sea level). The overall climate is hot and humid, with average maximum temperatures between 30 and 35 °C. The irradiation in the area fluctuates between 6.19 and 3.61 kWh<sup>-1</sup> m<sup>-2</sup> day<sup>-1</sup>. Precipitation from March to June 2018 ranged from 15.1 to 170 mm, maximum temperatures reached 33.6 to 33.9 °C and relative humidity was 66% in March and 89% in December (SMN-CONAGUA, accessed November 11, 2023).

During the spring/summer cycle of 2018, seeds were sown in 200cell polystyrene trays using Sunshine<sup>®</sup> Special fine No. 3 (Sun Gro Horticulture, Canada) as a substrate, with two seeds per cell. Seedlings were transplanted 28 days after sowing in an open field in completely randomized blocks with three repetitions, with 30 plants spaced 0.30 m apart with 1.20 m between rows. The experimental unit comprised 10 plants.

A 5/4 drip fertigation system (band) was implemented, featuring a caliber 6000 band with a flow rate of 1.5 liters per hour (LPH). The plants were trained using the Spanish method. The agriculture technology package was used for tomato cultivation as proposed by INIFAP (2013).

From 10 randomly selected plants for each accession, 1 or 2 fruits



Fig. 1: Sampling locations for 28 native tomato accessions in southeastern Mexico.

at the commercial maturity stage (USDA, 2005) was harvested during the third harvest of the plants (74–83 days after transplantation) to obtain 1 kg of fruits with uniform color, turgidity, and size, were sampled from the low, middle, and high strata of the plants.

Each sample was dried at 55 °C in a convection oven, then stored in a desiccator until analyzed for antioxidant activity using the DPPH and ABTS methods and content of phenols, flavonoids,  $\beta$ -carotene, lycopene, and other carotenoids.

## Determination and quantification of bioactive compounds Antioxidant Activity

The 2,2'-azino-bis (3-ethylbenzothialzoline-6-sulfonic acid (ABTS<sup>+</sup>) and 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) radicals are frequently used to assess antioxidant activity in food matrices (KUSKOSKI et al., 2005). They have excellent stability under certain conditions, providing precise and repeatable results, and measuring activity from somewhat different contributors. The DPPH radical measures activity by lipophilic molecules, and the ABTS radical measures activity from lipophilic and hydrophilic antioxidants (LI et al., 2014). In addition, the radicals used in alternative methods may provide results that are too low, poorly reproducible, and in some cases, inconsistent (ARNAO, 2000; CHRISTODOULOU et al., 2022).

The ABTS<sup>+</sup> radical is sufficiently soluble, allowing for the measurement of the activity of both lipophilic and hydrophilic compounds. It reacts rapidly with natural and synthetic antioxidant substances and offers the advantage of presenting multiple absorbance peaks (414, 654, 754, and 815 nm) in an alcoholic medium. Moreover, it can be evaluated over a broad pH range (MUNTEANU and APETREI, 2021). On the other hand, the method using DPPH is considered highly sensitive, rapid, practical, and stable for measuring lipophilic compounds (LIANG and KITTS, 2014). Obtaining the extract for the quantification of antioxidant activity. A 0.5 g subsample of each dried fruit sample was combined with 5 mL of 80% (v/v) aqueous methanol, the mixture sonicated for 20 min at room temperature and centrifuged at 4000 rpm for 5 min (Hermle centrifuge, Labortechnik, Z 326K, Germany). After separating the supernatant, the extraction procedure was repeated, and the two extracts were combined to measure the antioxidant activity.

Free radical method using (DPPH<sup>•</sup>). According to the method proposed by KUSKOSKI et al. (2005), in a 15 mL test tube, 3.9 mL of 100  $\mu$ M DPPH<sup>•</sup> in 80% methanol was mixed with 0.1 mL of the methanolic extract, which was then homogenized with a vortex at room temperature, then after 30 min and after 60 min in the dark, the absorbance at 517 nm was measured in a GCB brand UV-visible spectrophotometer, CINTRA model 1010 (Australia). The DPPH<sup>•</sup> concentration was calculated using a Trolox (6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid) calibration curve (100–800 ppm) at 30 min and at 60 min and expressed as micromoles of Trolox equivalent per 100 g of dry mass ( $\mu$ M TE·100 g<sup>-1</sup> dm).

*Free radical method using* (ABTS<sup>+</sup>). The methodology of RE et al. (1999) was followed; 2.45 mM ABTS<sup>+</sup> in ethanol was incubated for 16 h at room temperature in the dark to generate the free radical, then 1 mL of the ABTS<sup>+</sup> solution was mixed with the necessary volume of anhydrous ethanol to obtain an absorbance of  $0.7 \pm 0.1$  at 734 nm to stabilize the radical. Once stabilized, 1 mL of the ABTS<sup>+</sup> solution was mixed with 2 mL of the methanolic sample extract and the mixture incubated in the dark for 7 min. The absorbance was then measured at 734 nm in the spectrophotometer described above. The antioxidant activity was quantified as described above using a standard curve for Trolox (10–90 ppm) and expressed in milligram equivalents Trolox per 100 g of dry mass (mg ET·100 g<sup>-1</sup> dm).

Accession	Common name	Location	Latitude N	Longitude O	Altitude (asl)	Fruit color
		Chiapas				
Ch100	Rosa pa'ak	Zaragoza, Palenque	17°30'33"	91°58'56"	60	Red
Ch102	Rosa pa'ak	Nueva Galilea, Palenque	17°30'33"	91°58'56"	60	Pink
Ch103	Rosa pa'ak	Nueva Galilea, Palenque	17°30'33"	91°58'56"	60	Red
Ch105	Rosa pa'ak	Samaritano, Palenque	17°30'33"	91°58'56"	60	Red
		Tabasco				
T106	Bolita	Tecolutilla, Comalcalco,	18°16'55"	93°19'52"	2	Red
T107	Rosa pa'ak	Patastal, Comalcalco	18°35'48"	93°34'81"	6	Orange
		Campeche				
C108	Rosa pa'ak	Blanca Flor, Hecelchakán	20°10'00"	90°08'00''	10	Red
		Yucatán				
Y110	Zocato	Dzidzantún, Dzidzantún	21°14'45"	89°02'35"	2	Marbled
Y111	Flama	Cholul, Cholul	21°02'35"	89°33'23"	9	Orange
Y112	Manzano	Cholul, Cholul	21°02'35"	89°33'23"	9	Pink
Y113	Rosa pa'ak	Cholul, Cholul	21°02'35"	89°33'23"	9	Orange
Y114	Pera amarillo	Cholul, Cholul	21°02'35"	89°33'23"	9	Yellow
Y115	Cherry naranja	Cholul, Cholul	21°02'35"	89°33'23"	9	Red
Y116	Macizo	Conkal, Conkal	21°04'24"	89°31'15"	9	Red
Y117	Zocato	Conkal, Conkal	21°04'24"	89°31'15"	9	Red
Y118	Rosa pa'ak	Dzutoh, Tixméhuac	20°14'07"	89°06'30"	33	Red
Y119	Rosa pa'ak	Tahdziú, Tahdziú	20°12'08"	88°56'35"	32	Red
Y120	Cherry	Santa Eluteria, Cuncunul	20°38'29"	88°17'46"	29	Yellow
Y121	Rosa pa'ak	Santa Eluteria, Cuncunul	20°38'29"	88°17'46"	29	Red
Y122	Pera amarillo	Santa Eluteria, Cuncunul	20°38'29"	88°17'46"	29	Red
Y123	Perita	Chichimilá, Chichimilá	20°37'51"	88°13'02"	26	Red
Y124	Pera amarillo	Chichimilá, Chichimilá	20°37'51"	88°13'02"	26	Red
Y128	Rosa pa'ak	Xbox, Chacsinkín	20°12'14"	89°00'18"	28	Red
Y129	Macizo	Xbox, Chacsinkín	20°12'14"	89°00'18"	28	Red
Y130	Cherry naranja	Yaxcabá, Yaxcabá	20°31'26"	88°48'41"	27	Orange
Y131	Milpa	Yaxcabá, Yaxcabá	20°31'26"	88°48'41"	27	Red
Y132	País	Xoy, Peto	20°07'22"	88°58'15"	40	Red
Y133	Rosa pa'ak	Tixcacalcupul, Tixcacalcupul	20°32'12"	88°16'13"	27	Pink

Tab. 1: Common name, origin, geographic location and color of the tomato (Solanum lycopersicum L.) accessions studied.

asl: above sea level.

*Total phenols*. The total phenol content was determined using the Folin-Ciocalteau method reported by SINGLETON and ROSSI (1965) with modifications, where by a mixture of tungsten and phosphomolybdic acid in a basic medium is reduced by oxidizing the phenolic compounds, thus generating blue oxides of tungsten and molybdenum. The absorbance was measured at 765 nm in the spectrophotometer described above. Phenolics compounds were quantified using a calibration curve based on 2.5-25 ppm gallic acid and expressed in milligrams of total phenols gallic acid equivalent per 100 g dry mass (mg TP GAE·100 g<sup>-1</sup> dm).

*Flavonoids*. As outlined by CHANG et al. (2002), total flavonoids were quantified using calibration curves for reference standards, commonly quercetin, catechin, and rutin. In the present study, we used quercetin because it is the primary flavonoid in tomatoes and has the highest absorbance (SHRAIM et al., 2023). The absorbance of each methanolic extract was measured at 415 nm in the spectrophotometer described above and the flavonoid concentration calculated from the standard curve for quercetin (10 to 300 mg·L<sup>-1</sup>) and expressed as mg quercetin equivalents per 100 g of sample in dry mass (mg QE-100 g<sup>-1</sup> dm).

 $\beta$ -Carotene and lycopene. The determination of  $\beta$ -carotene and lycopene was carried out according to NAGATA and YAMASHITA (1992).

One gram of sample in 10 mL of a hexane-acetone mixture (2:3) was homogenized in an ultrasonic bath for 3 min (30 s on, 10 s off), then the mixture was centrifuged at 5000 rpm for 10 min, and the absorbance was measured at 663, 645, 505, and 453 nm in the spectro-photometer described above quantities were calculated as:

Lycopene (mg·100 mL<sup>-1</sup>) =  $-0.0485 - A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$ 

*Carotenoids*. The method of SOLTANI et al. (2019) was used. One gram of sample in 10 mL of 80% acetone plus 1% w/v sodium carbonate was sonicated for 10 min, centrifuged at 6000 rpm for 5 min, and the supernatant collected. These steps were repeated until no color remained in the starting sample. The supernatants were combined and the absorbance measured at 663, 645 and 470 nm using the spectrophotometer described above. The results were calculated with the following equations:

$$Ca = 12.21A_{665} - 2.81A_{649} \text{ (chlorophyll } a)$$

$$Cb = 20.13A_{649} - 5.03A_{665} \text{ (chlorophyll } b)$$

$$Cc = \frac{(1000A_{470} - 3.27Ca - 104Cb)}{245} \text{ (carotenoids)}$$

## Statistical analyses

Means for each group of compounds were compared for significant differences ( $\alpha = 0.05$ ) among the accessions using an analysis of variance (ANOVA); when significant effects of treatments were found, the Scott-Knott test was used to determine which treatment means contributed to the differences. A principal component analysis (PCA) was used to order the accessions by the level of diversity. InfoStat version 2020e statistical software was used for all analyses (DI RIENZO et al., 2012).

## **Results and discussion**

# Antioxidant activity: DPPH method

Antioxidant activity (AA) values after the 30-min reaction ranged from 34 (accession Y131) to 133.16 (accession Y115)  $\mu$ M Trolox equivalents per 100 g of sample (dm) ( $\mu$ M TE 100·g<sup>-1</sup>) (Tab. 2) and differed significantly among the analyzed accessions ( $P \le 0.05$ ). Accession Y115 differed the most compared to the mean for all accessions, followed by accessions Ch103, Ch105 and Y116.

Quantities after the 60-min reaction were similar to those obtained after 30 min. Significant differences ( $P \le 0.05$ ) were found among the samples, with Y115 having the highest antioxidant activity (144.23  $\mu$ M TE·100 g<sup>-1</sup>), followed by Ch103, Ch105 and Y116, which did not differ significantly in activity.

These accessions had higher activity than reported by VELA-HINOJOSA et al. (2019) for hybrid and native tomatoes (*S. lycopersicum*; 1.2–5.4  $\mu$ M TE·100 g<sup>-1</sup>) and RIVAS-NAVIA et al. (2020) for wild tomatillo (*S. pimpinellifolium* L.; 88.20  $\mu$ M TE·g<sup>-1</sup>).

#### Antioxidant activity: ABTS method

Antioxidant activity (AA) values were higher than those obtained by the DPPH method at both measurement times, 1 and 7 min (Tab. 2). Variation in AA among the accessions was significant ( $P \le 0.05$ ). After the 1-min reaction, values for AA for accessions Y120, CH100, T107, Ch103 and C108 did not differ significantly and were the highest measured (213.70–224.34  $\mu$ M TE·100 g<sup>-1</sup>). After 7 min, AA ranged from 202.84 to 269.05  $\mu$ M TE·100 g<sup>-1</sup> dm, with the highest AAs in the Y123, Y120, Ch100, C108, Ch103 and T107 accessions. These values were higher than those reported for wild tomatillo, 77.06  $\mu$ M TE·g<sup>-1</sup> dm; RIVAS-NAVIA et al., 2020) and for native tomato from southern Italy (40–70  $\mu$ M TE·g<sup>-1</sup> dm; SCARANO et al., 2020).

The differences in AAs among accessions can be attributed to the fact that the antioxidant activity of food matrices is influenced by the presence, levels and synergistic and antagonistic interactions of different compounds, especially phenolic species and ascorbic acid (SCARANO et al., 2020; SUMALAN et al., 2020). In addition, we did

Tab. 2: Antioxidant activity (μM TE·100 g<sup>-1</sup>, dm) in native tomato accessions from southeastern Mexico estimated using free radical DPPH and ABTS after different reaction durations.

Accession	DPPH	DPPH	ABTS	ABTS	
	30 min	60 min	1 min	7 min	
Ch100	48.04 ± 2.12c	$66.15 \pm 1.97c$	$216.45 \pm 4.57d$	$263.42 \pm 6.87$ d	
Ch102	$60.16 \pm 1.37 d$	$78.47 \pm 1.22$ d	$178.68 \pm 2.79b$	$242.21 \pm 7.28c$	
Ch103	$121.34 \pm 1.75g$	$133.67 \pm 1.40$ g	$222.10 \pm 7.71d$	$268.55 \pm 8.94d$	
Ch105	$121.66 \pm 1.34$ g	$132.85 \pm 1.06g$	$184.96\pm0.21b$	$233.90 \pm 8.74c$	
T106	$90.44 \pm 3.98e$	$104.50 \pm 3.64e$	$172.54 \pm 6.36b$	233.77 ± 13.55c	
T107	$103.46 \pm 2.88 f$	$115.14 \pm 3.07 f$	$222.10 \pm 7.71d$	$269.05 \pm 8.69d$	
C108	$106.04 \pm 2.84 f$	$118.82 \pm 2.84 f$	$224.34 \pm 0.64$ d	$267.66 \pm 6.73d$	
Y110	$108.71\pm0.35 \mathrm{f}$	$120.08 \pm 1.18 f$	$166.75 \pm 15.02a$	$232.87 \pm 22.69c$	
Y111	$90.57 \pm 2.82e$	$105.49 \pm 3.81e$	$160.79 \pm 1.34a$	$216.26 \pm 2.14b$	
Y112	$60.50 \pm 2.88$ d	$78.51 \pm 3.26d$	$166.85 \pm 1.66a$	$226.12 \pm 4.35b$	
Y113	$59.23 \pm 2.64$ d	$76.89 \pm 2.90$ d	$161.85 \pm 3.30a$	$208.19 \pm 2.64a$	
Y114	$59.64 \pm 3.46d$	$80.06 \pm 3.68$ d	$149.77 \pm 0.86a$	$204.71 \pm 0.35a$	
Y115	$133.16\pm1.37h$	$144.23 \pm 1.95h$	$170.94 \pm 26.57b$	$224.41 \pm 18.90b$	
Y116	$125.62 \pm 1.34$ g	$134.90 \pm 0.96$ g	$156.94 \pm 9.77a$	$207.87 \pm 10.71a$	
Y117	$43.71 \pm 2.48b$	$61.67 \pm 2.96c$	$153.76 \pm 9.76a$	$202.84 \pm 8.09a$	
Y118	$38.80 \pm 0.47a$	$58.61 \pm 0.32b$	181.41 ± 1.93b	$223.62 \pm 3.84b$	
Y119	$51.32 \pm 0.97c$	$70.13 \pm 0.51c$	151.54 ± 2.59a	$204.97 \pm 8.09d$	
Y120	$62.93 \pm 1.83d$	$81.10 \pm 1.60$ d	$213.70 \pm 3.45 d$	$261.58 \pm 3.74d$	
Y121	$62.48 \pm 0.73 d$	$79.23 \pm 0.43$ d	$162.33 \pm 1.42a$	$209.52 \pm 6.20a$	
Y122	$34.79 \pm 5.97a$	$64.47 \pm 5.68c$	$180.07 \pm 5.89$ b	$229.35 \pm 4.93b$	
Y123	$65.30 \pm 0.36d$	$83.2 \pm 0.69 d$	$189.60 \pm 11.28b$	$253.52 \pm 17.57d$	
Y124	$51.18 \pm 0.92c$	$69.56 \pm 0.97c$	195.98 ± 3.89c	$246.97 \pm 3.41c$	
Y128	$55.85 \pm 0.86d$	$73.41 \pm 0.19$ d	$183.13 \pm 3.15b$	$236.79 \pm 3.19c$	
Y129	$65.44 \pm 3.60$ d	$86.06 \pm 3.59 d$	$174.52 \pm 0.67$ b	$237.03 \pm 1.56c$	
Y130	$50.41 \pm 3.63c$	$67.38 \pm 3.27c$	$183.42 \pm 6.51b$	238.81 ± 5.79c	
Y131	$34.00 \pm 5.97a$	$49.82 \pm 5.55a$	$173.51 \pm 3.39b$	$224.96 \pm 4.29b$	
Y132	$47.52 \pm 4.24c$	65.96 ± 3.53c	$182.89 \pm 4.53b$	$237.39 \pm 4.04c$	
Y133	$44.85 \pm 5.40 b$	$62.38 \pm 5.14c$	$188.80 \pm 4.90 b$	243.31± 2.28c	

n = 3, means  $\pm$  standard deviation. Different letters in the same column indicate significant differences in the means between accessions ( $P \le 0.05$ ) based on comparison of means using the Scott-Knott test.

not measure activity by all components (e.g., anthocyanins, some vitamins, tocopherols, saponins and terpenoids) that can contribute antioxidant activity in these matrices were measured.

As we noted earlier, the method using the ABTS radical measures activity from hydrophilic antioxidants in addition to the lipophilic molecules measured using the DPPH radical (LI et al., 2014). In addition, the ABTS<sup>+</sup> radical method can be used at a wide pH range and is particularly advantageous for measuring highly pigmented and hydrophilic antioxidants (FLOEGEL et al., 2011) that are characteristic of tomato fruits that can vary greatly in acidity and are intensely colored.

## Total phenols

The total phenols varied significantly among accessions, ranging from 134.63 (accession Y122) to 242.01 (accession Y129) mg·100 g<sup>-1</sup> gallic acid equivalent (mg·100 g<sup>-1</sup> GAE; dm). But the content from accessions Y129, Y121, Y130, Y131, and Y116 from the state of Yucatán and Ch102 from Chiapas did not differ significantly and had the highest concentrations of phenols. Because phenolic compounds play a crucial role as antioxidants, tomatoes from the native accessions could potentially provide health benefits. Our findings are similar to the phenol levels of 188, 231, and 243 mg·100<sup>-1</sup> g GAE (dm) found in tomato pulp of genotypes 7711, FA-574, DTH-7, respectively (GEORGE et al., 2004) and slightly lower than those obtained for cherry tomato germplasm lines (from 156.97 to 317.93 mg·100 g<sup>-1</sup> GAE [dm]) and non-cherry tomato lines (ranging from 152.17 to 283.77 mg·100 g<sup>-1</sup> GAE [dm]) (BHANDARI et al., 2016). The dif-

ferences can be attributed to differing climatic and soil conditions; tomato plants thrive better in hot and semi-arid climates, leading to a higher production of total phenolic compounds (RAMÍREZ-FLORES et al., 2019).

Furthermore, the content and type of phenolic compounds in the fruits may vary with plant genotype, storage conditions, soil salinity, maturity stage, water availability, and light intensity during cultivation (COLLINS et al., 2022). For example, phenolic content increases in in the exocarp, during fruit growth (RANCÍC et al., 2010) can change when exposed to UV rays. The genes involved in phenol biosynthesis are activated by exposure to light, suggesting a sun-protection mechanism that shields tissues from potential damage by UV rays. Heat stress positively modulates the activity of phenylalanine ammonium lyase, affecting total phenols content, activating their biosynthesis, and inhibiting oxidation in tomato plants (SCARANO et al., 2020). In this context, the synthesis of total phenols in the studied accessions could also be associated with a defense mechanism against the stress from greater exposure to sunlight (UV radiation) and high temperatures. Our plants were grown during the spring/summer season, when temperatures are higher for an extended period and daylight lasts longer.

### Flavonoids

Flavonoids accumulate in tomato fruits during ripening, with quercetin and chlorogenic acid the most abundant, and concomitantly, chlorophyll content decreases and the epicarp matures (CHAUDHARY et al., 2018).

Tab. 3: Content of total phenols (mg·100 g<sup>-1</sup>), flavonoids (mg·100 g<sup>-1</sup>), β-carotene (mg·100 g<sup>-1</sup>), lycopene (mg·100 g<sup>-1</sup>), and carotenoids (mg·kg<sup>-1</sup>) in native tomato accessions from southeastern Mexico.

Accessions	Total phenols	Flavonoids	ß-carotene	Lycopene	Total carotenoids
Ch100	$183.34 \pm 0.57b$	$306.55 \pm 6.42a$	$165.52 \pm 3.09d$	$23.06 \pm 5.94a$	$105.90 \pm 7.3b$
Ch102	$226.26 \pm 8.38d$	$434.33 \pm 14.93d$	$126.89 \pm 7.67c$	$292.93 \pm 4.41j$	$127.81 \pm 15.0b$
Ch103	$212.12 \pm 10.80c$	$369.52 \pm 5.76b$	$107.30 \pm 8.46b$	$158.18 \pm 2.69d$	$114.11 \pm 1.07b$
Ch105	$180.86\pm2.92b$	$520.78\pm6.86f$	$171.59 \pm 3.80d$	$242.37 \pm 3.71$ g	$100.85 \pm 2.80b$
T106	$182.22 \pm 3.37b$	$317.65 \pm 17.10a$	$141.45 \pm 6.69c$	$83.12 \pm 4.35b$	$96.47 \pm 2.1b$
T107	$201.96 \pm 15.67c$	$548.29 \pm 17.50$ g	$66.7 \pm 1.79a$	$214.49\pm4.91f$	$98.96 \pm 2.7b$
C108	$181.40 \pm 3.65b$	$399.47 \pm 6.35c$	$69.33 \pm 1.78a$	$179.32 \pm 4.94e$	$85.91 \pm 14.6a$
Y110	$219.65 \pm 13.75c$	$559.05 \pm 5.73$ g	$167.05 \pm 2.15d$	$390.69 \pm 2.79 p$	$230.14 \pm 5.6c$
Y111	$210.01 \pm 12.66c$	$344.17 \pm 8.56b$	$184.08 \pm 4.04e$	$238.74 \pm 4.13g$	$77.72 \pm 2.5a$
Y112	$183.32 \pm 2.59b$	$428.53 \pm 11.28d$	$158.86 \pm 3.78d$	$484.51 \pm 0.71r$	$115.58 \pm 12.3b$
Y113	$179.93 \pm 3.50b$	$596.02 \pm 1.94h$	$198.90 \pm 1.90e$	$330.23\pm4.00m$	$94.96 \pm 6.3b$
Y114	192.64 ±1.52b	$473.45 \pm 8.31e$	$76.59 \pm 3.59a$	$94.45 \pm 0.50b$	$50.80 \pm 1.90a$
Y115	207.67 ±3.72c	$770.95 \pm 17.81j$	$248.1 \pm 1.55 f$	$378.23 \pm 5.43$ o	$118.30 \pm 3.3b$
Y116	$226.68 \pm 17.85d$	$357.37 \pm 8.86b$	$225.98 \pm 3.91e$	$401.49 \pm 5.32q$	$104.10 \pm 3.5b$
Y117	$210.92 \pm 13.22c$	$532.18\pm39.32f$	$156.17 \pm 3.68d$	$218.83\pm5.04f$	$64.35 \pm 1.0a$
Y118	$209.38 \pm 4.1c$	$436.16 \pm 17.95d$	$150.17 \pm 2.00c$	$249.32\pm2.85h$	$76.60 \pm 4.6a$
Y119	$199.35 \pm 6.78c$	$555.28 \pm 3.73g$	$226.84\pm14.13g$	$410.04 \pm 1.23q$	$377.22 \pm 15.8d$
Y120	$216.10 \pm 1.69c$	$622.74 \pm 10.13h$	$193.45 \pm 3.03e$	$241.11 \pm 1.24$ g	$108.96 \pm 3.30b$
Y121	$237.37 \pm 5.04d$	$560.59 \pm 22.48$ g	$167.40 \pm 10.04d$	$238.49\pm7.34g$	$98.88 \pm 3.1b$
Y122	$134.63 \pm 3.48a$	$568.78 \pm 35.97g$	$161.33 \pm 6.02d$	$307.30\pm6.13k$	$118.79 \pm 6.0b$
Y123	$178.31 \pm 3.39b$	$897.57 \pm 14.03 k$	$207.21 \pm 3.72e$	$267.26 \pm 1.35i$	$129.10 \pm 2.5b$
Y124	$201.12 \pm 12.9c$	739.61 ± 15.83j	$147.13 \pm 5.32c$	$181.18 \pm 4.88e$	$71.594 \pm 7.0a$
Y128	$216.93 \pm 4.50c$	$619.39 \pm 15.65h$	$204.70 \pm 3.28e$	$317.05 \pm 1.281$	$101.17 \pm 4.2b$
Y129	$242.00 \pm 15.36d$	$662.19 \pm 27.60i$	$484.33\pm68.20h$	$550.17\pm4.13s$	$131.01 \pm 6.0b$
Y130	$234.41 \pm 13.00d$	$427.42 \pm 4.48d$	$209.87 \pm 23.94e$	$296.44\pm2.97j$	$117.53 \pm 10.50b$
Y131	$227.27 \pm 2.98d$	$506.47 \pm 13.63e$	$275.50 \pm 2.86$ g	$86.59 \pm 1.84b$	$97.49 \pm 1.10b$
Y132	$193.68\pm14.93b$	$496.70 \pm 4.35e$	$241.68\pm2.10f$	$346.55\pm4.69n$	$59.22 \pm 1.60a$
Y133	$206.86 \pm 10.87c$	$596.09 \pm 11.28h$	$207.52 \pm 1.08e$	$40.83 \pm 1.24b$	$109.93 \pm 1.80b$

n = 3, means  $\pm$  standard deviation. Different letters in the same column indicate significant differences in the means between accessions ( $P \le 0.05$ ) based on comparison of means using the Scott-Knott test.

A highly significant phenotypic variation was found in flavonoids levels amoung our accessions (Tab. 3). Accession Y123 stood out for its extremely high flavonoid content (897.57 mg·100 g<sup>-1</sup> quercetin equivalent (QE), and Ch100 and T106 had the lowest (306.55 and 317.65 mg·100 g<sup>-1</sup> QE, respectively). All these levels, however, are higher than those reported by BHANDARI et al. (2016) for commercial cherry tomatoes (132.63 to 202.89 mg·100 g<sup>-1</sup> QE), cherry tomato germplasm lines (126 to 235.30 mg·100 g<sup>-1</sup> QE) and for non-cherry tomatoes (112.73 to 173.21 mg·100 g<sup>-1</sup> QE).

These results are promising because tomatoes are an important source of dietary flavonoids due to their high consumption worldwide. Therefore, accession Y123, with its much higher flavonoid content, could be used directly as a native variety or included in a genetic improvement program for flavonoid-rich tomatoes. In this regard, phenolic compounds, particularly flavonoids, are known to have beneficial health effects due to their antioxidant properties, which are associated with decreased risk of chronic, degenerative diseases (SLIMESTAD, 2009; ZANFINI et al., 2017). They are considered as potentially useful anti-inflammatory compounds and may help prevent cardiovascular diseases and cancer.

## **B-Carotene**

The β-carotene content differed significantly ( $P \le 0.05$ ) among the tomato accessions (Tab. 3). The extremely high content in accession Y129 (484.33 mg·100 g<sup>-1</sup> dm; equivalent to 29.06 fresh mass [fm]) differed significantly from the content in the rest of the accessions. The lowest concentrations, found in T107, C108 and Y114 (66.71, 69.33 and 76.59 mg·100 g<sup>-1</sup> dm, respectively; equivalent to 4.0, 4.16 and 4.59 mg·100 g<sup>-1</sup> fm) did not differ significantly.

Very wide ranges have been reported for the amount of  $\beta$ -carotene in creole or autochthonous tomatoes. For example, in fresh yellow tomatoes,  $\beta$ -carotene contents between 0.53 and 0.58 mg·100 g<sup>-1</sup> fm were reported (RAIOLA et al., 2016), which are much lower than in the present study. Contents between 0.26 and 6.481 mg·100 g<sup>-1</sup> fm were found in five different commercial tomato cultivars (ZANFINI et al., 2017).

 $\beta$ -carotene is an important compound for vision (a provitamin that is converted into retinol) and has important antioxidant action (GRUNE et al., 2010). Accession Y129 is thus a valuable germplasm for improving  $\beta$ -carotene content.

#### Lycopene

Lycopene contents (Tab. 3) also differed significantly (P < 0.05) among the accessions, and accession Y129 (550.17 mg·100 g<sup>-1</sup>) again had the highest content, followed by Y112 (484.51 mg·100 g<sup>-1</sup>), Y116 (401.49 mg·100 g<sup>-1</sup>), and Y119 (410.04 mg·100 g<sup>-1</sup>). Accessions from the state of Yucatán had the highest lycopene levels, surpassing levels in the pulp of 12 tomato accessions (*Lycopersicon esculentum*; 51.1 and 125 mg·100 g<sup>-1</sup> dm; GEORGE et al., 2004) and in 13 semi-wild tomato accessions from various regions of Mexico (between 194.8 and 369.8 mg·100 g<sup>-1</sup> dm; MÉNDEZ et al., 2011).

The high lycopene variability among accessions could be attributed to genotypic factors, where various genes may trigger increased enzymatic activity of phytoene synthase, leading to a massive production of lycopene precursors (KAUR et al., 2013). Another important aspect is that the biosynthesis and accumulation of lycopene in fruits are highly influenced by environmental factors present during their growth (ABDUL-HAMMED, 2022).

Lycopene has an antioxidant capacity 1.16 times higher than that of  $\beta$ -carotene and 2.19 times higher than that of vitamin C (MLADENOVIC et al., 2014). It also is an anti-inflammatory compound and inhibitory of lipid peroxidation (COLLINS et al., 2022) and is considered the most effective among natural carotenoids in tomatoes. The elevated levels of this pigment in tomatoes present an opportunity for improv-

ing health because this fruit can provide 85% of the total dietary lycopene (CHAUDHARY et al., 2018). While there is no ideal quantity for harnessing the nutraceutical benefits of tomatoes, various doses and supplementation durations of this bioactive compound can be suggested for individual needs. Studies propose that a daily intake of 6.5–30 mg of lycopene is effective against cancer in men, and 15 mg/ day over 12 weeks improved immune function in an elderly accession (IMRAN et al., 2020).

Based on our findings, accessions Y129, Y112, Y116, and Y119 are recommended for direct use and as germplasm for genetic improvement of commercial varieties, which tend to have less lycopene than in wild cultivars (FIGUEROA-CARES et al., 2018).

#### Carotenoids

Carotenoid levels ranged from 50.80 to 377.22 mg·kg<sup>-1</sup> and presented little significant variation ( $P \le 0.05$ ) among accessions. Accession Y119 had significantly more carotenoids than the other accessions (Tab. 3).

The tomato accessions had a broad range of colors (yellow, orange, pink, and red) that are characteristic of the presence of carotenoids. Carotenoids, the primary bioactive compounds in tomatoes, are lipophilic pigments that serve as photoprotectors, antioxidants, immunity enhancers, and precursors to vitamin A. Importantly, they may play a significant role in reducing the risk of insulin resistance and the development of diabetes (MAOKA, 2020). They can also protect against erythema caused by UVB-type solar radiation, which is associated with strong mutagenicity (COLLINS et al., 2022).

The extensive array of fruit colors is understood to stem from genetic diversity resulting from the domestication and enhancement of new varieties. These changes from the typical red color impact the biochemical composition, leading to modifications in various metabolites, including carotenoids, in the new varieties (KURINA et al., 2021).

Because humans cannot synthesize carotenoids de novo, they depend on their diet for carotenoids; therefore, accession Y119 has great potential as a source of chemoprotective carotenoids and as germplasm resource for breeding.

#### Principal component analysis

Principal component analysis (PCA) was employed to group native tomato accessions based on the types of bioactive compounds they might share. The PCA explained 68% of the total variation with the first two components (Tab. 4). The variable with the highest descriptive value for PC1 was associated with antioxidant capacity determined using ABTS for both durations (1 and 7 min). In contrast, for PC2, antioxidant activity had a more pronounced effect when determined using DPPH at 30 and 60 min (Tab. 5). In Fig. 2, nine accessions were identified based on their bioactive compound content, forming five groups. Group one, consisting of accessions Y110, Y115, and Y116, was characterized by the highest antioxidant activity determined using DPPH at 30 and 60 min. Group two, com-

Tab. 4: Characteristic value and variance of the main components of the 28 tomato accessions studied.

PC	Characteristic value	Variance ratio (%)	Cumulative variance	
PC1	2.46	29	27	
PC2	2.02	22	52	
PC3	1.50	17	68	

PC: principal component

Tab. 5:	Correlations	of the	original	variables	with	the	first	two	principal
	components	of the 2	8 tomato	accession	s stud	lied.			

Variable	PC1	PC2	
DPPH 30 min	0.48	0.85	
DPPH 60 min	0.49	0.85	
ABTS 1 min	0.82	-0.20	
ABTS 7 min	0.78	-0.14	
Phenols	-0.30	0.33	
Flavonoids	-0.30	$3.5 \cdot 10^{-3}$	
Lycopene	-0.53	0.55	
ß-carotene	-0.64	0.27	
Carotenoids	-0.12	-0.14	

prising accessions CH103, T107, and C108, represented the highest antioxidant activity using ABTS at 1 and 7 min. Accession Y129 had the highest levels of phenols and lycopene, and accession Y119 had the highest carotenoid content. On the other hand, PCA revealed that accession Ch100 was negatively correlated with lycopene, with the lowest lycopene content. Regarding the rest of the accessions, they appear to be closely grouped with minor differences among them. As seen in Fig. 2, the variables phenols and β-carotene were closely related, and carotenoids and flavonoids had similar patterns.

#### Conclusions

The profile of bioactive compounds in the native tomato accessions grown in the same location was highly variable. Because they were cultivated in the same conditions, this variation is attributed more to genotype than to environmental factors. Accession Y129 had the highest contents of  $\beta$ -carotene and lycopene, followed closely by Y119 with remarkable levels of carotenoids. Y115 had the highest antioxidant activity using the DPPH method. Accessions C108 and T107 were distinguished by having the highest antioxidant activity using the ABTS method. Accession Y123 had the highest concentrations of flavonoids. These accessions demonstrated great adaptability to the soil and climate in the area and synthesize high levels of bioactive compounds and can provide an excellent source of antioxi-

dants and other health benefits in rural areas where they are produced. The accessions are also excellent germplasm resources for selecting elevated levels of bioactive compounds content and provide broad genotypic diversity for developing new tomato cultivars.

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# **Conflict of interest**

No potential conflict of interest was reported by the authors.

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Fig. 2: Scatterplot of the principal component analysis of antioxidant activity (estimated using DPPH or ABTS), phenols, flavonoids, β-carotene, lycopene, and carotenoids, in 28 native tomato accessions in southeastern Mexico.

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