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Research article

Bioactive and nutritional compounds in fruits of pepper (*Capsicum annuum* L.) landraces conserved among indigenous communities from Mexico

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Abstract: Farmers' varieties or landraces of chili are regularly heterogeneous, selected and preserved by small traditional farmers and highly demanded by regional consumers. The objective of this study was to evaluate the variation in the content of phenolic compounds, vitamin C, carotenoids, capsaicinoids and antioxidant activity in fruits of a population collection of the landraces Huacle and De Agua, which originated in Oaxaca, Mexico, and a commercial variety of Jalapeño (control). The collection was grown in greenhouse conditions under a random block design. At harvest, a sample of ripe fruits was obtained to evaluate the content of phenolic compounds, vitamin C and antioxidant activity by UV–visible spectrophotometry and the concentration of capsaicin and dihydrocapsaicin was measured by high-resolution liquid chromatography. Significant differences were observed between the Huacle and De Agua landraces and between these and Jalapeño. The studied fruits exhibit the following pattern for flavonoid and carotenoid contents: Huacle > De Agua > Jalapeño. The opposite pattern was observed for total polyphenol and vitamin C contents: Jalapeño > De Agua > Huacle. The general pattern for capsaicinoids in fruits was Jalapeño > De Agua > Huacle. Huacle and De Agua populations showed high variability in all compounds evaluated, with positive correlations with antioxidant activity. The capsaicin content in Huacle populations varied ranging from 7.4 to 26.2 mg 100 g^{-1} and De Agua ranged from 12.4 to 46.8 mg 100 g^{-1}.

Keywords: antioxidant activity; bioactive compounds; indigenous food systems; underutilized landraces; plant genetic resources

1. Introduction

Current crop domestication is an ongoing dynamic process of coevolutionary nature involving plants and humans. It is influenced by anthropogenic disturbances, climate change, food transitions (e.g., diet changes) and market demands, among other factors, which Krug et al. [1] named as a new era of crop domestication in the interrelationship of genetic and phenotypic particularities of the crop species, including their wild relatives, agronomic and cultural practices (e.g., human selection, management) and ecogeographical factors. In this context, the chili pepper (*Capsicum annuum* L.) is an important crop, with a world production from 4.2 to 4.8 million tons [2]. This species continues in domestication and their perspectives or priorities for breeding have changed due to the new role for diet such as the addition of nutrients and bioactive compounds and facing climate changes, not only high yield [3]. In these cases, countries with high agrobiodiversity and centers of origin, domestication and diversification of cultivated plants have certain advantages regarding access to a greater diversity of food products [4], e.g., the diversity of *Capsicum annuum* L. in Mexico.

Perry and Flannery [5] showed different archaeobotanical evidence of the Mexican-Mesoamerican origin of *C. annuum* from pre-Columbian times, and Kraft et al. [6] indicated that the presence of phytoliths, pollen and starch grains in vessels provides multiple lines of evidence of domestication in Mexico. In this sense, evolution under domestication continues, resulting in various lines of genetic differentiation that we now identify as autochthonous varieties, fruit morphotypes or landraces [7]. Therefore, human selection in *C. annuum* has a significant effect on the differentiation among regional variants or types [8,9] and, consequently, significant effects on fruit composition, with high variation between and within landraces or groups of native populations [10]. Notably, some of these landraces are unknown outside their area of distribution and usually commercialized in regional markets.

Fresh or dried chili fruits provide sugars, vitamins, minerals, organic acids, ascorbic acid, tocopherols, carotenoids and several phenolic compounds, all of them important in the family diet, community health and global gastronomy. The most well-known and studied bioactive compounds are capsaicinoids, flavonoids and carotenoids, which, in addition to their biological function as a defense mechanism against biotic and abiotic factors in plants, and when they are consumed have functional and antioxidant activities that counteract diseases associated with eating disorders and chronic degenerative diseases between consumers [11]. For example, flavonoids and capsaicinoids participate in preventing cancer, inflammation, diabetes, obesity, hypertension and gastric system problems and act as analgesics and antimicrobials [12,13]. In addition, the specific sensory characteristics of each type of pepper are related to variations in the content of sugars, organic acids, capsaicinoids, carotenoids, phenolic compounds, vitamins and volatile compounds, among other compounds [10,14–19]. This complex of compounds in the fruits is released during processing stages such as drying, boiling, or toasting and confers different functional nutraceutical and nutritional properties to processed foods [20–23].

Pungency and color are some of the parameters used to determine the quality and commercial value of chili fruits based on their types or morphological group (e.g., Jalapeño, nonpungent variants, Ancho, Habanero, etc.). In the placenta and endocarp of the fruit, 20 or more capsaicinoid compounds responsible for itching or pungency are biosynthesized [24], among which capsaicin [(E)-N(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide)] and 6,7-dihydrocapsaicin represent more than 90% of the total [25]. Carotenoids, such as β-carotene, β-cryptoxanthin, capsanthin, antheraxanthin, capsorubin and zeaxanthin, confer different shades of color to the fruit and accumulate in the endocarp and pericarp when the fruit ripens [26,27]. However, the composition of the fruit changes due to management during production, environmental factors, genetic traits and genetic-environmental interactions, but little is known about the traditional varieties in the centers of origin that continue under domestication and facing climate changes. In this study, variation in phenolic compound, carotenoid, vitamin C, and capsaicinoid contents and antioxidant activity were evaluated in fruits of a collection of populations of *Capsicum annuum* native from Oaxaca, Mexico, grouped in the Huacle and De Agua landraces and cultivated under greenhouse conditions.

2. Materials and methods

2.1. Sampling of landraces and cultivation

Ten populations of Huacle landrace were collected in different communities of the municipality of San Juan Bautista Cuicatlan and eleven populations of De Agua landrace from five municipalities of Valles Centrales, both from Oaxaca, Mexico, between November 2016 and April 2017. In this work we use the landrace term to group all populations with similar fruit morphology, using the local name, such as referenced previously by Vera-Guzman et al. [10]. For experimental purposes, the commercial variety Jalapeño (Hortaflor® seed) was included as control in the experiment, considering their commercial importance and most know by consumers in the international market [28] (Figure 1, Table 1). In summary, 21 populations more one control were sown in polystyrene trays containing commercial substrate (peat moss, *Sphagnum* sp.) and later seedlings were transplanted in September 2017 under a random block design with three replicates in greenhouse conditions. Conventional management was provided for the plants, and fertilization was performed through the irrigation system using the commercial formulas Ultrasol® 15-30-15, 18-18-18 and 13-6-40 and with added calcium nitrate. For the control of pests and diseases, propamocarb (1 mL L^{-1} of water), imidacloprid (1 mL L^{-1} of water), cupric hydroxide (1 mL L^{-1} of water), diethyl-dithio-carbamate (1 g L^{-1} of water) and chlorothalonil (1 g L^{-1} of water), among other products, were used.

2.2. Sample preparation

At harvest, 250 g of mature fruits was randomly chosen from each experimental plot. The fruits were washed with distilled water and dried at room temperature until water residues were removed and then blended in a food processor (Nutribullet ®) to obtain a puree. Then, the sample was divided into three fractions. In the first fraction, total carotenoid and vitamin C contents were analyzed immediately to avoid degradation. The second fraction was stored at −20 °C until analysis for total polyphenol and flavonoid content and antioxidant activity. In the third fraction, whole fruits were dried in an oven at 45 °C until a constant weight was reached. The fruits were then processed and placed in amber plastic flasks for storage at 20 °C until analysis for capsaicinoid contents. Overall, the percent of moisture was determined gravimetrically by standard method 930.15 AOAC [29] and used to correct fresh weights to dry weights.

Figure 1. Sample of mature fruits of the A) Huacle and B) De Agua landraces collected and cultivated in Oaxaca, Mexico and the commercial variety Jalapeño (C).

Table 1. List of the native populations of the Huacle and De Agua landraces and origin municipalities in Oaxaca, Mexico.

Pop. ID	Landrace	Municipality (Oaxaca, Mexico)	Altitude (m)	Latitude N	Longitude W
CH-01	Huacle	San Juan Bautista Cuicatlán	649	17° 44’ 41.60”	96° 57’ 37.44”
CH-02	Huacle	San Juan Bautista Cuicatlán	655	17° 48’ 37.30”	96° 57’ 36.09”
CH-03	Huacle	San Juan Bautista Cuicatlán	625	17° 48’ 09.87”	96° 57’ 48.99”
CH-04	Huacle	San Juan Bautista Cuicatlán	623	17° 48’ 07.74”	96° 57’ 52.83”
CH-05	Huacle	San Juan Bautista Cuicatlán	620	17° 48’ 08.87”	96° 57’ 50.79”
CH-06	Huacle	San Juan Bautista Cuicatlán	600	17° 47’ 41.63”	96° 57’ 50.79”
CH-07	Huacle	San Juan Bautista Cuicatlán	605	17° 47’ 36.19”	96° 57’ 36.22”
CH-08	Huacle	San Juan Bautista Cuicatlán	615	17° 48’ 09.87”	96° 57’ 37.44”
CH-09	Huacle	San Juan Bautista Cuicatlán	622	17° 48’ 08.87”	96° 57’ 47.99”
CH-10	Huacle	San Juan Bautista Cuicatlán	620	17° 48’ 09.87”	96° 57’ 19.44”
CA-62	De Agua	San Jerónimo Tlacochahuaya	1582	17° 00’ 25.93”	96° 35’ 24.51”
CA-25	De Agua	San Bernardo Mixtepec	1643	16° 49’ 55.30”	96° 53’ 58.13”
CA-42	De Agua	Ejutla de Crespo	1495	16° 34’ 09.10”	96° 42’ 10.91”
CA-36	De Agua	Zimatlán de Álvarez	1646	16° 51’ 25.64”	96° 48’ 56.17”
CA-20	De Agua	Santa Cruz Zenzontepec	1040	97° 29’ 43.05”	16° 32’ 00.32”
CA-53	De Agua	Santa Cruz Zenzontepec	1040	16° 30’ 35.6”	97° 24’ 02.9”
CA-38	De Agua	Ejutla de Crespo	1483	16° 32’ 47.01”	96° 42’ 28.65”
CA-40	De Agua	Ejutla de Crespo	1491	16° 34’ 06.69”	96° 42’ 12.78”
CA-24	De Agua	San Bernardo Mixtepec	1697	16° 49’ 35.21”	96° 54’ 04.51”
CA-33	De Agua	San Bernardo Mixtepec	1649	16° 39’ 35.54”	96° 53’ 58.06”
CA-32	De Agua	San Bernardo Mixtepec	1649	96° 54’ 08.92”	16° 49’ 30.47”

2.3. Total carotenoids and vitamin C content analysis

Total carotenoids were measured using the method suggested by Vera-Guzmán et al. [10] with some modifications. The samples were ground using an extraction mix solution of ethanol, acetone and hexane at a ratio of 1:1:2 (v/v). The ground sample was placed in an ice bath and stirred for 20 min, distilled water was added and the mixture was allowed to rest at room temperature for 5 min but protected from light. An aliquot of the upper phase was taken to prepare a hexane-based dilution. The absorbance of the solution was measured with a UV/Vis spectrophotometer (Shimadzu UV 1800, Kyoto, Japan) at 446 nm. Total carotenoids in the sample were calculated using the measured absorbance and a calibration curve for a β-carotene standard (β-carotene with 97.0% purity; Fluka, Buchs SG, Switzerland) from 0.8 to 4.0 µg mL^{-1} ($r^2 = 0.999$). Total carotene concentrations were expressed as milligrams of β-carotene per gram of dry weight (mg βC g^{-1} dw).

The vitamin C content in fresh fruits was determined using the method described by Dürust et al. [30] with modifications. Samples were ground with oxalic acid (0.4%) at a ratio of 1:10 (w/v) and placed in a dark room for 20 min before centrifugation at 11500 rpm. Then, 1 mL of the supernatant was mixed with sodium acetate buffer and 2,6-dichlorophenol indophenol solution. The absorbance of the solution was measured at a wavelength of 520 nm, and vitamin C was calculated based on an adjusted calibration curve for a L-ascorbic acid standard (99% purity; Sigma, St Louis, Missouri, USA) from 1 to 5 µg mL^{-1} ($r^2 = 0.999$). The estimated concentration of vitamin C was reported as milligrams of ascorbic acid per gram of dry weight (mg AA g^{-1} dw).

2.4. Total polyphenol and flavonoid contents and antioxidant activity

To measure total polyphenol and flavonoid contents and antioxidant activity, 3 g of sample underwent 60% ethanol extraction or 80% methanol extraction, respectively. Then, total polyphenol content was determined using the method described by Singleton and Rossi [31]. First, 2.4 mL of deionized water and 0.2 mL of Folin-Ciocalteu reagent were added to 0.4 mL of the diluted extract and the solution was allowed to rest for 5 min. Subsequently, 2 mL of 7% Na_2CO_3 was added and the solution was incubated for 1 h at room temperature (23 ± 3 °C). Finally, the absorbance readings were measured at 750 nm. The total polyphenol content was estimated using a calibration curve for gallic acid (40 to 160 µg mL^{-1}, $r^2 = 0.995$) and the values were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw).

The flavonoid content was determined using the aluminum chloride colorimetric method [32]. A total of 0.5 mL of homogenate 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. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm. Flavonoid content was calculated using a calibration curve for a quercetin standard (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one; 98% purity; Sigma, St Louis, Missouri, USA) from 10 to 70 µg mL^{-1} ($r^2 = 0.996$). Data was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE g^{-1} dw).

Antioxidant activity was measured using the DPPH method (2,2-diephenyl-1-picrylhydrazyl) described by Brand-Williams et al. [33]. The DPPH radical was added to 100 µL of the methanol extract. Then, the solution was vortexed and allowed to stand for 30 min in the dark. Subsequent readings were performed in triplicate at 517 nm. Antioxidant activity was recorded based on a Trolox

(6-hydroxy-2,5,7,8-tetramethylchroman2-carboxylic acid) calibration curve (0.13 to 0.79 µmol mL^{-1}, $r^2 = 0.993$) and expressed in µmol Trolox equivalents per gram of dry weight (µmol TE g^{-1} dw).

Antioxidant activity was also determined by the FRAP method. The antioxidant capacity, expressed as iron reduction, was measured using the method described by Benzie and Strain [34]. A total of 3 mL of FRAP reagent (sodium acetate buffer, pH 3.6, 10 mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) and 10 mM $FeCl_3$ $6H_2O$) was added to 100 µL of the extract. This solution was incubated for 30 min at 37 °C, and the absorbance was read at 593 nm. The quantification of antioxidant activity was performed using a Trolox calibration curve (0.10 to 0.80 µmol mL^{-1}, $r^2 = 0.999$) and the results were expressed as µmol equivalents of Trolox per gram of dry weight (µmol TE g^{-1} dw).

2.5. Capsaicinoids analysis by high-performance liquid chromatography (HPLC) with diode-array detection (DAD)

Capsaicinoids were extracted from 5 g of the ground sample in 10 mL of acetonitrile and heated at 70 °C for 4 h with stirring every 30 min [35,36]. The suspended material was centrifuged at 11500 rpm for 15 min at 4 °C, and then the supernatant was transferred to a vial. The extract was filtered through acrodiscs (45 µm PTFE) and frozen at −20 °C until further analysis. Capsaicin (CAP) and dihydrocapsaicin (DIH) analyses were performed using a 1.00 mL min^{-1} flow rate on a Hypersil ODS column (length 250 mm, ID 4 mm and particle size 5 µm, Agilent, USA) in an HPLC (Model 1260 Infinity II; Agilent Technologies, CA USA) system with an acetonitrile/water (45:55%) mobile phase using a diode array detector in isocratic mode (280 nm wavelength). The analysis time was 20 min, and the injection volume was 20 µL. The retention times for capsaicin and dihydrocapsaicin were 11.5 and 17.1 min, respectively. Capsaicin and dihydrocapsaicin contents were estimated using an external standards calibration curve: capsaicin (8-methylN-vanillyl-trans-6-nonenamide of capsicum with 95% purity; Sigma, St. Louis, Missouri, USA) from 0.1 to 1 mg mL^{-1}, ($r^2 = 0.996$) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide of capsicum with 90% purity; Sigma, St. Louis, Missouri, USA) from 0.1 to 1 mg mL^{-1}, ($r^2 = 0.996$). Data was expressed as milligrams of capsaicin or dihydrocapsaicin per 100 g of dry weight (mg CAP or DIH 100 g^{-1} dw).

2.6. Statistical analysis

The databases were integrated from the evaluation of compounds in each experimental sample and subsequently subjected to analysis of variance to evaluate the differences between Huacle and De Agua landraces and between populations within each landrace, including the control (Jalapeño) or differences between and within landraces. The nesting effect of populations in landraces and the effect of laboratory replicates nested in greenhouse replicates were considered. Multiple comparisons of means were conducted using the Tukey test ($p < 0.05$). In addition, a simple Pearson correlation analysis between antioxidant activity and bioactive compounds was performed and a descriptive analysis of the main bioactive compounds and antioxidant activity by population was performed. Complementarily, a principal component analysis (PCA) was performed to describe the variation of populations within Huacle and De Agua landraces and control. All statistical analyses were performed with the SAS statistical package [37].

3. Results

3.1. Phenolic compounds, vitamin C, carotenoids and antioxidant activity

The analysis of variance indicated significant differences ($p \leqslant 0.01$) between landraces (Huacle and De Agua) and between populations within landraces in total polyphenols, flavonoids, vitamin C and carotenoid contents and antioxidant activity evaluated by DPPH and FRAP methods. Based on the magnitude of variance or square means for landrace and population effects, the variance due to the landraces effect was more than double and up to 19 times that of the variance due to populations within landraces for all the measured variables (Table 2), i.e., the differences between the Huacle and De Agua landraces were greater than those between the populations within each landrace.

Table 2. Significance of square means from the analysis of variance in phenolic compounds, vitamin C, carotenoids and antioxidant activity in chili fruits of the Huacle and De Agua landraces of *Capsicum annuum*.

Sources of variation	Total polyphenols	Flavonoids	Vitamin C	Carotenoids	Antioxidant activity	
					DPPH	FRAP
Landraces (L)	68.8**	11.0**	4.8**	9.39**	20.06**	15.40**
Populations/L[1]	24.2**	2.4**	0.3**	0.49**	3.6**	3.56**
Rep. (R)	149.5**	0.67^{ns}	1.7**	0.22^{ns}	5.60**	5.86*
Lab. replicates/R[1]	0.04^{ns}	0.06^{ns}	$<0.001^{ns}$	0.06^{ns}	0.01^{ns}	0.007^{ns}
Error	7.1	0.34	0.06	0.11	0.56	0.90
Coeff. var. (%)	24	27.8	12.8	19.8	15.5	13.6

^{ns} Not significant ($p > 0.05$); * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; [1] Indicate nesting of populations in landraces and nesting of laboratory replicates in repetitions of greenhouse cultivation, respectively.

The commercial variety Jalapeño used as the control differed significantly from the Huacle and De Agua landraces regarding total polyphenol, flavonoid and vitamin C contents and antioxidant activity evaluated by FRAP. The carotenoid contents in Jalapeño was similar to De Agua landrace. In antioxidant activity (DPPH method), Jalapeño and Huacle were similar. De Agua and Huacle showed equivalent total polyphenol contents and antioxidant capacity (FRAP method). Huacle had higher concentrations of flavonoids and carotenoids and antioxidant activity (DPPH) than did De Agua, but the trend for vitamin C was reversed. In this sense, the studied fruits exhibit the following pattern for flavonoid and carotenoid contents: Huacle > De Agua > Jalapeño. The opposite pattern was observed for total polyphenol and vitamin C contents: Jalapeño > De Agua > Huacle (Figure 2).

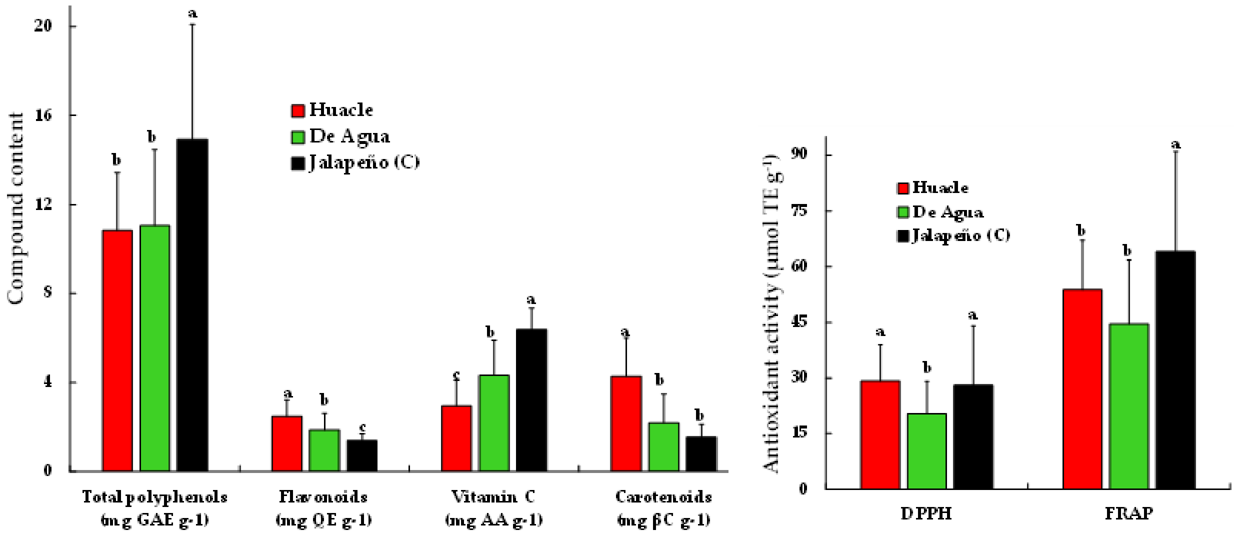

Figure 2. Differentiation between De Agua and Huacle chili landrace regarding total polyphenol, flavonoid, vitamin C, and carotenoid contents and antioxidant activity (DPPH and FRAP methods).

There was high variability between populations within each landrace. Among the Huacle populations, total polyphenols ranged from 7.9 to 13.8 mg of GAE g^{-1} and flavonoids ranged from 1.6 to 3.2 mg of QE g^{-1}. For De Agua, total polyphenols ranged from 9.0 to 14.1 mg of GAE g^{-1} and flavonoids ranged from 1.1 to 2.7 mg of QE g^{-1}. In Huacle, carotenoids ranged from 2.5 to 5.6 mg C g^{-1} and in De Agua, carotenoids ranged from 1.4 to 3.7 mg C g^{-1}. In this study, the Huacle populations exhibited slightly more variation than did De Agua populations. Among Huacle populations, vitamin C ranged from 1.8 to 4.3 mg AA g^{-1}. Among De Agua populations, vitamin C ranged from 3.3 to 5.9 mg AA g^{-1}. The variety of Jalapeño presented the highest total polyphenol and vitamin C contents. Regarding antioxidant activity, as evaluated by DPPH and FRAP, the populations of both landraces exhibited similar differential patterns (Table 3).

Table 3. Variation in phenolic compounds, vitamin C, carotenoids and antioxidant activity among populations of De Agua and Huacle landraces of chili pepper.

Pops. ID	Total polyphenols (mg GAE g^{-1})	Flavonoids (mg QE g^{-1})	Vitamin C (mg AA g^{-1})	Carotenoids (mg βC g^{-1})	Antioxidant activity (µmol TE g^{-1}) DPPH	FRAP
Huacle landrace:						
CH1	7.9 ± 2.3 e[1]	1.6 ± 0.3 efg	1.8 ± 0.4 h	2.5 ± 1.1 c-f	16.9 ± 4.2 ef	37.2 ± 12.5 c
CH2	10.5 ± 3.2 a-e	1.7 ± 0.2 d-g	2.9 ± 1.1 e-h	3.6 ± 1.4 a-f	35.5 ± 10.8 abc	53.9 ± 7.2 abc
CH3	9.3 ± 1.0 cde	2.3 ± 0.5 a-f	2.2 ± 0.2 gh	4.2 ± 2.0 a-d	23.6 ± 3.4 c-f	57.5 ± 5.3 abc
CH4	10.4 ± 2.6 a-e	2.1 ± 0.4 c-g	3.6 ± 1.4 d-h	3.7 ± 1.0 a-e	31.8 ± 2.0 a-d	59.3 ±6.4 abc
CH5	11.8 ± 3.1 a-e	2.9 ± 0.9 abc	2.8 ± 0.6 e-h	4.7 ± 1.3 a-c	33.8 ± 10.8 abc	63.3 ± 22.7 ab
CH6	12.5 ± 1.6 a-e	3.2 ± 0.5 a	4.3 ± 2.3 b-f	5.1 ± 1.0 ab	37.4 ± 10.0 ab	64.6 ± 5.6 a
CH7	13.8 ± 2.3 abc	3.1 ± 1.2 ab	3.5 ± 0.5 e-h	4.2 ± 0.6 a-d	38.8 ± 8.1 a	59.2 ± 7.8 abc

Continued on the next page

Pops.	Total polyphenols	Flavonoids	Vitamin C	Carotenoids	Antioxidant activity (µmol TE g^{-1})	
ID	(mg GAE g^{-1})	(mg QE g^{-1})	(mg AA g^{-1})	(mg βC g^{-1})	DPPH	FRAP
Huacle landrace:						
CH8	10.7 ± 1.3 a-e	2.5 ± 0.3 a-e	2.9 ± 0.5 e-h	4.8 ± 1.0 ab	27.0 ± 3.5 a-f	55.1 ± 12.9 abc
CH9	10.2 ± 1.9 b-e	2.5 ± 0.3 a-e	2.5 ± 0.4 fgh	4.2 ± 1.0 a-d	20.2 ± 3.4 def	44.0 ± 4.5 abc
CH10	11.0 ± 1.3 a-e	2.7 ± 0.2 a-d	3.0 ± 0.6 e-h	5.6 ± 3.6 a	27.1 ± 6.5 a-f	43.8 ± 7.5 abc
De Agua landrace:						
CCA20	14.1 ± 6.8 ab	2.6 ± 1.3 a-d	5.5 ± 0.6 abc	3.4 ± 2.2 a-f	25.8 ± 13.1 a-f	59.2 ± 20.2 abc
CCA24	12.8 ± 3.8 a-d	2.1 ± 0.3 b-g	4.2 ± 1.3 b-f	1.7 ± 0.2 e-f	25.1 ±6.9 b-f	45.4 ± 10.0 abc
CCA25	11.3 ± 0.6 a-e	1.9 ± 0.3 c-g	5.4 ± 2.1 a-d	3.1 ± 1.8 b-f	23.3 ± 3.5 c-f	49.1 ± 12.5 abc
CCA32	10.3 ± 1.1 a-e	1.3 ± 0.2 fg	3.3 ± 0.3 e-h	1.6 ± 0.4 ef	15.9 ± 5.8 ef	37.3 ± 10.5 c
CCA33	10.0 ± 2.4 b-e	1.8 ± 0.3 d-g	3.5 ± 0.8 e-h	1.5 ± 0.4 ef	13.9 ± 2.6 f	40.0 ± 3.3 bc
CCA36	13.3 ± 4.9 a-d	2.7 ± 1.1 a-d	5.9 ± 3.0 ab	3.7 ± 1.7 a-f	28.4 ± 11.5 a-e	63.4 ± 32.2 ab
CCA38	9.3 ± 2.5 cde	1.3 ± 0.5 g	3.4 ± 0.5 e-h	1.5 ± 0.5 ef	13.8 ± 5.7 f	36.9 ± 12.2 c
CCA40	10.4 ± 1.1 a-e	1.9 ± 0.5 c-g	3.7 ± 0.5 c-g	1.4 ± 0.4 def	24.8 ± 9.8 b-f	42.6 ± 18.9 abc
CCA42	10.5 ± 2.0 a-e	1.9 ± 0.4 c-g	4.1 ± 0.7 b-f	2.3 ± 0.5 def	19.3 ± 5.7 def	40.6 ± 8.8 abc
CCA53	10.6 ± 2.4 a-e	1.7 ± 0.4 d-g	4.6 ± 2.0 a-e	2.2 ± 0.8 def	16.2 ± 6.5 ef	39.7 ± 14.2 bc
CCA62	9.0 ± 1.8 de	1.1 ± 0.1 g	3.6 ± 0.4 c-h	1.6 ± 0.5 ef	17.4 ± 5.2 ef	36.6 ± 8.8 c
Jalapeño type (control):						
JAL	14.9 ± 5.2 a	1.4 ± 0.3 fg	6.4 ± 1.0 a	1.5 ± 0.6 e-f	28.0 ± 16.0 a-e	64.1 ± 27.0 ab

[1] In columns, means with the same letter are not significantly different (Tukey's test, $p \leq 0.05$); GAE = gallic acid equivalents; QE = Quercetin Equivalents; AA = Ascorbic Acid; βC = β-Carotene; TE = Trolox Equivalents.

Pearson correlation analysis (r) was used to identify positive and significant correlations between total carotenoid, vitamin C, total polyphenol and flavonoid contents in relation to antioxidant activity evaluated by DPPH method ($0.22 < r < 0.63$; Student's t test, $p \leq 0.01$, n = 66 samples evaluated) and FRAP method ($0.39 < r < 0.60$; Student's t test, $p \leq 0.01$, n = 66); in the latter case, the correlation with vitamin C was not significant ($r = -0.04$, Student's t test, $p > 0.05$, n = 66). These correlations suggest that the concentration of phenolic compounds and vitamin C influence the ability to capture free radicals and singlet oxygen.

3.2. Variation in capsaicinoid contents

The analysis of variance of the capsaicinoid contents indicated significant differences ($p \leq 0.05$, 0.01) in capsaicin (CAP), dihydrocapsaicin (DIH), total capsaicinoid (CAP + DIH) contents and the CAP/DIH ratio between landraces and populations within landraces. The mean square or variance due to landraces was 5 to 10 times more than due to differences between populations within landraces (Table 4). This indicates that the differences between landraces were significantly greater than the differences between populations.

Table 4. Significance of square means from the analysis of variance in capsaicin and dihydrocapsaicin in fruits of Huacle and De Agua landraces of *Capsicum annuum*.

Sources of variation	Capsaicin (CAP)	Dihydrocapsaicin (DIH)	CAP + DIH	CAP/DIH
Landraces (L)	46.4**	13.0**	55.5**	2.6**
Populations/L[1]	4.7**	2.5**	6.9**	0.3*
Replicates (R)	7.5**	7.9**	14.1**	2.2**
Lab. replicates/R[1]	0.01^{ns}	$<0.04^{ns}$	0.05^{ns}	0.08^{ns}
Error	0.8	0.5	1.2	0.1
Coeff. Var. (%)	20.6	21.3	20.0	20.0

^{ns} not significant ($p > 0.05$); * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; [1] Indicates nesting of populations in landraces and nesting of laboratory replicates in repetitions of greenhouse cultivation, respectively.

In the comparison of capsaicinoids between landraces and the control variety (Jalapeño), the capsaicin (CAP), dihydrocapsaicin (DIH) and total capsaicinoid (CAP + DIH) contents were higher in the Jalapeño control (Table 5). The CAP content was always higher than the DIH content, i.e., up to 2.1 times higher in De Agua and significantly different from that in Huacle (1.7 times). Significant differences were observed between landraces, with higher CAP and CAP + DIH contents in De Agua than in Huacle. DIH concentration was similar between the landraces. The general pattern for CAP and CAP + DIH content in fruits was Jalapeño > De Agua > Huacle and for DIH was Jalapeño > De Agua = Huacle; in all cases, the CAP content was always higher than the DIH content (Table 5). These patterns indicate significant differences between landraces with respect to the Jalapeño control.

Table 5. Differences in capsaicin and dihydrocapsaicin contents between Huacle and De Agua landraces and the commercial variety Jalapeño (control) of chili pepper.

Landraces and control	Capsaicin (CAP, mg 100 g^{-1})	Dihydrocapsaicin (DIH, mg 100 g^{-1})	CAP + DIH (mg 100 g^{-1})	CAP/DIH
Huacle	14.1 ± 7.4 c[1]	9.4 ± 5.8 b	23.5 ± 12.6 c	1.7 ± 0.6 b
De Agua	27.0 ± 14.4 b	13.1 ± 6.7 b	40.0 ± 21.0 b	2.1 ± 0.3 a
Jalapeño	44.0 ± 10.6 a	24.1 ± 6.4 a	68.1 ± 16.9 a	1.8 ± 0.1 ab

[1] In columns, means with the same letter are not significantly different (Tukey's test, $p \leq 0.05$).

Between Huacle and De Agua populations, there was high variability in capsaicin (CAP), dihydrocapsaicin (DIH), total capsaicinoid (CAP + DIH) content and in the CAP/DIH ratio (Table 6). In Huacle, a similar pattern was observed between CAP and DIH. Populations with higher or lower CAP values also had high or low DIH values. The trend was similar in De Agua. However, the De Agua populations with higher CAP (32.8 to 46.8 mg 100 g^{-1}) and DIH (21.2 mg 100 g^{-1}) contents significantly surpassed Huacle populations with higher values of CAP (26.2 mg 100 g^{-1}) and DIH (18.0 mg 100 g^{-1}) contents. The populations with the highest total capsaicinoid content (> 40 mg 100 g^{-1}) were Huacle CH1 and De Agua CCA24, CCA25, CCA33, CCA40 and CCA62. The content in Jalapeño was 68.2 mg 100 g^{-1}. In eight De Agua populations, the CAP/DIH ratio was greater than double, unlike in Huacle populations, among which only one population presented this pattern (Table 6).

Table 6. Variation in capsaicin, dihydrocapsaicin, total capsaicinoid (CAP + DIH) contents and CAP/DIH ratio in fruits of populations from the Huacle and De Agua landraces of *C. annuum*.

Pop. ID	Capsaicin (CAP, mg 100 g^{-1})	Dihydrocapsaicin (DIH, mg 100 g^{-1})	CAP + DIH (mg 100 g^{-1})	CAP/DIH
Huacle landrace:				
CH1	26.2 ± 6.1 b-f[1]	18.0 ± 5.9 abc	44.3 ± 10.6 a-e	1.5 ± 0.4 abc
CH2	15.3 ± 7.8 d-g	11.5 ± 6.6 b-g	26.8 ±14.3 c-g	1.4 ± 0.1 bc
CH3	11.5 ± 10.4 efg	11.5 ± 6.7 b-g	27.0 ± 16.2 d-g	1.2 ± 1.1 c
CH4	7.4 ± 3.2 g	3.5 ± 1.0 g	10.8 ± 3.6 g	2.2 ± 0.9 a
CH5	15.5 ± 7.0 d-g	8.4 ± 3.8 c-g	23.9 ± 10.7 d-g	1.8 ± 0.2 abc
CH6	14.5 ± 3.4 efg	8.0 ± 0.8 d-g	22.5 ± 3.2 d-g	1.8 ± 0.5 abc
CH7	15.1 ± 6.6 d-g	10.6 ± 7.1 c-g	25.7 ± 13.6 d-g	1.6 ± 0.3 abc
CH8	14.7 ± 1.9 d-g	9.6 ± 2.5 c-g	24.3 ± 4.0 d-g	1.6 ± 0.4 abc
CH9	11.7 ± 5.2 efg	7.9 ± 4.6 d-g	19.6 ± 9.2 efg	1.6 ± 0.5 abc
CH10	9.4 ± 3.0 fg	5.0 ± 2.2 fg	14.4 ± 5.1 fg	1.9 ± 0.4 abc
De Agua landrace:				
CCA20	21.1 ± 5.5 c-g[1]	11.1 ± 3.2 c-g	32.3 ± 8.6 b-g	1.9 ± 0.2 abc
CCA24	32.8 ± 16.8 a-d	16.7 ± 9.5 a-e	49.5 ± 25.8 a-d	2.2 ± 0.6 a
CCA25	37.5 ± 22.2 abc	17.7 ± 9.6 a-d	55.2 ± 31.7 ab	2.1 ± 0.1 ab
CCA32	26.5 ± 6.3 b-f	13.3 ± 4.9 b-f	39.8 ± 11.2 b-f	2.1 ± 0.4 ab
CCA33	28.3 ± 14.2 b-e	13.9 ± 6.9 b-f	42.2 ± 20.7 a-e	2.1 ± 0.5 ab
CCA36	21.6 ± 5.1 c-g	11.4 ± 3.3 b-g	33.1 ± 8.5 b-g	1.9 ± 0.1 abc
CCA38	14.9 ± 2.0 d-g	7.4 ± 0.8 efg	22.4 ± 2.7 efg	1.9 ± 0.2 abc
CCA40	46.8 ± 8.6 a	21.2 ± 3.3 ab	68.0 ± 11.9 a	2.2 ± 0.1 a
CCA42	12.4 ± 8.4 efg	6.0 ± 4.5 fg	18.4 ± 12.9 efg	2.1 ± 0.3 ab
CCA53	17.6 ± 4.0 d-g	8.5 ± 1.4 c-g	26.0 ± 5.3 d-g	2.1 ± 0.3 abc
CCA62	37.0 ± 9.9 abc	16.5 ± 4.1 a-e	53.5 ± 13.6 abc	2.2 ± 0.3 a
Jalapeño type (control):				
JAL	44.1 ± 10.6 ab	24.1 ± 6.4 a	68.2 ± 16.9 a	1.8 ± 0.1 abc

[1] In columns, means with the same letter are not significantly different (Tukey's test, $p \leq 0.05$)

In the principal component analysis (PCA), the first two components explained 94.5% of the total variation in terms of fruit composition (Figure 3). The differences between populations of each landrace were associated with antioxidant activity evaluated by DPPH and FRAP methods, indirectly reflecting the ability to capture free radicals by bioactive compounds, and capsaicin and dihydrocapsaicin contents helped differentiate landraces and populations within each landrace. The populations of the Huacle landrace were associated with low concentrations of capsaicinoids and higher antioxidant activity. Conversely, the populations of the De Agua landrace were associated with higher capsaicinoids content and lower antioxidant activity, as shown in Figure 3.

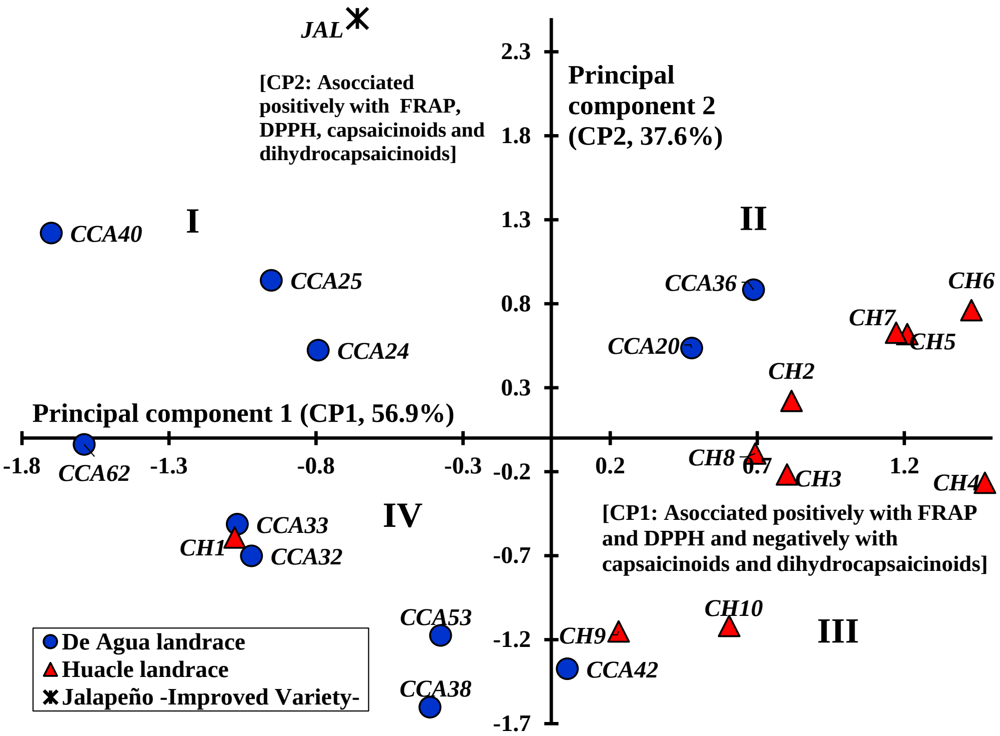

Figure 3. Scatterplot of pepper landrace populations as a function of the two principal components, on base phenolic compounds, vitamin C and capsaicinoid contents and antioxidant activity.

4. Discussion

The composition of chili fruit and its perception by consumers through aroma, flavor and texture determines, in part, the preferences and demands in regional, national and international markets and the nutritional-nutraceutical benefits for health. Consumers demand landraces of chili to recreate flavors is a medium that allows the conservation of these populations or traditional varieties by farmers and help researchers to differentiate plants and/or fruits between landraces and allow monitor the evolution process under domestication [7,11,17]. In this sense, Huacle and De Agua are names of pepper landraces cultivated, selected and preserved on-farm by traditional farmers in Oaxaca, Mexico but with demand local and regional of combinations of flavor and aroma.

In this study, the Huacle and De Agua landraces with different fruit morphologies were significantly different regarding flavonoids and vitamin C content and antioxidant activity evaluated by DPPH, capsaicin (CAP) and total capsaicinoid (CAP + DIH) contents and CAP/DIH ratio, with substantial differences with respect to the control (Jalapeño, Tables 3 and 6). In this study, the capsaicinoids content was higher in Jalapeño than in both landraces, i.e., twice that in Huacle and 50 to 60% more than in De Agua (Table 5), indicating that the pungency of Huacle and De Agua is very low or almost null compared to the Jalapeño. In Oaxaca, Mexico, Huacle is frequently used dry, and De Agua is usually roasted. In contrast, Jalapeño is consumed fresh or in sauces. Composition analyses help to propose a chemotaxonomy of *Capsicum annuum* landraces based on the chemical structure of the secondary metabolites in fruit [38]. Hervert-Hernandez et al. [39] obtained similar results in the differential composition of fruits between five spicy varieties of *C. annuum*, and Martínez-Ispizua et al. [40] obtained similar patterns among landraces from Valencia, Spain.

The variation in total polyphenol contents (7.9 to 14.9 mg GAE g^{-1}) between Huacle, De Agua and Jalapeño (control) was slightly higher than the values reported in different landraces and commercial varieties of *C. annuum* –2.47 to 8.331 mg GAE g^{-1}– [10,40–42] but lower than those in wild populations of *C. annuum* var. *glabriusculum* (26.2 to 42.4 mg GAE g^{-1}) and for hot pepper varieties (23.2 to 28.4 mg GAE g^{-1}) [39,43]. Even when there are certain methodological differences in laboratory protocols, in each investigation, different germplasm sources or genetic sources are evaluated and fruit composition can differ due to genetic, environmental or agroecological effects, genetic-environmental interactions and management practices, in addition to the selection of diverse landraces by farmers [8,44].

Huacle and De Agua landraces and Jalapeño exhibited high variation in flavonoid contents (1.1 to 3.2 mg QE g^{-1}), but the values were within the range for average flavonoid content reported by Ionică et al. [41] for five commercial varieties of chili (1.42 to 5.46 mg QE g^{-1}) but, in certain cases, lower than the specific variation in quercetin equivalents for 14 commercial varieties of chili (0.2 to 7.9 mg QE g^{-1}) [45]. However, the estimated variation in this study was slightly lower than those for wild chili based on catechin equivalents (3.53 to 4.14 mg EC g^{-1}) [43]. This indicates that the native populations of Huacle or De Agua traditionally consumed by communities within Oaxaca, Mexico, have a total flavonoid content similar to commercial or traditional varieties from other regions and that, perhaps, the combination of flavonoids and other secondary metabolites confer flavors and specific aromas that are preferred by consumers [46,47]. In general, in different countries there are not breeding programs to improve landraces. So, based on this work, we suggest a breeding program of plant and fruit selections in order to maintain the characteristic fruit composition, which can promote their demand not only at regional/national level even international.

The vitamin C content in fruits of Huacle and De Agua varied from 1.8 to 4.3 and from 3.4 to 5.9 mg AA g^{-1}, respectively, and was 6.4 mg AA g^{-1} in Jalapeño, indicating significant differences where variation that slightly exceeded that reported by Martínez-Ispizua et al. [40] for 18 sweet or non spicy chili landraces (0.6 to 2.47 mg AA g^{-1}). The vitamin C content in Huacle populations was within the range reported by Vázquez-Flores et al. [43] for wild populations of chili (2.58 to 3.07 mg AA g^{-1}) but lower than those reported for Jalapeño and De Agua. Ionică et al. [41] reported values of 0.17 to 1.60 mg AA g^{-1} in five commercial varieties, concentrations lower than those found in this study. In this context, the chili fruits evaluated herein are excellent sources of vitamin C. For example, the consumption of 100 g per day can meet the basic vitamin C consumption needs of an adult [11,48,49]. Besides, ascorbic acid is a nutritional-functional compound with antioxidant activity and is abundant in immature and mature chili [50–54].

The variation in carotenoid content in Huacle and De Agua landraces and Jalapeño was 1.5 to 5.6 mg βC g^{-1}, values lower than the range reported by Vázquez-Flores et al. [43] for wild populations (5.7 to 6.03 mg βC g^{-1}) but higher than the range reported by Vera-Guzmán et al. [10] for landraces from Oaxaca, Mexico, 0.034 to 1.329 mg βC g^{-1}. However, the final carotenoid content in fruit depends on the ripening color, e.g., red, yellow or orange and brownish yellow. In the populations evaluated in this study, mature Huacle fruits were dark brownish yellow and those for De Agua and Jalapeño were red. Thus, certain populations of Huacle have a higher carotenoid content than do De Agua and Jalapeño (Figure 1).

The variation in capsaicin (CAP) and dihydrocapsaicin (DIH) contents in Huacle, De Agua and Jalapeño ranged from 7.4 to 46.8 mg 100 g^{-1} and from 3.5 to 24.1 mg 100 g^{-1}, respectively. These values are within the range (0.49 to 222.9 mg 100 g^{-1} of CAP) reported by Ionică et al. [41] in fruits of commercial varieties, and by Paredes-Andrade et al. [55] in a germplasm of *C. annuum* from Central America (20 to 260 mg 100 g^{-1} of CAP) and also in the range reported by Cisneros-Pineda [25] (7.5 to 631.89 and 3.7 to 50.4 mg 100 g^{-1} of CAP and DIH, respectively) in fruits of landraces of *C. annuum* which differ from those reported by Vázquez-Flores et al. [43], 661 to 710 mg 100 g^{-1} of CAP and 149 to 212 mg 100 g^{-1} of DIH. These estimates for capsaicin and dihydrocapsaicin allow differentiating levels of pungency. For example, low values (<10 mg 100 g^{-1} of CAP or DIH) are considered sweet to slightly pungent, and high concentrations of CAP and/or DIH (> 10 mg 100 g^{-1} of CAP or DIH) are moderately to highly pungent [41]. Therefore, the Huacle and De Agua landraces vary from sweet to moderately pungent based on the values recorded. The De Agua populations with the highest CAP content (32.8 to 46.8 mg 100 g^{-1}) were CCA24, CCA25, CCA40 and CCA62, and the CH4 and CH10 populations of Huacle (7.4 to 9.4 mg 100 g^{-1} of CAP and 3.5 to 5.0 mg 100 g^{-1} of DIH) can be used as sweet or non-pungent peppers in several gastronomic preparations.

The antioxidant activity evaluated by DPPH and FRAP methods provided complementary information to differentiate populations within each landrace and allow to understand the complexity in the composition of the fruits evaluated. Antioxidant activity reflects the combined effect of total polyphenols, flavonoids, vitamin C, capsaicinoids, phenolic acids and other compounds on the reducing capacity or capture of free radicals [20,56]. The positive correlations between antioxidant activity and compounds are explained by their ability to donate hydrogen atoms from a hydroxyl group to the benzene ring and their redox properties, which increase their ability to adsorb and sequester free radicals [57,58]. In this sense, gallic acid, quercetin and capsaicinoids in isolated fractions of chili fruits have high DPPH antioxidant activity [59]. Capsaicin can sequester or eliminate DPPH radicals when the reaction site is located at the C7 position of the benzyl carbon [60]. In addition, capsaicin, dihydrocapsaicin and total polyphenols in chili peppers are associated with high antioxidant activity, as evaluated by the FRAP method [61].

All evaluated compounds were useful to find similitudes and differences between Huacle and De Agua landraces, and consequently to define distinctive traits from a phytochemical point of view to formulate on-farm conservation strategies and support a denomination of origin from a vegetal product. Currently, chili fruits are a source from vitamins, minerals and bioactive and antioxidant compounds, which contribute to improve the nutritional health of consumers and communities of small-scale farmers. For agronomic purposes, the populations evaluated are a source of useful traits or genes for pepper breeding. For example, there are non-pungent populations with a common trait in many commercial varieties, but also it was evident that many populations have a combination of polyphenols, flavonoids, vitamin C, carotenoids and capsaicinoids more convenient in the international cuisine.

5. Conclusions

The results indicate significant differentiation between Huacle and De Agua regarding flavonoids, vitamin C, carotenoids, capsaicin and total capsaicinoids contents and antioxidant activity evaluated by DPPH. The Huacle and De Agua landraces differed from the Jalapeño control in total polyphenol, flavonoid and vitamin C contents, antioxidant activity as evaluated by FRAP, capsaicin (CAP), dihydrocapsaicin (DIH) and total capsaicinoid (CAP + DIH) contents. The differentiation between Huacle and De Agua landraces can contribute to increase their value added and a start point for a breeding program focused on fruit quality because the evaluation of fruit composition allowed the differentiation of populations with higher total polyphenol, flavonoid, vitamin C, carotenoid, capsaicin and dihydrocapsaicin contents (in Huacle, CH1, CH6 and CH7; in De Agua, CCA24, CCA25, CCA40 and CCA62). Populations with non-pungent fruits were identified within Huacle and De Agua landraces, which can contribute to the demand for this type of product. In addition, this variation can be exploited directly by consumers through potential nutritional-nutraceutical value. The results provide useful phytochemical composition information that can be used to formulate conservation strategies and promote diversity.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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