

Correlation between Some Nutritional Components and the Total Antioxidant Capacity Measured with Six Different Assays in Eight Horticultural Crops

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The contents of antioxidant nutritional compounds, total soluble phenolics (TSP), vitamin C, vitamin E, β -carotene, and total carotenoids (TC), were correlated with the total antioxidant capacity (AOC) of hydrophilic (HPE) and lipophilic extracts (LPE) from eight horticultural crops, namely, guava, avocado, black sapote, mango, papaya, prickly pear fruit, cladodes, and strawberry. AOC was measured using six different assays: 2,2'-diphenyl-1-picrylhydrazyl (DPPH), *N,N*-dimethyl-*p*-phenyldiamine (DMPD), ferric-ion-reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), and total oxidant scavenging capacity (TOSC). AOC values from HPE were about 95 times higher than LPE values. HPE of guava had the highest AOC value when evaluated with DMPD, DPPH, FRAP, TEAC, and TOSC assays, whereas with ORAC assay, black sapote had the highest value. HPE of papaya and prickly pear fruit presented the lowest AOC values with all assays. From HPE, vitamin C and TSP contents were highly correlated with AOC for all assays, while from LPE, TC and β -carotene contents possessed a high correlation with AOC only in the DMPD assay.

KEYWORDS: Antioxidant capacity; fruits; vegetables; carotenoids; vitamin C; vitamin E; total soluble phenolics; tocopherol; ascorbic acid

INTRODUCTION

Several clinical and epidemiological studies have shown that the consumption of natural antioxidant-rich foods, such as fruits and vegetables, are beneficial to health because of their capacity to prevent some health problems, such as heart diseases and cancer, cataracts, macular degeneration, and neurological diseases (1, 2). The beneficial effects of plant foods are attributed to their high content of antioxidant compounds that diminish the free-radical oxidative stress, which is responsible for cellular damage (3). However, there is a lack of information regarding the bioactive compounds content and antioxidant capacity (AOC) of fruits cultivated in the tropics. Different studies have demonstrated that the individual contribution of the bioactive compounds to AOC varies with the type and concentration present in the tissue (4). Various methods have been developed to measure the AOC of fruits and vegetables, but results usually vary depending upon the type of product and method used (5). There is still a lack of information on the appropriate method(s) that need to be used for different products. For this reason, it is

recommended that at least two different methods be used to measure AOC in any given sample. In this study, the content of antioxidants, such as total soluble phenolics (TSP), vitamin C, vitamin E, β -carotene, and total carotenoids (TC), in eight horticultural crops (avocado, black sapote, guava, mango, papaya, prickly pear fruit, cladodes, and strawberries) were correlated with the AOC of hydrophilic (HPE) and lipophilic extracts (LPE) being measured using six different antioxidant assays.

MATERIALS AND METHODS

Chemicals and Solvents. Reagents were obtained from Sigma-Aldrich (St. Louis, MO), unless stated otherwise, and standard purity was 97% for β -carotene and 95 and 90% for α - and δ -tocopherol, respectively. High-performance liquid chromatography (HPLC)-grade methanol, acetone, *n*-hexane, and ethanol were purchased from J.T. Baker (Baker Mallinckrodt, Mexico). HPLC-grade water was prepared by a Milli-Qplus purification system (Millipore Corp., Bedford, MA).

Materials. About 3–5 kg (depending upon the fruit size) each of eight horticultural crops were selected on the basis of ripeness, freedom of damage, and uniformity of size and color. These were strawberries (*Fragaria ananassa* L. cv Sweet Charlie), guava (*Psidium guajava* L. cv Media China), mango (*Mangifera indica* L. cv Ataulfo), papaya (*Carica papaya* cv Maradol), prickly pear fruit (*Opuntia ficus-indica* M. cv Reina), prickly pear cladodes, known in Mexico as “nopal” (*Opuntia ficus-indica* M. cv Milpa Alta), avocado (*Persea americana*

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M. cv Hass), and black sapote (*Diospyros digyna* J.). Guava, mango, papaya, black sapote, and avocado were obtained from the local markets in Querétaro, Mexico, at different times during the period of March–May 2006, while strawberries, prickly pear fruit, and nopal were harvested from Guanajuato and San Luis Potosi, Mexico, on July 2006. All products were taken to the Laboratory of Phytochemicals and Nutrition of the Faculty of Natural Sciences of the Autonomous University of Querétaro. All products were used at the maturity preferred by consumers for their fresh consumption. Guava, mango, papaya, avocado, strawberries, and prickly pear fruit were at full ripe stage, while black sapote was at its overripe stage.

Sample Preparation and Measurements of Physiological and Quality Indices. Upon arrival to the laboratory, fruits were washed and physically and chemically characterized by measuring weight, internal and external color, and total soluble solids content (TSS, °Brix), and representative samples were freeze-dried until reaching a constant weight, for moisture content analysis. °Brix was measured in the juice obtained from a representative portion of each fruit, using a temperature-adjusted hand refractometer (ATAGO, Co. Ltd., Osaka, Japan). Color was measured with a Minolta spectrophotometer (Minolta, Co. Ltd., Osaka, Japan), which was calibrated with the white pattern during each sampling time. External color was longitudinally determined on three points of each flat side of the fruit (six points for each fruit). For flesh (internal) color, a big slice from a flat side of each fruit was obtained and color was determined longitudinally on three equidistant points. L^* , a^* , b^* , C^* , and h° values were recorded. All different samples were then frozen with liquid nitrogen and kept at -80°C until freeze-dried within about 1–4 weeks. Freeze-dried samples were kept in the dark at -80°C during the whole period of analysis, which lasted about 8 weeks.

Analysis of Antioxidant Compounds. TSP was extracted as reported (6), with some modifications. A total of 1 g of freeze-dried powder samples was homogenized in 20 mL of 80% acetone using an Ultra Turrax model T25 basic homogenizer (IKA Works, Willmington, NC) at room temperature. The homogenate was sonicated for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT) and then centrifuged at 19000g for 15 min at 2°C . The supernatant was collected, and the residue was subjected to an additional extraction process under the same previously mentioned conditions. Both supernatants were mixed and evaporated at 20°C and low pressure in a rotary evaporator. The concentrate was diluted with 25 mL of methanol. This was further increased to 50 mL with HPLC-grade water and filtered through a $0.45\ \mu\text{m}$ membrane, and aliquots were taken for analysis. The extraction process was performed in triplicate. For TSP quantification, aliquots were diluted 1:10 with HPLC-grade water, and 30 μL of diluted sample per hole was placed in 96-hole plates, and 150 μL of Folin–Ciocalteu reagent (dilution 1:10) and 120 μL of 7.5% Na_2CO_3 were added. The plates were incubated for 2 h in the dark, and absorbance at 630 nm was measured using a Dynex MRX microplate reader (Dynex Technol. Chantilly, VA). Results were expressed as milligrams of gallic acid equivalents (GAE)/100 g of fresh weight (fw).

Vitamin C determination was carried out as reported (7), with some modifications. Samples of 0.5 g of freeze-dried powder were homogenized in 10 mL of extraction solution [0.1 M citric acid and 0.05% ethylenediaminetetraacetic acid (EDTA) at pH 2.35–2.40] using an IKA T25 basic homogenizer (IKA Works, Willmington, NC). Then, the homogenate was centrifuged at 15000g for 10 min at 2°C . The supernatant was separated and filtered through Sep-Pak C18 Vac 3 mL cartridge (Waters Co., Milford, CT). The first 5 mL was discarded, and the next 3 mL was analyzed. The cartridge had been previously conditioned with 10 mL of ethanol and then with 10 mL of HPLC-grade water. The residual water was expelled with air. A total of 3 mL of the sample was collected, and 1 mL (0.832 mg/mL) of 1,2-phenylenediamine prepared in methanol/water (5:95, v/v) was added. The samples were incubated for 37 min in the dark and filtered through a $0.45\ \mu\text{m}$ nylon membrane. Aliquots of 40 μL were injected in a HP 1100 Series HPLC (Hewlett-Packard/Agilent Technologies Co., Palo Alto, CA). A $300 \times 3.9\ \text{mm i.d.}$, 10 μm , $\mu\text{Bondapak C18}$ column and a $20 \times 3.9\ \text{mm i.d.}$, 10 μm C18 guard column (Waters Co., Milford, CT) were used. The mobile phase consisted of 5 mM hexadecyltrimethylammonium bromide (cetrimide) and 50 mM KH_2PO_4 in methanol/

water (1:99, v/v) at pH 4.6. The flow rate was 1.5 mL/min. Ascorbic acid was monitored at 261 nm, and dehydroascorbic acid was monitored at 348 nm. Calibration curves were prepared from standards and used for quantification. The concentration range and correlation coefficients (r^2) for the calibration curves were 0–0.4 mg/mL and 0.9979 for ascorbic acid and 0–0.3 mg/mL and 0.9999 for dehydroascorbic acid.

Vitamin E was determined as reported previously (8). Samples of 0.5 g of freeze-dried powder were homogenized in 10 mL of HPLC-grade methanol, stirred at 55 rpm in a water bath at 30°C for 15 min, and centrifuged at 5000g for 5 min. The supernatant was filtered through a $0.45\ \mu\text{m}$ nylon membrane, and 20 μL of filtered sample was injected into the HPLC. A $150 \times 4.6\ \text{mm i.d.}$, 3.5 μm , Symmetry C18 column (Waters Co., Milford, CT) was used. HPLC-grade methanol (100%) was employed as a mobile phase, at a flow rate of 0.5 mL/min. For α - and γ -tocopherols detection, a model FLD G1321A fluorescence detector (Agilent Technologies Co., Palo Alto, CA) at an excitation wavelength of 294 nm and emission wavelength of 325 nm was used. Calibration curves for quantification were prepared using standards of α - and γ -tocopherols, respectively. The concentration range and correlation coefficients (r^2) for the calibration curves were 0–10 $\mu\text{g/mL}$ and 0.9999 and 0–10 $\mu\text{g/mL}$ and 0.9999 for α - and γ -tocopherols, respectively.

β -Carotene and TC extractions were performed using the 970.64-AOAC method (9), with some modifications (10). Samples of 0.5 g of freeze-dried powder were mixed with 10 mL of hexane/acetone/toluene/ethanol (10:7:7:6, v/v/v/v) solution and 1 mL of 40% KOH in methanol, stirred at 56°C for 20 min, and cooled with faucet water, and 10 mL of 10% Na_2SO_4 was added, stirred, and incubated in the dark until phase separation (approximately 10 min). The top phase was separated and used for analysis. TC quantification was measured on top-phase aliquots in a Beckman DU-65 spectrophotometer at 450 nm. A calibration curve was established using β -carotene in hexane as the standard and hexane as the blank. For β -carotene quantification, one top-phase aliquot was filtered through a $0.45\ \mu\text{m}$ nylon membrane (Pall Co., Ann Arbor, MI) and injected (10 μL) to the HPLC, equipped with a diode-array detector. A $150 \times 4.6\ \text{mm i.d.}$, 3.5 μm , Symmetry C18 column (Waters Co., Milford, CT) was used. Acetonitrile/methanol/dichloromethane (82:10:8, v/v/v) was employed as the mobile phase at a flow rate of 2 mL/min. Analysis were monitored using HP ChemStation software version A.06.03 (Hewlett-Packard/Agilent Technologies Co., Palo Alto, CA). A calibration curve (from 0 to 10 $\mu\text{g/mL}$) was prepared with β -carotene, and measurements were again performed in triplicate.

Extracts Preparation and AOC Analysis. LPE and HPE were obtained as reported (11), with some modifications. Samples of 1 g of freeze-dried powder were homogenized in 10 mL of hexane/dichloromethane (1:1, v/v) using an Ultra Turrax model T25 Basic homogenizer (IKA Works, Willmington, NC). The homogenate was sonicated for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT) and then centrifuged at 15000g for 10 min at 4°C . The supernatant was collected, and the residue was again subjected to the same extraction process. Both supernatants were mixed and dried at 40°C and low pressure. The dried extract was resuspended in 10 mL of HPLC-grade acetone, filtered through a $0.45\ \mu\text{m}$ nylon membrane, and designated as LPE. The residue, after the second extraction process, was dried with nitrogen, and the dried residue was homogenized in 20 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v), then sonicated, and centrifuged as previously described for LPE. The supernatant was collected, and the extraction process was repeated with the pellet. Both supernatants were mixed and designated as hydrophilic extract (HPE). LPE and HPE were used for AOC analysis, except for ORAC and TEAC.

DPPH assay was performed as reported (12), with some modifications, using a microplate reader. Aliquots of 280 μL of 100 μM DPPH/methanol solution per well were placed in the same row of a 96-well plates, and then, 20 μL of extracts, diluted to different concentrations, was added to each well to complete 300 μL . Aliquots of 300 μL of methanol were placed in the first row as a blank. The plates were incubated for 30 min in the dark, and measurements were taken at 490 nm in a MRX microplate reader (Dynex Technology, Chantilly, VA).

Table 1. TSS (°Brix), Weight, Moisture Content, and Internal and External Color of Eight Horticultural Crops^a

	products							
	nopal	papaya	guava	black sapote	avocado	mango	strawberry	prickly pear
TSS (°Brix)	4.13 ± 0.10	10.96 ± 1.30	11.73 ± 0.46	17.87 ± 0.98	9.63 ± 0.20	21.14 ± 1.64	9.52 ± 0.34	13.33 ± 1.01
weight (g)	49.3 ± 9.80	1997.2 ± 409	71.35 ± 6.25	179.97 ± 17.34	211.7 ± 33.70	345.5 ± 67.10	9.74 ± 2.90	109.61 ± 18.70
moisture (%)	93.51 ± 0.10	90.04 ± 1.36	80.09 ± 0.12	71.35 ± 6.25	65.62 ± 2.58	75.63 ± 0.75	88.84 ± 0.20	80.53 ± 0.59
internal color								
croma (C)		35.34 ± 2.40	23.37 ± 2.98	16.83 ± 3.77	36.79 ± 2.78	55.19 ± 6.50	35.16 ± 3.60	14.68 ± 2.21
hue (°h)		48.13 ± 2.49	83.83 ± 2.56	10.25 ± 4.75	93.14 ± 1.12	64.21 ± 4.28	30.64 ± 1.23	88.77 ± 4.65
external color								
croma (C)	15.23 ± 3.10	36.54 ± 0.27	47.44 ± 3.93	7.85 ± 1.12	4.27 ± 0.89	43.27 ± 2.20	21.69 ± 2.86	27.64 ± 4.62
hue (h*)	103.7 ± 3.40	58.68 ± 1.39	87.48 ± 1.42	53.13 ± 14.64	31.69 ± 12	62.20 ± 2.72	14.44 ± 3.11	105.70 ± 3.29

^aData are means of three repetitions and two duplicates ± standard deviation (SD).

Results (AOC) were expressed as Trolox equivalents (TE) in $\mu\text{mol}/100\text{ g fw}$ (13).

DMPD assay was carried out as reported (14), with some modifications, using a microplate reader. An aliquot of 1 mL of 100 mM DMPD solution, prepared with HPLC-grade water, was added to 100 mL of 100 mM acetate buffer (pH 5.25), and then 200 μL of 50 mM FeCl_3 was added to generate the $\text{DMPD}^{+\cdot}$ radical solution. Aliquots of 280 μL of $\text{DMPD}^{+\cdot}$ radical solution and 20 μL of different extract dilutions (or 20 μL Trolox) were added to 96-well plates. The plates were then incubated in the dark for 30 min and read at 490 nm in a MRX microplate. $\text{DMPD}^{+\cdot}$ radical solution was used as a control, and results (AOC) were expressed as TE in $\mu\text{mol}/100\text{ g fw}$.

For FRAP assay (15), aliquots of 280 μL of FRAP reagent were placed in 96-well plates, and 20 μL of extracts, diluted to different concentrations, was added in each well. The plates were incubated for 30 min in the dark and read using a filter of 630 nm in a MRX microplate reader. Calibration curves were prepared using Trolox as a standard, and results were expressed as TE in $\mu\text{mol}/100\text{ g fw}$. FRAP reagent was prepared with 50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 10 mM 2,4,6-tripyridyl-2-triazine (TPTZ) in 40 mM of HCl, and 5 mL of 20 mM FeCl_3 .

For the TOSC assay (16), vials with product extracts were prepared, adding 700 μL of phosphate buffer (100 mM, pH 7.4) and 100 μL of 2 mM KMBA. Hydrophilic (diluted at 1:50) or lipophilic (nondiluted) extracts (100 μL) were added; vials were sealed; and 100 μL of 200 mM ABAP was injected through septum into each vial. The vials were then stirred for 5 s and incubated at 39 °C for 90 min. Next, 100 μL of vial-headspace gas was taken and injected in a HP 5890 Series II gas chromatograph (Agilent Technologies, Palo Alto, CA), equipped with a 30 m \times 0.53 mm i.d. capillary column HP-PLOT Q (J&W Scientific, Agilent Technologies, CA). Operating conditions were 180 and 230 °C in the injection port and detector, respectively. Oven temperature started at 130 °C for 1 min, increased to 180 °C at a rate of 70 °C/min, and finally maintained at 180 °C for 3 min. Nitrogen was used as the carrier gas at 1.76 kg/cm²; a flame ionization detector was employed; and the analysis of the data was carried out using HP Chemstation software version A.08.03 (Agilent Technologies).

For ORAC assay, samples of 0.1 g of freeze-dried powder in 10 mL of water was vigorously stirred and sonicated for 5 min at 37 °C and centrifuged at 14000g for 20 min (17). The reaction mixture was prepared with the following preincubated (at 37 °C for 15 min) solutions: 1.7 mL of 75 mM phosphate buffer (pH 7.0), 100 μL of fluorescein (0.1 mg/L), 100 μL of 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH) (0.16 mg/L), and 100 μL of extracts. The reaction was initiated by adding AAPH, and the fluorescein depletion was monitored every 5 min until the residual was less than 5% (~60 min). Excitation and emission wavelengths were 484 and 515 nm, respectively. A LS 55 spectrofluorometer (Perkin-Elmer, Norwalk, CT) was used. The equation

$$\text{AUC} = (0.5 + f_5/f_0 + f_{10}/f_0 + f_{20}/f_0 + f_{25}/f_0 + f_{30}/f_0 + \dots f_{60}/f_0) \times 5 \quad (1)$$

was used to calculate the area under the curve (AUC), where f_0 is the fluorescein concentration at time zero and f_i is the fluorescein concentration at time i , expressed as μmol of TE/100 g fw. The net

AUC was obtained by subtracting the blank AUC from sample AUC. A phosphate buffer was used as the blank, and a calibration curve was established on the basis of Trolox dilutions.

For TEAC assay (18), samples of 0.2 g of freeze-dried powder were mixed with 10 mL of dichloromethane and 10 mL of water. The mixture was stirred for 2 min and centrifuged at 1000 rpm for 15 min. Two phases were obtained, and the extraction process was repeated for the aqueous phase. Both dichloromethane extracts were mixed and subjected to evaporation at 30 °C in a rotary evaporator. The dried extract was resuspended in 1 mL of dichloromethane and designated as LPE, which was used for AOC analysis. The precipitated pellet from the aqueous phase was treated with 20 mL of methanol and sonicated for 30 min. The sample was then filtered and evaporated to expel the solvent. The methanol-free aqueous extract was designated as HPE and used for AOC analysis. The $\text{ABTS}^{+\cdot}$ radical was generated by mixing 5 mL of 7 mM 2,2'-azobis-(3-ethylbenzotriazoline-6-sulfonic) acid (ABTS) with 88 μL of 140 mM $\text{K}_2\text{S}_2\text{O}_8$ and incubating it for 16 h in the dark. Then, 970 μL of $\text{ABTS}^{+\cdot}$ solution was mixed with 30 μL of extracts or Trolox standard solution in methanol. Absorbance was monitored at 734 nm at 1 and 6 min; the percentage of inhibition was calculated; and results were expressed as μmol of TE/100 g fw.

Statistical and Correlation Analysis. The contents of the antioxidant components were analyzed according to a completely randomized experimental design. Analysis of variance and LSD test ($\alpha = 0.05$) were used for comparison of means. Results were presented as means of three replications and two duplicates. For AOC analysis, a block design was used, in which every product was a block and every method was a treatment. Correlation analyses were performed for the contents of TSP, vitamin C, vitamin E, β -carotene, and TC in the eight horticultural products, and the AOC of HPE and LPE was measured using the six different antioxidant assays.

RESULTS AND DISCUSSION

Characterization of the Horticultural Products. Physical and chemical characteristics of the eight tested horticultural products were determined at the beginning of the experiment to establish the ripeness stage and quality based on internal and external color, total soluble solids, and moisture content (Table 1). Avocado and nopal had the lowest (65.6%) and highest (93.5%) moisture contents, respectively. Mango and black sapote had the highest TSS values (21.1 and 17.8%, respectively), while nopal had the lowest value (4.13%). All moisture and TSS values were similar to others previously reported (19, 20). Color data (Table 1) correspond to the stage of maturity for each product, indicating that papaya, guava, avocado, mango, strawberries, and prickly pear fruit were ripe, black sapote was overripe, and nopal was at its horticultural maturity stage as commonly used for human consumption.

Antioxidant Compounds. Vitamin C, vitamin E, β -carotene, TSP, and TC were the antioxidant compounds quantified. Guava had the highest TSP content (459.8 mg of GAE/100 g fw), followed by strawberries (256.1), black sapote (247.8), nopal (209.1), avocado (118.3), and prickly pear fruit (72.3), and

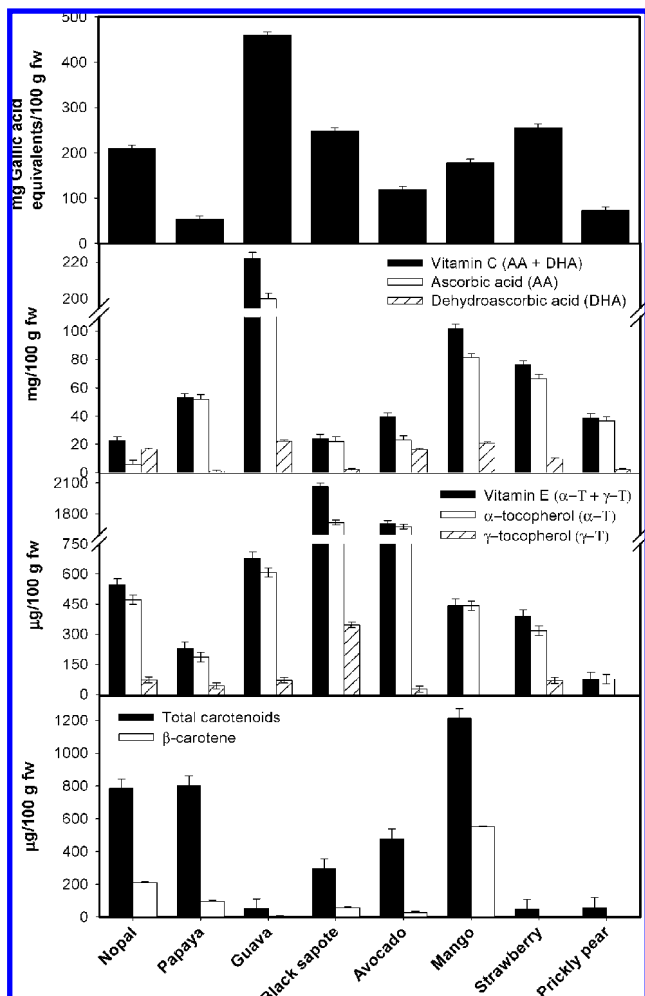


Figure 1. Total soluble phenolics, vitamin C, vitamin E, and total carotenoids and β -carotene content in eight horticultural crops.

papaya (53.8) had the lowest contents among the products tested (**Figure 1**). Some of the results obtained in this investigation were similar to other published reports, but others are different, depending upon the product in question. TSP content for some tropical fruits marketed in Florida, where 231.67 mg/100 g fw for red guava (cv. Sardina), 50.89 mg/100 g fw for mango (cv. Keitt), and 44.22 mg/100 g fw for papaya (cv. Red Lady) (21). Guava, avocado, mango, and papaya had 247.3, 24.2, 56, and 57.6 mg/100 g fw, respectively (22). Papaya had the lowest TSP content among the group of products tested in several reports, which agrees with the results herein (21, 22).

Because L-dehydroascorbic acid shows biological activity and it can easily be converted to L-ascorbic acid by humans, the sum of ascorbic acid and L-dehydroascorbic acid was regarded as vitamin C. Guava fruit had the highest levels of vitamin C content (222 mg/100 g fw), whereas the lowest levels were found in black sapote (24.1 mg/100 g fw) and nopal (22.2 mg/100 g fw); the remaining fruits had low contents of vitamin C ranging from 38.5 to 104.1 mg/100 g fw (**Figure 1**). L-Ascorbic acid was the main contributor to the total vitamin C content (>85%), with the exception of nopal and avocado, which had a contribution of 26 and 58%, respectively. Similar results for ascorbic acid content were found in papaya (456.2 mg/100 g fw) and strawberry (53.9 mg/100 g fw) (23). The daily recommended intake of vitamin C for the Mexican population (from 19 years of age and older) is 60 mg/day (24), and therefore, a portion of 100 g of strawberries, guava, or mango would satisfy this recommended daily intake.

Vitamin E content was calculated from the sum of α - and γ -tocopherol content, because both compounds exhibit vitamin E activity, with α -tocopherol as the main contributor (higher than 80%). All of the evaluated products showed significant differences ($p < 0.05$) in vitamin E content, with the exception of mango and strawberries, which showed similar contents. The highest vitamin E content was found in black sapote (2064 $\mu\text{g}/100 \text{ g fw}$), followed by avocado (1704 $\mu\text{g}/100 \text{ g fw}$), guava (677 $\mu\text{g}/100 \text{ g fw}$), nopal (544 $\mu\text{g}/100 \text{ g fw}$), mango (442 $\mu\text{g}/100 \text{ g fw}$), strawberries (388 $\mu\text{g}/100 \text{ g fw}$), papaya (229 $\mu\text{g}/100 \text{ g fw}$), and prickly pear fruit (77 $\mu\text{g}/100 \text{ g fw}$). γ -Tocopherol was not detected in mango and prickly pear fruit (**Figure 1**). Similar results were reported by ref 25 for a nonspecified variety of avocado and for strawberry. The daily recommended intake of vitamin E for the Mexican population, from 19 years of age and older, is 10 mg/day (24), and therefore, a portion of 100 g of the studied products covers between 0.8 and 20.6% of these recommended daily intakes. Black sapote and avocado can be considered as good sources of vitamin E.

β -Carotene and TC Contents. The content of β -carotene was highest in “Ataulfo” mango but was not detected in strawberries and prickly pear fruit (**Figure 1**). “Ataulfo” mango also had the highest content of TC, while prickly pear fruit, guava, and strawberry had the lowest contents (**Figure 1**). A high correlation ($r = 0.887$, $p < 0.01$) was observed between TC and β -carotene, and β -carotene was the main component of total carotenoids in the products studied. However, there are variations between the results reported in our work and those reported by others. For example, 1050 $\mu\text{g}/100 \text{ g fw}$ of β -carotene content was reported for “Maradol” papaya (20), a value 10 times higher than what was obtained in our investigation. This variability could be due to several factors, such as type of product, cultivar, differences in food constituencies, stage of ripeness, and methods used for extraction and quantification (5).

AOC Measured by Six Different Assays. Results of AOC from HPE (**Figure 2**) ranged from 78.9 to 6230.6 μmol of TE/100 g fw with DPPH assay, from 798.4 to 15420.4 μmol of TE/100 g fw with DMPD assay, from 257.1 to 7482.9 μmol of TE/100 g fw with FRAP assay, from 330.4 to 8591 μmol of TE/100 g fw with ORAC assay, from 222.1 to 2243.8 μmol of TE/100 g fw with TEAC assay, and from 2055.5 to 8235.6 μmol of TE/100 g fw with TOSC assay. LPE values ranged from 0 to 91.2 μmol of TE/100 g fw with DPPH assay, from 0 to 145.2 μmol of TE/100 g fw with DMPD assay, from 8.4 to 31.8 μmol of TE/100 g fw with TEAC assay, and from 13.7 to 61 μmol of TE/100 g fw with TOSC assay. For HPE, three response levels for AOC can be established: high, intermediate, and low, with DMPD corresponding to the high level, DPPH, FRAP, ORAC, and TOSC corresponding to the intermediate level, and TEAC corresponding to the low level.

In general, values of HPE for AOC were much higher than values of LPE; AOC from HPE was about 95 times higher than AOC from LPE. The HPE from guava had the highest AOC value among the products tested when evaluated with DMPD, DPPH, FRAP, TEAC, and TOSC assays, whereas in black sapote, HPE had the highest AOC but only when measured with the ORAC assay. On the other hand, HPE of papaya and prickly pear fruit showed the lowest AOC values for all of the assays.

DMPD assay exhibited the highest AOC among all of the assays, in both HPE and LPE, being up to 6 times higher than that of the other assays in the case of HPE. However, caution must be taken when evaluating organic-acid-rich products by

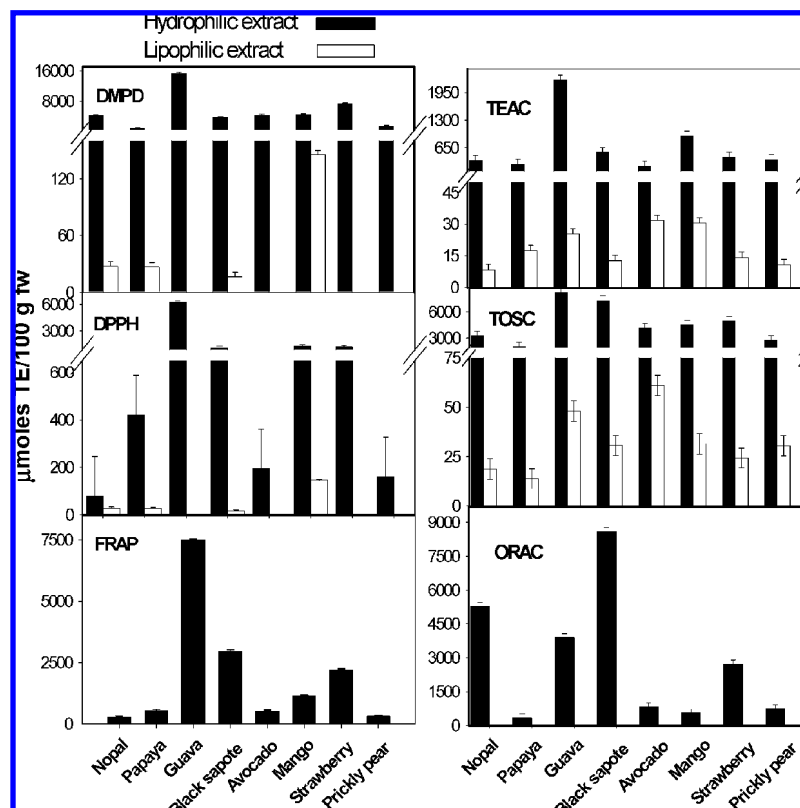


Figure 2. Antioxidant capacity of hydrophilic and lipophilic extracts in eight horticultural crops measured by six different assays. DPPH, 2,2'-diphenyl-1-picrylhydrazyl; DMPD, *N,N*-dimethyl-*p*-phenylenediamine; FRAP, ferric ion reducing antioxidant power; ORAC, oxygen radical absorbance capacity; TEAC, Trolox equivalent antioxidant capacity; and TOSC, total oxidant scavenging capacity.

DMPD assay because interferences have been reported for these compounds, mainly by citric acid (26).

Apparently, the high AOC results from DMPD assay found herein can be explained by organic acid interferences, because fruits are normally rich in organic acids, especially in the case of young cladodes of nopal (27). Therefore, unless organic acids are removed or neutralized from the HPE, the DMPD procedure should be avoided when measuring AOC in fruits. However, DMPD exhibited the highest AOC value for LPE, being only 1.6 times higher than the second highest value (DPPH), which does not seem to be out of proportion. This fact is in favor of the use of DMPD assay to determine AOC from LPE, because organic acids are removed during the lipophilic extraction procedure. Although FRAP assay presented acceptable results for AOC measurement of HPE, this method did not show AOC for LPE. This is a limitation that may be due to restrictions of the method to be able to detect compounds acting under mechanisms of transference of hydrogen atoms, such as thiols, proteins, and carotenoids (15).

TEAC assay showed the lowest levels of AOC for both HPE and LPE, among the assays tested, showing about a third part of AOC as compared to the other assays (excluding DMPD assay), while DPPH, FRAP, ORAC and TOSC showed approximately the same AOC values for HPE. These results can be due to the fact that the TEAC antioxidant compound extraction procedure is based on an aqueous dichloromethane solution, which is different from the other methods. If this is true, the capacity of different antioxidant compound extraction procedures for different types of compounds (hydrophilic and lipophilic) should be tested to standardize an extraction method. Methods for extraction and quantification are suggested to be a source of variability for AOC (5).

Table 2. Correlation Coefficients of Linear Regression among the Individual Antioxidant Constituents and the Antioxidant Activity Tested by Different Methods^a

	total soluble phenolics	vitamin C	vitamin E	β -carotene	total carotenoids
DMPD	0.94 ^b	0.88 ^b	0.00	0.98 ^{b,c}	0.87 ^{b,c}
DPPH	0.88 ^b	0.95 ^b	0.00	0.02	0.12
FRAP	0.92 ^b	0.85 ^b	0.02	0.07	0.22
ORAC	0.94 ^b	0.79	0.33	0.03	0.06
TEAC	0.84 ^b	0.95 ^b	0.00	0.00	0.05
TOSC	0.94 ^b	0.90 ^b	0.50 ^c	0.02	0.15

^a DPPH, 2,2'-diphenyl-1-picrylhydrazyl; DMPD, *N,N*-dimethyl-*p*-phenylenediamine; FRAP, ferric ion reducing antioxidant power; ORAC, oxygen radical absorbance capacity; TEAC, Trolox equivalent antioxidant capacity; and TOSC, total oxidant scavenging capacity. All data correspond to hydrophilic extracts, except for footnote c. ^b Significant at $p \leq 0.01$. ^c From lipophilic extracts.

DPPH exhibited its measurement response of AOC at a level similar to FRAP, ORAC, and TOSC levels for HPE. Furthermore, DPPH showed an acceptable level of AOC measurement for LPE. The DPPH method has been widely used, because of its stability, simplicity, and reproducibility (28). These results indicate that DPPH is recommended for analyzing AOC of fruits and vegetables, along with TOSC or DMPD assays for LPE.

Correlation Analysis between AOC and Antioxidant Compounds. DPPH Method. From HPE and LPE, correlation analyses were performed between AOC and vitamin C, TSP, vitamin E, β -carotene, and TC. For HPE, results showed a high correlation between AOC and TSP ($r = 0.88$, $p < 0.01$) and between AOC and vitamin C ($r = 0.95$, $p < 0.01$) (Table 2). For LPE, there was no correlation between AOC and vitamin E, β -carotene, or TC. From these results for this method, it is clear that AOC in HPE is mainly due to TSP and vitamin C.

Some studies performed with DPPH assay have established a high correlation between AOC and TSP but a low correlation with vitamin C (3, 22, 29).

DMPD Method. AOC for HPE was highly correlated with TSP ($r = 0.938$, $p < 0.001$) and vitamin C ($r = 0.88$, $p < 0.001$), indicating that these two compounds are very much responsible for the AOC in HPE (Table 2). No similar reports have been found in the literature for this method. On the other hand, in LPE, AOC showed a high correlation with β -carotene and TC content. Similar results were obtained for different tomato cultivars (30). DMPD assay was the one unique method showing high correlation between AOC and TC or β -carotene (Table 2).

FRAP Method. For HPE, results showed a high correlation between AOC and TSP ($r = 0.92$, $p < 0.01$) and also between AOC and vitamin C ($r = 0.85$, $p < 0.01$). Other investigators have reported similar results (4), while others (31) showed a high correlation only between AOC and vitamin C.

TOSC Method. For HPE, results showed a high correlation between AOC and TSP ($r = 0.88$, $p < 0.01$), but there was no correlation between AOC and vitamin C ($r = 0.604$, $p > 0.05$). However, when black sapote data was excluded from the regression analysis because of its atypical behavior, the correlation was significant for both compounds (Table 2). For LPE, AOC showed a weak correlation with vitamin E ($r = 0.50$, $p < 0.05$). No reports have been found on AOC in relation to vitamin C using this method or regarding the relationship of lipophilic antioxidant compounds in fruits and vegetables.

ORAC Method. For HPE, no correlation between AOC and TSP and vitamin C was observed. However, excluding data from black sapote and nopal, the correlation was significant for TSP ($r = 0.94$, $p < 0.01$). Black sapote and nopal exhibited a high AOC by ORAC (Figure 2). These results could be due to the differences in extraction procedures between the ORAC method and the rest of the assays. The ORAC assay uses a very simple aqueous extraction procedure, while the other methods (except for TEAC) use an extraction procedure based on acetone/water/acetic acid (70:29.5:0.5) solution. Furthermore, compounds different than TSP and vitamin C can be important contributors to the AOC of black sapote and nopal. Most studies demonstrate a high correlation between AOC and TSP by ORAC, but with regard to vitamin C, results have been contradictory (21, 29, 32).

TEAC Method. For HPE, results showed a high correlation between AOC and TSP ($r = 0.84$, $p < 0.01$) and between AOC and vitamin C ($r = 0.95$, $p < 0.01$). For LPE, results did not show a correlation between AOC and any of the evaluated antioxidant compounds.

In conclusion, for hydrophilic extracts, total soluble phenols and vitamin C contents were highly correlated with total antioxidant capacity when evaluated using the six assays. On the other hand, for lipophilic extracts, β -carotene and total carotenoids contents were highly correlated with the total antioxidant capacity only when measured with the DMPD assay.

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