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Antioxidant Activity of the Phenolic and Oily Fractions of Different Sweet Bell Peppers

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Abstract. The content of phenols and ascorbic acid of the phenolic fraction, and carotenoids, tocopherols and capsaicinoids of the oily fraction from sweet bell peppers from northwest Mexico was determined. Antioxidant activity in both fractions was evaluated (ABTS and DPPH methods). Green cultivar had the highest content of phenols, flavonoids, and ascorbic acid and highest antioxidant activity. α-Tocopherolwas found in the four cultivars; however, capsaicinoids were not detected. The phenolic fraction had higher antioxidant activity than the oily fraction.
Key words: *Capsicum annuum*, bell pepper, antioxidant activity, phenols, carotenoids, tocopherols, ascorbic acid.

Resumen. Se determinó el contenido de fenoles y ácido ascórbico de la fracción fenólica y carotenoides, tocoferoles y capsaicinoides de la fracción oleosa de chiles bell del noroeste de México. Se evaluó la actividad antioxidante de ambas fracciones (métodos ABTS y DFPH). El cultivar verde tuvo el mayor contenido de fenoles, flavonoides y ácido ascórbico y la más alta actividad antioxidante. Se encontró α-tocoferol en los cuatro cultivares; sin embargo, no se detectaron capsaicinoides. La fracción fenólica presentó mayor actividad antioxidante que la oleosa.
Palabras clave: *Capsicum annuum*, chile bell, actividad antioxidante, fenoles, carotenoides, tocoferoles, ácido ascórbico.

Introduction

Peppers (*Capsicum annuum* L.) are important vegetables that are used fresh or as a spice. The international market for peppers is continuously growing and world production grew from 17'289,616 metric tons in 1997 to 26'056,900 metric tons in 2007 [1]. México is the second largest producer of peppers in the world, with more than one hundred varieties comprising 22 groups of fresh peppers, either pungent or sweet [2]. Among the sweet peppers, the bell variety is one of the most important; most of the bell peppers grown in México are exported to the USA. In 2009, the USA purchased 98.3% of the Mexican production of peppers (705 million dollars) [2].

Bell peppers are some of the most popular fresh vegetables in the world, because of the combination of color, taste, and nutritional value. They are considered a good source of bioactive compounds, such as vitamins, pro-vitamins, and antioxidant compounds [3, 4, 5]. Peppers contain high levels of vitamin C comparable to the levels in citrus fruits, and other vegetables recognized as good sources of this vitamin [3, 6]. The intake of these bioactive compounds provides beneficial effects to health, due to antioxidant properties that offer protection to cells against oxidative damage, and thus prevent the development of common degenerative conditions such as cancer, cardiovascular disease, cataract, diabetes, Alzheimer's and Parkinson's [5, 7, 8, 9].

At present, the most studied phytochemicals in plants are phenolic compounds because it has been observed that they have different properties, primarily as antioxidants. Many studies have focused on the antioxidant activity of the phenolic fraction of the plants [4,8,9], disregarding the antioxidant activity of the oily fraction, in which compounds, such as vitamins A and E, have been shown to be effective free radical scavengers [5,10], can be found.

In northwest México, bell peppers of high quality (green, Orion; red, Mazurka; orange, Simpaty; and yellow, Taranto) are produced, primarily for exportation to USA. These cultivar s have not been studied yet. The present work aimed to determine the content of phenols and ascorbic acid of the phenolic fraction, and carotenoids, tocopherols, and capsaicinoids of the oily fraction; and antioxidant activity in both fractions of different cultivars of sweet bell peppers (green, red, orange, and yellow) harvested in northwest México.

Results and Discussion

Phenolic compounds

Green bell pepper had the highest total phenol content, and no significant differences ($p > 0.05$) between red, yellow, and orange were observed (Table 1). The total flavonoid content of green bell peppers was the highest, followed by the red cultivar (Mazurca). The value of the total phenol content [fresh weight (fw), data not shown] of pepper cultivars harvested in México was similar to the values reported for peppers from Yunnan China by Zhuang *et al*. [11] with the exception of sweet red pepper, in which the level of total phenol (2.10 ± 0.08 mg/g GAE fw) was slightly higher than that of the Mexican cultivar. However, the values found in our study were two fold higher than that of the total phenol content (0.045 mg/g dw) reported by Deepa *et al*. [12] in the same cultivar (Mazurca) grown in Holland.

Table 1. Phenolic contents in the different sweet bell peppers.

	Green	Red	Orange	Yellow
Total Phenols (mg GAE/g dw)	14.80 ± 0.58[b]	12.89 ± 0.73[a]	12.35 ± 0.13[a]	12.90 ± 0.22[a]
Total Flavonoids (mg CE/g dw)	7.53 ± 0.08[c]	4.80 ± 0.13[b]	4.26 ± 0.23[a]	4.27 ± 0.10[a]
Caffeic Acid (μg/g dw)	108.82 ± 3.73[c]	67.78 ± 3.14[b]	38.03 ± 3.43[a]	52.42 ± 0.26[b]
Chlorogenic Acid (μg/g dw)	290.08 ± 4.24[d]	221.53 ± 6.53[c]	117.54 ± 4.54[a]	136.51 ± 8.29[b]
Myricetin (μg/g dw)	658.19 ± 2.95[d]	244.33 ± 8.67[c]	100.62 ± 3.47[a]	151.35 ± 7.50[b]
Quercetin (μg/g dw)	Nd	9.97 ± 0.34	Nd	Nd
Luteolin (μg/g dw)	Nd	Nd	154.03 ± 1.61	Nd
Resveratrol (μg/g dw)	174.34 ± 2.00[c]	111.57 ± 2.49[b]	89.72 ± 1.09[a]	90.78 ± 1.72[a]

[a-d] Significant differences (p<0.05) are expressed by different letters in the same row.
Nd= not detected.
Detection limit of quercetin and luteolin: ≤5 μg/mL and 7 μg/mL, respectively.

In green and red bell peppers, the total phenol content was 1.2- and 4.9-fold higher, respectively, compared with the values reported by Gorinstein *et al*. [8]. The total flavonoid content of green and red bell pepper was 9.9- and 1.8-fold greater, respectively, than that reported by Gorinstein *et al*. [8]. Ninfali *et al*. [13] reported 1.58 mg of caffeic acid equivalents (CAE)/g fw of total phenols in red pepper (*Capsicum frutescens*) from Italy. These results agreed with the content of total phenols of red, orange, and yellow bell peppers harvested in México (data not shown).

Caffeic acid, and chlorogenic acid were the hydroxycinnamic acids identified in the different cultivars, in addition to the flavonoids myricetin, quercetin, and luteolin; the latter two were present only in the red, and orange cultivars. The green cultivar had the highest content of caffeic and chlorogenic acids, and myricetin, followed by the red cultivar. Sakakibara *et al*. [14] reported these compounds, in addition to quercetin, and luteolin, in peppers from a market in Japan.

The accumulation of bioactive compounds is determined by factors internal to the organism (genotype), but it can be strongly modified by the conditions of the growing season [15, 16]. However, environmental factors contributing significantly to the differences among cultivars can be minimized when the fruits are grown in semi-controlled conditions. Therefore, the differences in the phenolic levels of the studied bell peppers were mainly due to genetic differences because the conditions of growth for the four cultivars analyzed were similar. The genetic differences of each cultivar can lead to differences in the biosynthetic pathways and fruit composition [17].

Ascorbic acid

Green bell pepper contained the most amount of ascorbic acid, followed by the red and yellow bell peppers ($p < 0.05$) (Table 2). The red and yellow cultivars were not significantly different ($p > 0.05$). Matsufuji *et al*. [5] and Marín *et al*. [6] reported higher ascorbic acid content in other bell pepper cultivars.

Factors that may influence to the ascorbic acid content are the genetic and environmental factors. Manthey and Perkins-Veazie [18] and Olsson *et al*. [15] found that harvest date had no significant effect on the ascorbic acid content. Chassy *et al*. [19] found that genotype has the greatest influence on the level of phytochemicals in fruits and vegetables. For these reasons, in this study, the genotype is the factor that may have greater impact on the ascorbic acid content because the environmental factors and growth conditions were the same for all cultivars.

Carotenoids and chlorophylls in bell peppers

Red cultivar showed the highest content of total carotenoid, while green cultivar showed the lowest (Table 3). The green cultivar contained the most amounts of chlorophyll-a, and -b; the latter is responsible for the color of green pepper. Three important carotenoids were detected: zeaxanthin, lutein, and β-carotene. Orange and red bell peppers contained the highest level of β-carotene, followed by the yellow pepper; while the green cultivar had the lowest level ($p < 0.05$). In contrast, Sun *et al*. [4] found higher contents of β-carotene in the green and red cultivars of bell pepper. These differences could be attributed to the different weather and growing conditions prevailing in the two studies. With the methodology [20] used in the present work lutein and zeaxanthin were not separated; however, it has been reported that lutein is absent in red peppers and zeaxanthin in green peppers [21]. The color of sweet bell peppers is the attribute most appreciated by consumers. The green color is due to the chlorophyll and carotenoids typical of the chloroplasts [6].

The higher or lower carotenoid content for a given cultivar depends on various factors: greater or lesser expression of the genes governing carotenogenesis, physiological and morphological characteristics intrinsic to the cultivar, and growth conditions [17]. The last factors can be ignored in the present study because the conditions of growth for the four cultivars analyzed were similar.

Tocopherols

Only α-tocopherol was detected in the pericarp of bell pepper cultivars. The content varied between 0.98, and 3.65 mg/g dw (Table 2), with the highest amount in the red cultivar, and the

Table 2. Ascorbic acid and α-tocopherol contents in the different sweet bell peppers (mg/g dw).

	Green	Red	Orange	Yellow
Ascorbic Acid	1.74 ± 0.03[c]	0.58 ± 0.03[b]	0.49 ± 0.01[a]	0.58 ± 0.03[b]
α -tocopherol	0.98 ± 0.04[a]	3.65 ± 0.03[d]	1.92 ± 0.03[c]	1.23 ± 0.04[b]

[a-d] Significant differences ($p < 0.05$) are expressed by different letters in the same row.

Table 3. Carotenoid contents in the different sweet bell peppers (μg/g dw).

	Green	Red	Orange	Yellow
Carotenoids				
Total carotenoids	1513.5 ± 59.2[a]	7137.0 ± 50.7[d]	5292.7 ± 55.8[c]	2236.3 ± 40.6[b]
Zeaxanthin	Nd	8.8 ± 0.2	Nd	Nd
Lutein	70.2 ± 1.2	Nd	Nd	Nd
Zx + Lt			36.4 ± 1.5[b]	5.7 ± 0.2[a]
β-carotene	12.2 ± 0.8[a]	43.9 ± 1.6[c]	56.6 ± 1.0[d]	15.9 ± 1.6[b]
Chlorophylls				
Chl a	409.1 ± 12.9[d]	284.7 ± 7.6[c]	198.3 ± 15.9[b]	34.5 ± 3.6[a]
Chl b	72.1 ± 1.5[c]	12.2 ± 0.8[a]	31.2 ± 1.8[b]	11.5 ± 1.8[a]

[a-d] Significant differences ($p < 0.05$) are expressed by different letters in the same row.
Zx: zeaxanthin. Lt: lutein.
Nd = Not detected.
Detection limit for lutein and zeaxanthin: 1.1 μg/mL and 0.019 μg/mL, respectively.

lowest in the green. Other studies have identified α-tocopherol as the major component of the pericarp of other pepper varieties unlike other isomers, such as γ-tocopherol, which is found mainly in pepper seeds [22]. Other authors have reported the lowest amounts of α-tocopherol (0.0049 to 0.078 mg/g fw; 0.138 mg/g dw) in different bell cultivars [5] and other varieties [22]. A given plant growing in different geographical regions may have different vitamin contents [23].

Genotype or variety differences could also contribute to the magnitude of differences observed. Fanasca *et al*. [24] evaluated the effect of cultivar, and fertilization on the content of tocopherols in tomato plants; they found that only the cultivar had a significant effect on the α-tocopherol content.

Therefore, the differences in α-tocopherol levels of the bell pepper cultivars analyzed in this study are mainly due to the genotype. The environmental factors are minimal because the fruits were grown under the same conditions.

Capsaicinoids

Capsaicinoids were not detected in the bell pepper cultivars (detection limit ≤ 0.08 mg/L of extract for capsaicin and dihydrocapsaicin). Tanaka *et al*. [25] did not find capsaicinoids or capsinoids in different cultivars of sweet bell peppers. Unlike hot peppers, sweet bell peppers lack pungency. The pungency is the result of the accumulation of capsaicinoids.

Antioxidant activity

All fractions (phenolic and oily) showed antioxidant activity by ABTS and DPPH methods. Deepa *et al*. [12] did not find antioxidant activity in lipophilic extracts measured by the ferric reducing/antioxidant power (FRAP) method, which requires acidic (pH = 3.6) conditions to maintain iron solubility [26]. It is known that some compounds, such as carotenoids, may undergo isomerization in an acidic environment, and consequently lose their activity [27]. There are no significant differences between the ABTS assay and FRAP assay, except that ABTS is performed at neutral pH [26]. The ABTS cation radical can be solubilized in both aqueous and organic media, and it is not affected by ionic strength; thus, the antioxidant capacity can be measured due to the hydrophilic and lipophilic nature of the compounds. In contrast, DPPH is a stable hydrophobic radical [28] that can only be dissolved in organic media (especially in alcohol), not in aqueous media. Consequently, this method is suitable for measuring the antioxidant activity of hydrophobic compounds (*e.g.*, carotenoids and tocopherols) [26].

TEAC assay

The phenolic fraction of the green cultivar showed higher antioxidant activity than the other cultivars (red, orange, and yellow), whereas the oily fraction (carotenoids + tocopherols) of the orange cultivar had the highest antioxidant activity (Figure 1A).

Rochín-Wong *et al*. [29] reported a correlation coefficient of R = 0.93 among total phenols and antioxidant activity (ABTS) of chiltepín; while in this study we obtained a correlation coefficient of R = 0.76. Such differences could be due to the type and content of phenolics present. A significant correlation between chlorogenic acid (R = 0.91), resveratrol (R = 0.89), caffeic acid (R = 0.84), myricetin (R = 0.84), vitamin C

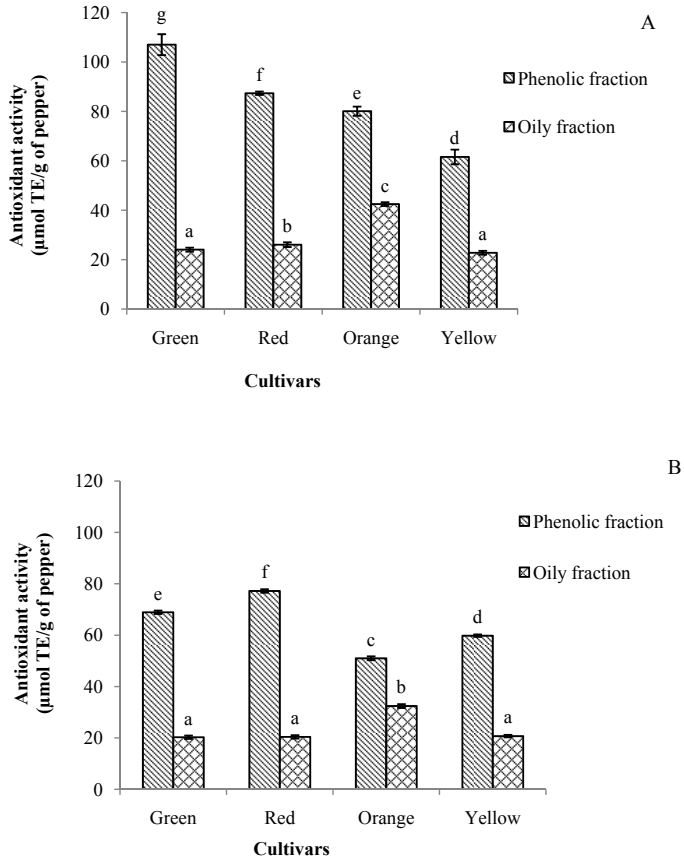

Fig. 1. Antioxidant activity of extracts of different bell pepper cultivars measured by ABTS (A) and DPPH (B) radicals. Data are the mean of three determinations. Different letters within each cultivar mean statistical difference by Tukey's least significant difference test ($p < 0.05$).

(R = 0.81), total carotenoids (R = 0.85), and β-carotene (R = 0.85) with antioxidant activity was found. This indicates that all of these compounds have a significant effect on the antioxidant activity. Hervert-Hernández *et al*. [9] reported activity in the range of 26.6-44.4 µmol TE/g dw for guajillo, morita, chipotle, and árbol peppers. Álvarez-Parrilla *et al*. [30] reported antioxidant activity ranging from 28.64 to 55.41 µmol TE/g dw for chipotle, serrano, and jalapeño peppers from México. The results of both investigations were lower than most of the results obtained in the present investigation.

DPPH• radical scavenging activity

The phenolic fractions showed a higher antioxidant activity than the oily fractions (carotenoids + tocopherols) (Figure 1B). The activity of the phenolic extracts of the peppers was significantly different. The red pepper extract showed the highest antioxidant activity, and the orange pepper extract showed the lowest. In contrast, in the oily extracts, the orange pepper extract showed the highest ability to quench free radicals. Sun *et al*. [4] reported lower values (2.1-3.9 µmol TE/g fw) for the antioxidant activity of phenolic extracts of similar cultivars of bell peppers (green, red, orange, and yellow) than those found in this study (4.91-8.80 µmol TE/g fw). However, a similar trend was noted in that the highest values of antioxidant activity were obtained for the red cultivar. The same trend was observed by others [5].

In this study, a correlation of R = 0.44 between total phenols and antioxidant activity (DPPH) was observed. Nsimba *et al*. [31] reported weak correlations between total phenolic content and antioxidant activity (DPPH) in amaranth and quinone extracts. However, the correlation between total flavonoids and antioxidant activity was higher (R = 0.95) than for total phenols [32]. Flavonoid activity is based on the number and location of hydroxyl groups present, as well as the presence of a 2-3 double bond, and 4-oxo function [33].

Conclusions

Bell peppers studied in this work possess antioxidant activity, and are a good source of phenolics, carotenoids, and tocopherols. The green pepper contains the highest levels of phenolic compounds and ascorbic acid. The red and orange peppers contain the highest levels of total carotenoids and α-tocopherol. The results of the antioxidant activity assays indicated that the phenolic fraction had the highest activity compared with the oily fraction (carotenoids and tocopherols); thus, these results may suggest a higher potential of the bell peppers for the maintenance of health. In addition, it can be concluded that the oily fraction also showed antioxidant activity.

Material and Methods

Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, caffeic acid, myricetin, chlorogenic acid, zeaxanthin, lutein, β-carotene, chlorophyll-a, chlorophyll-b, α-tocopherol, ABTS [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)], capsaicin, and dihydrocapsaicin were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). All of the solvents were analytical grade, and purchased from JT Baker (Xalostoc, México State, México), and EM Science (Gibbstown, New Jersey, USA).

Samples

Four commercially important cultivars of pepper (*Capsicum annuum* L.), green, red, orange, and yellow (Orion, Mazurca, Simpaty, and Taranto, respectively), were selected from Guadalupe de Guaymas S.P.R. de R. L. agricultural field in northwest México (28° 14' 37.08" N; 110° 39' 03.82" W) on December 2009. Batches of 10 kg, for each cultivar, from different boxes stored in the agricultural field were kindly donated.

Peppers studied were grown in a greenhouse with similar conditions of temperature, humidity and fertilizer. Peppers were harvested at the same time. The peppers were transported from

the greenhouse to the Department of Scientific and Technological Research, Universidad de Sonora, México. Peppers without visible damage were selected and washed with distilled water. The pericarp of each cultivar was cut into slices (5 × 0.5 cm). The samples for carotenoids, tocopherols, and capsaicinoids analyses were lyophilized. Fresh and lyophilized samples were stored at –20°C until the analyses.

Phenolic compounds extraction

Approximately 5 g (fresh weight, fw) of minced bell pepper fruit from each cultivar was placed in conical tubes with 10 mL of methanol:water (70:30, v/v). The mixture was then sonicated (Sonic 1510 R-DTH, Branson Ultrasonics Corporation, Danbury, Connecticut, USA) for 30 min, and centrifuged (7,000 *g*) at 4°C for 15 min (Centrifuge IEC CL3 IR, Thermo Electron Industries SAS, Château-Gontier, Mayenne, France). The supernatant was filtered through Whatman N° 2 paper. The methanolic extraction occurred in the dark at room temperature (20 ± 2 °C), and was repeated twice to ensure maximum extraction of all the compounds. The extracts were frozen at –20°C until analysis [34].

Total phenol and flavonoid determinations

The phenolic compounds in the extracts were spectrophotometrically determined at 765 nm using the Folin-Ciocalteau 1N reagent, and gallic acid as standard. The results are reported as mg of gallic acid equivalents (GAE) per g of dry sample [32].

Flavonoid content was determined by a colorimetric assay [34]. Two mL of extract was mixed with 4 mL of deionized water, and 300 µL of $NaNO_2$:water (5:95, w/v). After 5 min, 300 µL of $AlCl_3$:water (10:90, w/v) was added, and after another minute, 2 mL of 1 M NaOH was added. The final volume was brought up to 10 mL with deionized water and stirred, and read at 510 nm (UV-Vis spectrophotometer Cary 100, Varian, Australia PTY LTD, Melbourne, Victoria, Australia). Total flavonoids were expressed on a dry weight (dw) basis as mg of catechin equivalents (CE) per g dw [8].

Phenolic compounds quantification

The identification of phenolic compounds was performed according to the procedure suggested by Cantos *et al*. [35]. Fifty µL of methanolic extract (0.25 g fresh sample mL^{-1} of methanol:water (70:30, v/v) was analyzed by liquid chromatography (HPLC Pro Star 230, Varian, Palo Alto, California, USA) using a Supelcosil™ LC18 column (30 × 0.4 cm × 5 µm particle size, Supelco, Bellefonte, Pennsylvania, USA), and an ultraviolet detector (model 9050, Varian, Palo Alto, California, USA). The solvents were water:formic acid (95:5, v/v) (solvent A), and HPLC grade methanol (solvent B). Elution was performed with a gradient starting with 2% of B to reach 32% of B at 30 min, 40% of B at 40 min, and 95% of B at 50 min and then isocratic for 5 min at a flow rate of 1.5 mL/min. The identification of individual phenolic compounds was performed by comparison with the retention times of standards and the absorption spectra. For quantification, calibration curves for each identified phenolic compound were established. Detection was at 280 nm for flavanones, flavanols, and hydroxycinnamic acids, at 320 nm for flavones, and at 360 nm for flavonols [14, 36].

Ascorbic acid quantification

Fruit tissue (10 g) was homogenized for 2 min with 50 mL of an aqueous solution containing 30g/L of metaphosphoric acid, and 80 mL/L of acetic acid. The homogenate was filtered, and then centrifuged for 15 min at 7000 *g*. The supernatant was filtered through filter paper (0.22 µm). The ascorbic acid content was quantified by an HPLC system equipped with a UV-Vis detector, water bondapack-NH_2 analytical column (3.9 × 300 mm, 10 µm), and 10 µL loop injector. The mobile phase was acetonitrile:KH_2PO_4 (75:25, w/w) at a flow rate of 1.5 mL/min. The absorbance was read at 268 nm [37]. The ascorbic acid concentration was calculated using an external standard, and expressed as mg ascorbic acid per g of dry weight.

Carotenoids and tocopherols extraction

Preliminary tests were run using lyophilized samples, fresh samples, and different solvent systems [acetone, chloroform: methanol (1:1, v/v), chloroform:methanol (1:2, v/v), ethanol 96%, ethanol 80%, and chloroform:methanol (2:1, v/v)] to ensure the optimum extraction of carotenoids, and tocopherols from bell peppers. Chloroform:methanol (1:1, v/v) gave the highest efficiency for the extraction of carotenoids and tocopherols, as well as the extracts with the highest antioxidant activity. For that reason, in the present study, a sample of 0.5 g of each pepper was extracted with chloroform:methanol (1:1, v/v). The mixture was then sonicated for 30 min, and centrifuged (7,000 *g*) at 4°C for 15 min. The supernatant was filtered through Whatman N° 2 paper. The oily extraction was conducted in the dark at room temperature (20 ± 2°C), and repeated twice to ensure maximum extraction of all the compounds. The extracts were stored (–20°C) until the analyses of carotenoids and tocopherols.

Carotenoids quantification

Total carotenoids were quantified at 460 nm [38]. Identification and quantification of carotenoids and chlorophylls were carried out with a gradient program [20] at a flow rate of 1.7 mL/min. Fifty µL of oily extract (0.025 g/mL solvent) was analyzed by liquid chromatography using a Supelcosil™ LC18 column and an ultraviolet detector. The identification of individual compounds was accomplished by comparison with the retention times of standards and the absorption spectra. For quantification, calibration curves were developed for each identified compound. The absorbance was read at 450 nm.

Tocopherols quantification

Five milliliters of oily extract were refluxed with nitrogen, and resuspended in 1 mL of hexane for the HPLC analysis. The tocopherol content was determined by HPLC according to the Ce 8-89 method [39]. The samples (100 µL) were directly injected into a chromatograph equipped with an ultraviolet detector. A normal-phase Supelco LC-Si column (15 mm×4.6 mm, 0.5 µm), Sigma-Aldrich Química, Toluca, México state, México) was used and operated at room temperature. The mobile phase was hexane:isopropanol (99.5:0.5, v/v) at a flow rate of 1.5 mL/min. Tocopherols were measured at 292 nm. The peaks of the chromatogram were identified by comparing their retention times to those of standards. Quantification was carried out using calibration curves for α-tocopherol, γ-tocopherol, and δ-tocopherol.

Capsaicinoids quantification

A sample of 0.5 g of lyophilized pepper was extracted with acetonitrile (5 mL), heated at 80°C for 4 h, and then centrifuged for 15 min at 7000 g and filtered (0.20 µm) [40]. Identification and quantification of capsaicinoids were carried out according to Tanaka *et al*. [25]. Fifty µL of extract (0.1 g/mL of acetonitrile) was analyzed by liquid chromatography using a Supelcosil^{TM} LC18 column, and an ultraviolet detector. The mobile phase was methanol:water (70:30, v/v) at a flow rate of 1.0 mL/min. Calibration curves were established for capsaicin and dihydrocapsaicin at 280 nm.

Antioxidant capacity evaluation

For the measurement of the antioxidant activity of the pepper extracts, two methods were used. The first method evaluated the trolox equivalent antioxidant capacity (TEAC) is based on the reduction of green/blue coloration produced by the reaction of $ABTS^{\bullet+}$ with the antioxidant present. A volume of 0.1 mL of each extract was mixed with 3.9 mL of the radical solution, and then the absorbance was read at 754 nm after 7-min of reaction at room temperature using ethanol as the control. The absorbance differential (Absi – Absf) was converted to the inhibition percentage, and the antioxidant activity was calculated in umoles of trolox equivalent (TE) per g of dry sample using a calibration curve of trolox from 0.00 to 0.89 µm/mL [34]. ABTS is one of the most effective methods for evaluating antioxidant activity in food due to the hydrophilic and lipophilic nature of antioxidant components present in fruits [28].

The second method, which is based on the reduction of DPPH in the presence of antioxidants, the antioxidant activity is detected as a change from purple to yellow color in the solution. A volume of 3.9 mL of DPPH solution and 0.1 mL of each extract were mixed. The reaction was carried out for 30 min, and the absorbance was measured at 515 nm. The changes in absorbance at the beginning and at the end of the reaction were transformed to percentage of inhibition. The results were expressed as µm trolox equivalents/g of dry sample using a calibration curve of trolox from 0.00 to 0.93 µm/mL [34].

Statistical analysis

The results are presented as the mean of triplicates of determinations of bioactive compounds and antioxidant activity± SD. Significant differences between means were detected by one-way analysis of variance (ANOVA), followed by multiple comparisons using Tukey's least significant difference test. Differences were considered significant when $p < 0.05$. A statistical analysis was performed to establish the correlation between the antioxidant activity determined by the ABTS and DPPH assays with the different bioactive compounds in each cultivar. All statistical analyses were conducted using the SPSS 17.0 statistical package (IBM, New York, New York, USA).

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References

1. http://usda.mannlib.cornell.edu/MannUsda/homepage.do,accessed in October, 2012.
2. http://www.siap.gob.mx/images/stories/ infogramas/100705-monografia-chile.pdf, accessed in October, 2012.
3. Martínez, S.; Curros, A.; Bermúdez, J.; Carballo, J; Franco, I. *Int. J. Food Sci. Tech.* **2007**, *58*, 150-161.
4. Sun, T.;Xu, Z.; Wu, C.T.; Janes, M.; Prinyawiwatkul, W.; No, H. K. *J. Food Sci.* **2007**, *72*, S98-S102.
5. Matsufuji, H.; Ishikawa, K.; Nunomura, O.; Chino, M.; Takeda, M. *Int. J. Food Sci. Tech.* **2007**, *42*, 1482-1488.
6. Marín, A.; Ferreres, F.; Tomas-Barberán, F.A.; Gil, M.I. *J. Agric. Food Chem.* **2004**, *52*, 3861-3869.
7. Kaur, C.H; Kapoor, H.C. *Int. J. Food Sci. Tech.* **2001**, *36*, 703–725.
8. Gorinstein, S.; Yong-Seo, P.; Buk-Gu, H.; Namiesnik, J.; Leontowicz, H.; Leontowicz, M.; Kyung-Sik, H.;Ja-Yong, C.; Seong-Gook, K. *Eur. Food Res. Tech*. **2009**, *228*, 903-911.
9. Hervert-Hernández, D.; Sáyago-Ayerdi, S.G.; Goñi, I. *J. Agric. Food Chem.* 2010, *58*, 3399-3406.
10. Seddon, J.M.; Ajani, U.A.; Sperduto, R.D; Hiller, R.; Blair, N.; Burton, T.C.; Farber, M.D.; Gragoudas, E.S.; Haller, J.; Miller, D.T.; Yannuzzi, L.A.; Willet, W. *JAMA, J. Am. Med. Assoc*. **1994**, *272*, 1413-1420.
11. Zhuang, Y.; Chen, L.; Sun, L.; Cao, J. *J. Funct. Foods.* **2012**, *4*, 331-338.
12. Deepa, N.; Kaur, C.; George, B.; Singh, B.; Kapoor, H.C.*LWT-Food Sci. Technol*. **2007**, *40*, 121-129.
13. Ninfali, P.; Mea, G.; Giorgini, S.; Rocchi, M.; Bacchiocca, M. *Br. J. Nutr.* **2005**, *93*, 257-266.
14. Sakakibara, H.; Honda, Y.; Nakagawa, S.; Ashida, H.; Kanazawa, K. *J. Agric. Food Chem*. **2003**, *51*, 571-581.
15. Olsson, M.E.; Ekvall, J.; Gustavsson, K.E.; Nilsson, J.; Pillai, D.; Sjöholm, I.; Svensson, U.; Akesson, B.; Nyman, M.G. *J. Agric. Food Chem.* **2004**, *52*, 2490-2498.

16. Lata, B.; Tomala, K. *J. Agric. Food Chem*. **2007***, 55*, 10795-10802.
17. Hornero-Méndez, D.; Gómez-Ladrón de Guevara, R.; Mínguez-Mosquera, M.I.*J. Agric. Food Chem*. **2000**, *48*, 3857-3864.
18. Manthey, J.A.; Perkins-Veazie, P. *J. Agric. Food Chem*. **2009**, *57*, 10825-10830.
19. Chassy, A.W.; Bui, L.; Renaud, E.N.C.; Van Horn, M.; Mitchell, A.E. *J. Agric. Food Chem*. **2006**, *54*, 8244-8252.
20. Mínguez-Mosquera, M.I.; Hornero-Méndez, D. *J. Agric. Food Chem*. **1993**, *41*, 1616-1620.
21. Marín, A.; Gil, M.I.; Flores, P.; Hellín, P.; Selma, M.V. *J. Agric. Food Chem*. **2008**, *56*, 11334-11341.
22. Hitasomi, E.; Matsui, M.; Kubota, K.; Kobayashi, A. *J. Agric. Food Chem*. **2000**, *48*, 4924-4928.
23. Ching, L.S.; Mohamed, S. *J. Agric. Food Chem*. **2001**, *49*, 3101-3105.
24. Fanasca, S.; Colla, G.; Maiani, G.; Venneria, E.; Rouphael, Y.; Azzini, E.; Saccardo, F. *J. Agric. Food Chem*.**2006**, *54*, 4319-4325.
25. Tanaka, Y.; Hosokowa, M.; Otsu, K.; Watanabe, T.; Yazawa, S. *J. Agric. Food Chem*. **2009**, *57*, 5407-5412.
26. Karadag, A.; Ozcelik, B.; Samim, S. *Food Anal. Method.* **2009**, *2*, 41-60.
27. Meléndez-Martínez, A.; Vicario, I.; Heredia, F. *Arch. Latinoam. Nutr*. **2004**, *54*, 209-215.
28. Arnao, M.B. *Trends Food Sci. Technol*. **2000**, *11*, 419-421.
29. Rochín-Wong, C.S.; Gámez-Meza, N.; Montoya-Ballesteros, L.C.; Medina-Juárez, L.A. *Rev. Mex. Ing. Quim*. In press.
30. Álvarez-Parrilla, E.; De la Rosa, L.A.; Amarowicz, R.; Shahidi, F. *J. Agric. Food Chem*. **2011**, *59*, 163-173.
31. Nsimba, R.Y.; Kikuzaki, H.; Konishi, Y. *Food Chem*. **2008**, *106*, 760-766.
32. Xu, B.J.; Chang, S.K.C.*J. Food Sci*. **2007**, *72*, S159-S166.
33. Rice-Evans, C.A.; Miller, N.J.; Paganga, G. *Free Radical Biol. Med*. **1996**, *20*, 933-956.
34. Molina-Quijada, D.M.A.; Medina-Juárez, L.A.; González-Aguilar, G.A.; Robles-Sánchez, R.M.; Gámez-Meza, N. *CyTA- Journal of Food*. **2010**, *8*, 57-63.
35. Cantos, E.; García-Viguera, C.; De Pascual-Teresa, S.; Tomás-Barberán, F.A. *J. Agric. Food Chem*. **2000**, *48*, 4606-4612.
36. Shan, B.; Cai,Y.Z.; Sun, M.; Corke, H. *J. Agric. Food Chem*. **2005**, *53*, 7749-7759.
37. González-Aguilar, G.A.; Ruiz-Cruz, S.; Soto-Valdéz, H.; Vázquez-Ortiz, F.; Pacheco-Aguilar, R.; Wang, C.Y. *Int J. Food Sci. Tech*. **2005**, *40*, 377–383.
38. Davies, B.H., in: Chemistry and Biochemistry of Plant Pigments, Vol. 2, Goodwin, T.W., Ed., London Academic Press, London, **1976**, 38-165.
39. AOCS. Official Methods and Recommended Practices of the American Oil Chemists' Society (6th edn). AOCS Press, Urbana, IL (**2009**).
40. Estrada, B.; Bernal, M.; Díaz, J.; Pomar, F.; Merino, F. *J. Agric. Food Chem*. **2000**, *48*, 6234-6239.