

**ANTIOXIDANT ACTIVITY OF PEPPERS (*Capsicum annuum* L.)
EXTRACTS AND CHARACTERIZATION OF THEIR PHENOLIC
CONSTITUENTS**

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SUMMARY

The aim of this study was to characterize the phenolic constituents and evaluate the antioxidant activity of five pepper (*Capsicum annuum* L.) cultivars harvested in the same season, geographic area and climatic conditions. Phenols, flavonoids and ascorbic acid of Anaheim, Bell, Caribe, Jalapeno and Serrano peppers were quantified, and antioxidant activity of their extracts were evaluated by the method of radical scavenging of DPPH• and ABTS•+. It was found that Serrano pepper had the highest ascorbic acid content, followed by Bell and Caribe, whereas the lowest values were found in Jalapeno and Anaheim. The highest contents of phenolic compounds

were in Caribe and Bell peppers. The total flavonoid contents ranged from 25.38 ±3.44 (Anaheim) to 60.36 ±9.94 mg QE/100g fw (Caribe). The Bell and Caribe extracts showed the highest ($p < 0.05$) stabilization of ABTS•+. The highest oxidation inhibition percentage for radical DPPH• was observed in Caribe extract, coinciding with the highest levels of gallic acid, chlorogenic acid, epicatechin, rutin, luteolin, resveratrol ($r \geq 0.85$) and ascorbic acid. In conclusion, among the pepper cultivars studied, Caribe and Bell showed to have the best antioxidant properties and can be suggested as preferable for human consumption.

Introduction

Epidemiological studies have found an inverse correlation between consumption

of fruits, vegetables and grains, and the risk of cardiovascular diseases and cancer (Ferrari and Torres, 2003; Ilow *et al.*, 2008). This is

attributed to the wide variety of nutrients and phytochemicals with beneficial biological effects to the human body. Among these effects is the

ability to stabilize reactive oxygen species (ROS) that are commonly generated in normal metabolism as well as under various types of stress.

KEYWORDS / Antioxidant Activity / *Capsicum annuum* / Flavonoids / Pepper / Polyphenolic Compounds /

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CAPACIDAD ANTIOXIDANTE DE EXTRACTOS DE PIMIENTOS (*Capsicum annuum* L.) Y CARACTERIZACIÓN DE SUS CONSTITUYENTES FENÓLICOS

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RESUMEN

El objetivo de este estudio fue caracterizar los constituyentes fenólicos y evaluar la actividad antioxidante de cinco cultivares de pimiento (*Capsicum annuum* L.) cosechados en la misma estación y región geográfica, y en semejantes condiciones climáticas. Se cuantificaron los fenoles, flavonoides y ácido ascórbico de los cultivares Anaheim, Bell, Caribe, Jalapeño y Serrano, y se evaluó la capacidad antioxidante por el método de estabilización de los radicales DPPH y ABTS⁺. Se encontró que el pimiento Serrano tuvo el contenido más alto de ácido ascórbico, seguido por Bell y Caribe, mientras que los valores más bajos se encontraron en Jalapeño y Anaheim. El mayor contenido de compuestos fenólicos fue en los pimientos Caribe

y Bell. El contenido de flavonoides totales fue de 25,38 ±3,44 (Anaheim) a 60,36 ±9,94 (Caribe) mg EQ/100g pf. Los extractos de Bell y Caribe fueron los que mostraron la estabilización más alta ($p < 0,05$) del ABTS⁺. El mayor porcentaje de inhibición de la oxidación de radicales DPPH se presentó en el extracto de Caribe, que coincidió con los niveles más altos de ácido gálico, ácido clorogénico, epicatequina, rutina, luteolina, resveratrol ($r \geq 0,85$) y el ácido ascórbico. En conclusión, entre los chiles estudiados, Caribe y Bell mostraron tener las mejores propiedades antioxidantes y pueden ser sugeridos como los preferibles para consumo humano.

CAPACIDADE ANTIOXIDANTE DE EXTRACTOS DE PIMENTA (*Capsicum annuum* L.) Y CARACTERIZAÇÃO DE SEUS CONSTITUÍNTES FENÓLICOS

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RESUMO

O objetivo deste estudo foi caracterizar os constituintes fenólicos e avaliar a atividade antioxidante de cinco cultivares de pimenta (*Capsicum annuum* L.) colhidos na mesma estação e região geográfica, e em semelhantes condições climáticas. Quantificaram-se os fenóis, flavonóides e ácido ascórbico dos cultivares Anaheim, Bell, Caribe, Jalapenho e Serrano, e se avaliou a capacidade antioxidante pelo método de estabilização dos radicais DPPH e ABTS⁺. Achou-se que a pimenta Serrano teve o conteúdo mais alto de ácido ascórbico, seguido por Bell e Caribe, enquanto que os valores mais baixos se encontraram em Jalapenho e Anaheim. O maior conteúdo de compostos fenóli-

cos foi nas pimentas Caribe e Bell. O conteúdo de flavonóides totais foi de 25,38 ±3,44 (Anaheim) a 60,36 ±9,94 (Caribe) mg EQ/100g pf. Os extratos de Bell e Caribe foram os que mostraram a estabilização mais alta ($p < 0,05$) do ABTS⁺. A maior porcentagem de inibição da oxidação de radicais DPPH se apresentou no extrato de Caribe, que coincidiu com os níveis mais altos de ácido gálico, ácido clorogênico, epicatequina, rutina, luteolina, resveratrol ($r \geq 0,85$) e o ácido ascórbico. Concluindo, entre os chiles estudados, Caribe e Bell mostraram ter as melhores propriedades antioxidantes e podem ser sugeridos como os preferíveis para consumo humano.

The ROS provoke damage to biomolecules such as proteins, RNA and DNA (Johnson and Loo, 2000). Hence the importance of the determination of the antioxidants present in different foods, and their possible role in human health.

Up to recent years, the protection against various diseases was attributed only to vitamin C (ascorbic acid), E (tocopherols) and carotenoids contained in vegetal foods. However, clinical and epidemiological studies have demonstrated that other phytochemicals present in fruits and vegetables are implicated

as antioxidants (Kaur and Kapoor, 2001).

At present, the most studied phytochemicals in plants are the phenolic compounds, because they have various functions in the human body, mainly as antioxidants (Kroon and Williamson, 2005). However, the composition and levels of individual phytochemicals with antioxidant potential present in vegetables do not necessarily reflect the total antioxidant capacity, which depends on the type and concentration of phytochemicals, as well as the synergistic or inhibitory interaction of molecules in the matrix. Therefore, it is important to

study the phytochemicals present in vegetables of high consumption in some countries, such as peppers, in order to generate information about their potential health benefits. The fruits of pepper (*Capsicum annuum* L.), are highly consumed in fresh form, processed or as spice in various dishes around the world. It has also been found that peppers are a good source of polyphenolic compounds with antioxidant properties (Howard *et al.*, 2000; Marin *et al.*, 2004).

The objective of this study was to identify and quantify the contents of phenolic acids, flavonoids and ascorbic acid

present in the pericarp of five pepper (*Capsicum annuum* L.) cultivars grown in Northwestern Mexico, and to evaluate their antioxidant activity.

Materials and Methods

Chemicals

The 6-hydroxy-2,5,7,8-tetraethyl-2-carboxylic acid (trolox), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical (ABTS⁺), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), potassium persulfate, Folin-Ciocalteu reagent and standards (gallic acid, caffeic

acid, catechin, chlorogenic acid, epicatechin, rutin, resveratrol, luteolin, and ascorbic acid) were obtained from Sigma-Aldrich Co. (Mexico). The solvents used were analytical and HPLC grade.

Sample collection

Five commercially important pepper (*Capsicum annuum* L.) cultivars (SNITT, 2003): Anaheim (green pepper), Bell (bell pepper), Caribe (blond pepper), Jalapeno (typical) and Serrano (typical) were selected for this study and harvested in November 2008. Fruits of commercial size, green color and physiologically mature stage were harvested in Zamora, Sonora, Mexico (29°16'30.16"N, 110°53'13.93"O and 285masl). After harvest, fruits were transported to the Department of Scientific and Technological Research, Universidad de Sonora, Mexico. Peppers (5kg) without visible damage or physiological defects were selected, washed with distilled water, cut crosswise in halves to remove seeds and the pericarp was cut in strips 5×0.5cm. The samples were stored up to one month at -20°C, until analysis.

Ascorbic acid determination

Ascorbic acid was determined as described by Doner and Hicks (1981). Fruit tissue (10g) was homogenized for 2min with 50ml of an aqueous solution containing 30g·l⁻¹ metaphosphoric acid and 80ml·l⁻¹ acetic acid. The homogenate was filtered and centrifuged for 15min at 10000rpm. The supernatant was filtered through filter paper (0.22µm). The ascorbic acid content was determined by means of a HPLC system (Varian 9012, Palo Alto, CA, USA) equipped with a UV-Vis detector (Varian 9050, Palo Alto, CA, USA), a water bondapack-NH₂ analytical column (3.9×300mm, 10µm), and a 10µl loop injector. The mobile phase was acetonitrile:KH₂PO₄ 0.05M (75:25

v/v) at a flow rate of 1.5ml·min⁻¹. Absorbance was read at 268nm. The ascorbic acid concentration was calculated using an external standard and expressed as mg ascorbic acid per 100g of fresh weight (fw; Robles-Sánchez *et al.*, 2009). All tests were performed in triplicate.

Methanolic extracts

Ten grams of homogeneous samples from 5kg of each cultivar of fresh pepper were placed in conical tubes with 20ml of aqueous methanol (70:30, v/v) and homogenized in an Ultra-Turrax T 25 basic S1 (IKA-Works Inc. Wilmington, NC, USA). The homogenate was then sonicated (Sonic 1510 R-DTH, Branson Ultrasonics Corporation, CT, USA) for 30min and centrifuged (16800g) (Centrifuge IEC CL3 IR, Thermo Electron Industries SAS, France) at 4°C for 15min. The supernatant was filtered thru Whatman N° 2 paper. The methanolic extraction was done in darkness at room temperature (20 ±2°C) and was repeated twice to ensure maximum extraction of all the compounds. The extracts (0.25g·ml⁻¹) were frozen at -20°C for 48h until to analysis (Chitindingu *et al.*, 2006).

Total phenols and flavonoids

The phenolic compounds in the extracts were spectrophotometrically determined at 750nm using the Folin-Ciocalteu 1N reagent and gallic acid as standard. The results are reported as mg of gallic acid equivalents (GAE) per 100g fresh sample (Molina-Quijada *et al.*, 2010). Flavonoid content was determined by colorimetric assay (Molina-Quijada *et al.*, 2010). In brief, 1ml of extract was mixed with 4ml of deionized distilled water and 0.3ml of 5% NaNO₂. After 5min, 0.3ml of 10% AlCl₃ was added, and after another minute, 2ml of 1M NaOH were added. The final volume was brought up to 10ml with deionized water,

stirred, and lectures were taken at 415nm (UV-VIS spectrophotometer Cary 100, Varian Australia PTY LTD, Australia). Total flavonoids were expressed on a fresh weight (fw) basis as milligrams of quercetin equivalents per gram.

Phenolic compounds identification

The identification of phenolic compounds was performed according to the procedure described by Ramamurthy *et al.* (1992). Twenty µl of methanolic extract (0.25g fresh sample/ml of methanol 70%) were analyzed by liquid chromatography (HPLC ProStar 230, Varian, CA, USA), using a Supelcosil™ LC18 column (30×0.4cm×5µm particle size, Supelco, Bellefonte, PA, USA) and an ultraviolet detector (model 9050, Varian, CA, USA). The elution started with 90% solvent A (acetic acid 2%) and 10% solvent B (acetic acid:acetonitrile:water, 2:30:68, v/v/v) up to 100% solvent B at 30min, with a flow rate of 1.5ml·min⁻¹. The identification of individual phenolic compounds was performed comparing the retention times of standards and its absorption spectrum.

For quantification, calibration curves were made for each of the identified phenolic compounds. Gallic acid, caffeic acid, catechin, and epicatechin were quantified at 280nm (wavelength at which maximum absorbance is present); resveratrol and chlorogenic acid at 320nm; and quercetin-3-rutinoside, known as rutin (flavonol), and luteolin (flavone) at 360nm.

Evaluation of antioxidant activity

For the measurement of the antioxidant activity of the pepper extracts, two methods were used. The first one evaluates the trolox equivalent antioxidant capacity (TEAC) and is based on the reduction of green/blue coloration produced by reaction of ABTS^{•+}

with the antioxidant present in the sample. A volume of 0.1ml of each methanolic extract (0.25g·ml⁻¹) was mixed with 3.9ml of the radical solution (3.84mg·ml⁻¹) and the absorbance was read at 754nm (UV-VIS spectrophotometer Cary 100, Varian Australia PTY Ltd., Australia) after 1min of reaction at room temperature, using ethanol as a control. The absorbance differential (Absi-Absf) was converted to inhibition percentage and antioxidant activity was calculated in µmoles equivalent trolox (ET) per g of fresh sample, using a calibration curve of trolox (an analogue of vitamin E water soluble) from 0.00 to 0.99mmol·ml⁻¹ (Kuskoski *et al.*, 2005).

The second method, the reduction of DPPH[•] in presence of antioxidants, is detected as a change of color (from purple to yellow) in the solution. A volume of 3.9ml of DPPH[•] solution (0.25mg·ml⁻¹ methanol) with 0.1ml of each of the dilutions of methanolic extracts (0.00 to 0.25g·ml⁻¹) were mixed. The reaction was carried out for 30min and the absorbance was measured at 515nm in the UV-VIS spectrophotometer. The changes in absorbance at the beginning and the end of the reaction were transformed into percentage of inhibition (Materska and Perucka, 2005).

Statistical analysis

All determinations were carried out in triplicate and data were subjected to analysis of variance (ANOVA). Statistical analyses were performed using SigmaStat version 3.5 (Point Richmond, CA, USA). Significant differences among means were determined by the Tukey test; p values <0.05 were considered statistically significant.

Results and Discussion

Ascorbic acid

Ascorbic acid contents of the various peppers ranged

from 121.14 to 251.60mg/100g fw (Table I). These results confirm that the consumption of 100g of fresh peppers provides the recommended daily administration of ascorbic acid (100-200mg; Lee and Kader, 2000). Serrano pepper presented higher ascorbic acid content than Bell and Caribe, whereas the lowest values were found in Jalapeno and Anaheim peppers. Zhuang *et al.* (2012) reported that the ascorbic acid content of peppers is mainly dependent on the cultivars. Peppers are considered an important source of ascorbic acid (170-280mg/100g); therefore, they have been attributed health benefits (Kumar and Tata, 2009). However, it seems that other compounds with high antioxidant potential such as polyphenols may also contribute in this context.

Total phenols

In general, high levels of total phenols were found in all the pepper extracts studied. Caribe pepper presented the highest value and Jalapeno pepper the lowest (Table I). Vinson *et al.* (1998) and Sun *et al.* (2007) reported lower levels of total phenols in Bell peppers than those found in the present study. Contrarily, Helmja *et al.* (2007) reported a higher content of these compounds in pungent pepper (480mg/100g fw). Similarly, Kevers *et al.* (2007) reported higher levels of total phenols in red, yellow and green peppers (296, 284 and 215mg/100g, respectively), even higher than those found in spinach, broccoli, cucumbers and carrots. It is well known that content of phytochemicals, including phenolic compounds present in vegetables, is affected by the specie and type of pepper, agronomic conditions, maturity (Righetto *et al.*, 2005), posthar-

TABLE I
PHENOLICS, FLAVONOIDS AND ASCORBIC ACID CONTENTS OF
FRESH PEPPERS (*Capsicum annum* L.)

Concentration (mg/100g)	Peppers				
	Anaheim	Bell	Caribe	Jalapeno	Serrano
Total phenolics (GAE)	97.99 b	103.26 b	154.30 c	59.34 a	94.85 b
Gallic acid	69.30 b	81.80 c	101.30 d	49.10 a	94.60 d
Caffeic acid	2.20 c	1.10 b	1.00 b	0.20 a	0.20 a
Chlorogenic acid	0.97 d	0.49 b	1.79 e	0.20 a	0.70 c
Flavonoids (QE)	25.38 a	38.64 b	60.36 c	28.77 a	32.73 a
Catechin	3.68 d	1.85 c	3.47 d	0.11 a	1.03 b
Epicatechin	7.35 d	3.70 c	7.93 d	0.10 a	1.17 b
Rutin	0.38 b	1.90 c	7.90 d	0.20 a	1.98 c
Luteolin	0.35 b	4.75 d	5.09 d	0.20 a	0.57 c
Stilbene					
Resveratrol	0.38 a	1.22 c	1.45 c	Nd	0.80 b
Ascorbic acid (AAE)	121.14 a	220.42 c	210.81 c	156.36 b	251.60 d

Values in each column with different letters (a-e) show a significant difference ($p < 0.05$). GAE: gallic acid equivalents, QE: quercetin equivalents, AAE: ascorbic acid equivalents. Nd: not detected.

vest handling and pre and postharvest treatments applied to the fruit (Howard *et al.*, 2000). A close correlation between total phenols and antioxidant activity ($r = 0.914$ for DPPH[•], and $r = 0.837$ for ABTS^{•+}) was found.

Total flavonoids

The total flavonoids content (Table I) was significantly higher ($p < 0.05$) in the Caribe pepper (60.36 \pm 9.95mg QE/100g fw), followed by Bell (38.64 \pm 0.37mg QE/100g fw). No significant differences ($p < 0.05$) were observed among Anaheim, Serrano and Jalapeno peppers. According to the botanical classification scale of Peterson and Dwyer (1998) for flavonoid concentration, peppers were classified as having high flavonoid content. Howard *et al.* (2000) reported flavonoid contents from 17.17 to 85.49mg QE/100g fw in similar pepper varieties (*Capsicum annum* L.). They observed a content of total flavonoids of 31.71mg QE/100mg fw for Bell pepper, similar to than those found in this study. Sun *et al.* (2007) reported total flavonoids from not detectable to 80mg QE/100mg fw in Bell peppers of different colors. Marinova *et al.* (2005) found a range of flavonoids between 13.7 (red pepper) and 27.4 (green pepper) mgQE/100g fw. These values contrast with those re-

ported by Materska and Perucka (2005), who observed greater contents of flavonoids in red peppers than in green ones. These variations in flavonoid and phenol contents have been associated to pepper maturity, type of cultivar and growing conditions (Howard *et al.*, 2000). The strongest correlation was between total flavonoids and antioxidant activity (DPPH[•], $r = 0.885$). There were also a significant positive but weaker correlation between total flavonoids and antioxidant activity (ABTS^{•+}, $r = 0.629$).

Identification of phenolic compounds

The main phenolic compounds identified in the five pepper cultivars were gallic acid, caffeic acid and chlorogenic acid, among phenolic acids; catechin, epicatechin, rutin and luteolin among flavonoids; and the stilbene, resveratrol (Table I). Gallic acid was the main phenolic compound found in all the pepper cultivars studied, with higher levels ($p < 0.05$) than the other compounds. Caribe and Serrano peppers showed the highest levels ($p < 0.05$) of gallic acid, followed by Bell, Anaheim and Jalapeno peppers.

Marín *et al.* (2004) reported similar levels of caffeic acid (1.37mg/100g fw) than those found in this study (1.10

\pm 0.50mg/100g fw) for Bell pepper. Estrada *et al.* (2000) found that phenolic acids content decreases with fruit ripening. It is possible that during this process, these compounds may participate in various metabolic reactions to form new compounds or retard oxidation reactions, thereby preventing the deterioration of the fruit. It is possible that the low presence of these compounds in advanced stages of fruit ripening makes it more vulnerable to oxidation reactions and could be

one of the causes of high susceptibility to microbial attack and deterioration (González-Aguilar *et al.*, 2010).

The major components in the flavonoid fraction were catechin, epicatechin and luteolin, which presented the highest levels ($p < 0.05$) in the extracts from Bell and Caribe peppers (Table I). Materska and Perucka (2005) found that luteolin levels ranged from 2.11 to 4.99mg/100g fw in four types of pepper with different maturity stages. Marín *et al.* (2004) reported similar levels of luteolin (4.22mg/100g fw) than those observed in this study for green Bell pepper. However, Kevers *et al.* (2007) found quercetin (56mg/100g fw) and kaempferol (26mg/100g fw) as the major flavonoids in green peppers stored for 34 days at 4°C. Probably these differences could be attributed to the type of pepper and conditions to which the fruits were exposed.

The Bell and Caribe extracts presented the highest resveratrol content ($p < 0.05$), while in jalapeno extract this product was not detected. These values of resveratrol may be responsible of high antioxidant capacity of Bell and Caribe peppers. Fabris *et al.* (2008) found an antioxidant activity of resveratrol similar to butylhydroxytoluene and α -tocopherol. This com-

pound is commonly found in higher concentrations in other fruits, such as grapes, cultivated in North-western Mexico (Molina-Quijada et al., 2010).

Antioxidant activity by ABTS^{•+} method

Bell and Caribe extracts showed a higher antioxidant activity (34.44 ± 0.43 and $33.60 \pm 1.35 \mu\text{M ET/g fw}$, respectively) than that observed in Anaheim, Serrano and Jalapeno extracts ($p < 0.05$; Figure 1). Sun and Tanumihardjo (2007) reported a lower antioxidant capacity (about $8.40 \mu\text{M ET/g fw}$) for Bell extract than that found in this study. Navarro et al. (2006) conducted a study on antioxidant activity under salt stress at different stages of Bell pepper ripening; they concluded that a moderate salinity causes a significant increase in the antioxidant capacity (ABTS^{•+}) of the peppers, as a natural defense mechanism of tissue against the stress. Pellegrini et al. (2003) reported that the highest antioxidant activity (ABTS^{•+}) of various studied vegetables, corresponded to Bell red pepper, with $8.40 \mu\text{M ET/g fw}$. This means that the antioxidant capacity depends of growing conditions, fruit maturity and the pepper type. A significant lineal correlation was found between resveratrol ($r = 0.873$), epicatechin ($r = 0.852$), luteolin ($r = 0.846$), and rutin ($r = 0.842$) with the free radical scavenging activity (ABTS^{•+}).

Antioxidant activity by DPPH[•] radical test

Figure 2 shows the inhibition percent values of peppers extracts ($0.25 \text{g} \cdot \text{ml}^{-1}$) on the radical DPPH[•]. Bell and Caribe extracts presented 60.41 and 83.44% inhibition, respectively, while Serrano, Anaheim and Jalapeno (44.66, 29.08 and 8.45%, respectively) did not reach 50% inhibition, despite the high content of ascorbic acid (Table I). Materska and

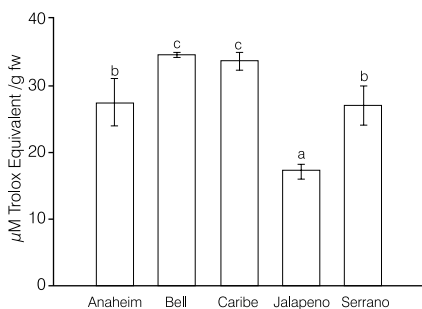


Figure 1. Antioxidant activity of phenolic extracts from pepper (*Capsicum annuum* L.) measured in micromoles equivalents trolox per gram fresh weight ($\mu\text{M TE/g pf}$).

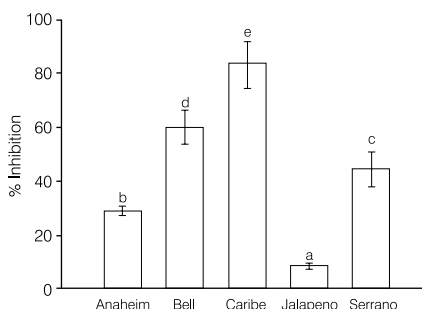


Figure 2. Antioxidant activity of phenolic extracts from pepper (*Capsicum annuum* L.) as inhibition percentage of DPPH[•] radical.

Perucka (2005) reported activities of 15 to 77% radical scavenging activity in the flavonoid fraction from hot pepper (*C. annuum*). Helmja et al. (2007) found similar values to this study (50% inhibition of DPPH[•] radical) in pungent peppers. Guil-Guerrero et al. (2006) evaluated the DPPH[•] radical inhibition of *C. annuum* varieties and a synthetic antioxidant (butylated hydroxyanisole; BHA); they found percentages of 40 and 90%, respectively. This variation may be due to differences either in the potency or in the concentration of reducing substances (mainly phenolics; Sim and Sil, 2008). The strongest significant lineal correlations were found between chlorogenic acid ($r = 0.910$), catechin ($r = 0.954$), epicatechin ($r = 0.943$), rutin ($r = 0.893$) and resveratrol ($r = 0.881$) with the free radical scavenging activity (DPPH[•]).

The caffeic acid, chlorogenic acid, catechin, epicatechin, luteolin and resveratrol contents correlated positively with the antioxidant activity

by ABTS^{•+} and DPPH[•] tests. The highest positive correlations among phenolic contents and antioxidant activities were found for catechin ($r = 0.96$), epicatechin ($r = 0.94$), and rutin ($r = 0.89$) with the DPPH[•] radical; and resveratrol ($r = 0.88$) with the ABTS^{•+} radical. Commonly, a good correlation between the values of specific groups of antioxidants and the antioxidant activity could determine the real contribution that is in function of the type and concentration of the individual antioxidant compounds presented in the sample (Jacobo-Velázquez and Cisneros-Cevallos, 2009).

The antioxidant activity of phenols is related to the number and position of the hydroxyl groups present in their chemical structure and its relative concentration in the matrix (Rice-Evans et al., 1996). The concept of specific antioxidant capacity (ratio of antioxidant capacity/total soluble phenols) has been considered as a new parameter to understand the activity of a mixture of phenols leading to the neutralization of free radicals; due to the synergistic, additive or antagonistic behavior of phenolic compounds to inhibit reactive oxygen species (Jacobo-Velázquez and Cisneros-Zevallos, 2009). Further studies should be conducted on this concept.

Conclusions

According to the results obtained, the pepper cultivars studied have levels of phenolic constituents that contribute to a high antioxidant activity and may be considered as a good source of natural antioxidants. Caribe and Bell peppers had the highest antioxidant capacity, which correlated with the highest levels of total phenols and flavonoids. The highest positive correlations of phenolic contents and antioxidant activities were for catechin, epicatechin, rutin and resveratrol. However, other compounds (e.g. ascorbic acid) present in

the peppers could contribute to the antioxidant activity and therefore should be considered in order to understand know the individual contribution of each group of phytochemicals to the total antioxidant activity. The information presented in this study can help promote the consumption of peppers in fresh form. To complement this work, studies of the effect of different technological processes on the phytochemical content of peppers extracts are in process.

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REFERENCES

- Chitindingu K, Ndhala AR, Chapano C, Benhura MA, Muchuweti M (2007) Phenolic compounds content, profiles and antioxidant activities of *Amaranthus hybridus* (pigweed), *Brachiaria brizantha* (upright brachiaria) and *Panicum maximum* (guinea grass). *J. Food Biochem.* 31: 206-216.
- Doner LW, Hicks KB (1981) High-performance liquid chromatographic separation of ascorbic acid, erythorbic acid, dehydroascorbic acid, dehydroerythorbic acid, dikelogulonic acid, and dikeloglunonic acid. *Anal. Biochem.* 115: 225-230.
- Estrada B, Bernal MA, Díaz J, Pomar F, Merino F (2000) Fruit development in *Capsicum annuum*: changes in capsaicin, lignin, free phenolics, and peroxidase patterns. *J. Agric. Food Chem.* 48: 6234-6239.
- Fabris S, Momo F, Ravagnan G, Stevanato R (2008) Antioxidant properties of resveratrol and piceid on lipid peroxidation in micelles and monolamellar liposomes. *Biophys. Chem.* 135: 76-83.
- Ferrari CKB, Torres EAFS (2003) Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. *Biomed. Pharmacother.* 57: 251-260.
- González-Aguilar GA, Ayala-Zavala JF, De la Rosa LA, Álvarez-Parrilla E (2010) Phytochemical

- changes in the postharvest and minimal processing of fresh fruits and vegetables. In De la Rosa LA, Álvarez-Parrilla E, González-Aguilar GA (Eds.) *Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value and Stability*. Wiley-Blackwell. Ames, IA, USA. pp. 309-315.
- Guil-Guerrero JL, Martínez-Guirado C, Rebolloso-Fuentes MM, Carrique-Pérez A (2006) Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annuum*) varieties. *Eur. Food Res. Tech.* 224: 1-9.
- Helmja K, Vaher M, Gorbatšova J, Kaljurand M (2007) Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. *Proc. Eston. Acad. Sci. Chem.* 56: 172-186.
- Howard LR, Talcott ST, Brenes CH, Villalon B (2000) Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J. Agric. Food Chem.* 48: 1713-1720.
- Ilow R, Regulska-Ilow B, Walkiewicz G, Biernat J, Kowalisko A (2008) Evaluation of bioflavonoid intake in the diets of 50-year-old inhabitants of Wrocław. *Adv. Clin. Exp. Med.* 17: 327-336.
- Jacobo-Velázquez DA, Cisneros-Zevallos L (2009) Correlations of antioxidant activity against phenolic content revisited: a new approach in data analysis for food and medicinal plants. *J. Food Sci.* 74: R107-113.
- Johnson MK, Loo G (2000) Effects of epigallocatechin gallate and quercetin on oxidative damage to cellular DNA. *Mutat. Res/ DNA Repair* 459: 211-218.
- Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables - the millennium's health. *Int. J. Food Sci. Technol.* 36: 703-725.
- Kevers C, Falkowski M, Tabart J, Defraigne JO, Dommès J, Pincemail J (2007) Evolution of antioxidant capacity during storage of selected fruits and vegetables. *J. Agric. Food Chem.* 55: 8596-8603.
- Kroon P, Williamson G (2005) Polyphenols: dietary components with established health benefits? *J. Sci. Food. Agric.* 85: 1239-1240.
- Kumar OA, Tata SS (2009) Ascorbic acid contents in chili peppers (*Capsicum* L.). *Not. Sci. Biol.* 1: 50-52.
- Kuskoski EM, Asuero GA, Troncoso AM, Mancini-Filho J, Fett R (2005) Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. *Ciênc. Tecnol. Aliment.* 25: 726-732.
- Lee SK, Kader AA (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharv. Biol. Technol.* 20: 207-220.
- Marín A, Ferreres F, Tomás-Barberán FA, Gil MI (2004) Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *J. Agric. Food Chem.* 52: 3861-3869.
- Marinova D, Ribarova F, Atanassova M (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Univ. Chem. Technol. Metallurgy* 40: 255-260.
- Materska M, Perucka I (2005) Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *J. Agric. Food Chem.* 53: 1750-1756.
- Molina-Quijada DMA, Medina-Juárez LA, González-Aguilar GA, Robles-Sánchez RM, Gámez-Meza N (2010) Compuestos fenólicos y actividad antioxidante de cáscara de uva (*Vitis vinifera* L.) de mesa cultivada en el noroeste de México. *CyTA - J. Food.* 8: 57-63.
- Navarro JM, Flores P, Garrido C, Martínez V (2006) Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chem.* 96: 66-73.
- Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, Brighenti F (2003) Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* 133: 2812-2819.
- Peterson J, Dwyer J (1998) Taxonomic classification helps identify flavonoid-containing foods on a semiquantitative food frequency questionnaire. *J. Am. Diet. Assoc.* 98: 677-685.
- Ramamurthy MS, Maiti B, Thomas P, Nair PM (1992) High performance liquid chromatography determination of phenolic acids in potato tubers (*Solanum tuberosum*) during wound healing. *J. Agric. Food Chem.* 4: 569-572.
- Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 20: 933-956.
- Righetto AM, Netto FM, Carraro F (2005) Chemical composition and antioxidant activity of juices from mature and immature acerola (*Malpighia emarginata* DC). *Food Sci. Tech. Int.* 11: 315-321.
- Robles-Sánchez RM, Rojas-Graü MA, Odriozola-Serrano I, González-Aguilar GA, Martín-Belloso O (2009) Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut "Kent" mango (*Mangifera indica* L.). *Postharv. Biol. Technol.* 51: 384-390.
- Sim KH, Sil HY (2008) Antioxidant activities of red pepper (*Capsicum annuum*) pericarp and seed extracts. *Int. J. Food Sci. Technol.* 43: 1813-1823.
- SNITT (2003) *Caracterización e Identificación de las Demandas de Investigación y Transferencia de Tecnología del Sistema Producto Chile Verde*. Sistema Nacional de Investigación y Transferencia Tecnológica para el Desarrollo Rural Sustentable. Baja California Sur, Mexico. www.snitt.org.mx/pdfs/demanda/chile-verde.pdf
- Sun T, Tanumihardjo SA (2007) An integrated approach to evaluate food antioxidant capacity. *J. Food Sci.* 72: R159-R165.
- Sun T, Xu Z, Wu CT, Janes M, Prinyawiwatkul W, No HK (2007) Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). *J. Food Sci.* 72: S98-S102.
- Vinson JA, Hao Y, Su X, Zubik L (1998) Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.* 46: 3630-3634.
- Zhuang Y, Chen L, Sun L, Cao J (2012) Bioactive characteristics and antioxidant activities of nine peppers. *J. Funct. Foods* 4: 331-338.