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Antioxidant Activity of Fresh and Processed Jalapeño and Serrano Peppers

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In this research, total phenols, flavonoids, capsaicinoids, ascorbic acid, and antioxidant activity (ORAC, hydroxyl radical, DPPH, and TEAC assays) of fresh and processed (pickled and chipotle canned) Jalapeño and Serrano peppers were determined. All fresh and processed peppers contained capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, even though the latter could be quantified only in fresh peppers. Processed peppers contained lower amounts of phytochemicals and had lower antioxidant activity, compared to fresh peppers. Good correlations between total phenols and ascorbic acid with antioxidant activity were observed. Elimination of chlorophylls by silicic acid chromatography reduced the DPPH scavenging activity of the extracts, compared to crude extracts, confirming the antioxidant activity of chlorophylls present in Jalapeño and Serrano peppers.

KEYWORDS: *Capsicum annuum*; total phenols; flavonoids; ascorbic acid; capsaicinoids; antioxidant activity; chlorophylls

INTRODUCTION

Hot peppers (*Capsicum annuum*) are widely produced and consumed in Mexico as raw, cooked, or processed products. The northern state of Chihuahua is among the main producers of hot peppers in Mexico, especially of Jalapeño varieties, with >35% of the national production (*1*). Hot peppers are known to be good sources of different phytochemicals, including vitamins A and C, phenolic compounds, flavonoids, and carotenoids, among others (*2*−*5*). They are the only plants that are able to produce capsaicinoids, responsible for their characteristic hot taste. The concentrations of these compounds depend on cultivar, maturity, growing conditions, and postharvest manipulation (*6*). They have been described as the vegetables with the highest vitamin C content (*7*). Thus, they are known to present high antioxidant activity (*4*). Green peppers are also known to serve as a good source of chlorophylls, which, under certain conditions, may act as radical scavengers, increasing the antioxidant activity of high chlorophyll content vegetables (*8*).

It has been estimated that hot peppers are the second most consumed vegetable by the Mexican population after tomatoes (*1*), with a consumption of approximately 7−9 kg/person per year (*9*). From this amount, approximately 75% is consumed as fresh product for the preparation of different dishes. Other commonly consumed presentations of hot peppers include pickled, dried, and smoked and in sauces. There are several varieties of hot peppers consumed in Mexico; two of the most popular varieties are Jalapeño and Serrano, in both fresh and processed forms. The main processed presentations are pickled and chipotle. Chipotle is a red Jalapeño pepper (last ripe state) that has been dried and smoked for a period of 4−7 days. Even though it can be sold dried, typically it is rehydrated and canned. It has been described that heat processing has a great impact on the content of phytochemicals and, consequently, on the antioxidant activity of fresh peppers (*2*). To determine the beneficial effect of the consumed fruits and vegetables in each region, it is important to characterize the main phytochemicals consumed in the diet, paying special attention to the loss of these phytochemicals in the products due to food manipulation. For this reason, the aim of the present work was to evaluate total phenols, flavonoids, ascorbic acid, capsaicinoids, and antioxidant activity of fresh and processed (pickled and chipotle canned) Jalapeño and Serrano peppers. Considering that Chihuahua is the main Jalapeño producer in the country, fresh Jalapeños from three of the main production regions were also analyzed. Finally, the effect of chlorophylls on DPPH scavenging activity of all samples was analyzed.

MATERIALS AND METHODS

Chemicals. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{2-}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin−Ciocalteu phenol reagent, sodium carbonate, monobasic potassium phosphate, dibasic potassium phosphate, gallic acid, human low-density lipoprotein (LDL) cholesterol, ethylenediaminetetraacetic acid

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(EDTA), L-ascorbic acid, ferric chloride, fluorescein, ferrous chloride, sodium bicarbonate, metaphosphoric acid, trichloroacetic acid, dinitrophenyl hydrazine, thiourea, capsaicin, dihydrocapsaicin, Tween 40 emulsifier, β-carotene, and linoleic acid were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada). All solvents, unless otherwise specified, were purchased from Fisher Scientific (Nepean, ON, Canada) and were of ACS grade or better.

Materials. Seven different fresh and processed hot peppers were used in the present research. Canned peppers, namely, chipotle (smoked and canned Jalapeño pepper) and pickled Jalapeño and Serrano peppers were from the brand La Costeña; fresh Serrano peppers (*C. annuum*) were purchased at a local supermarket at Ciudad Juarez Chihuahua, Mexico; fresh Jalapeño peppers (*C. annuum*) from three growing regions in the state of Chihuahua, Mexico (Ascencion, 31° 06′ N, 107° 59′ W, As pepper; Flores Magon, 29° 55′ 60″ N, 106° 57′ W, FM pepper; Meoqui, 28° 76′ N, 105° 29′ W, Mq pepper) were kindly donated by local producers. Canned peppers were drained to remove any excess liquid. Fresh peppers were sorted to eliminate damaged, poor-quality fruit, washed with chlorinated water (200 ppm sodium hypochlorite), and drained. In all samples (fresh and canned), peduncles of the peppers were removed, weighed, cut in four parts, frozen at −80 °C for 1 day, freeze-dried for 48 h (Labconco freeze-dry/shell freeze system, Labconco Corp., Kansas City, MO), milled in a laboratory miller, and stored at −80 °C. Moisture was determined from the difference in weight before and after lyophilization. In all cases placenta and seeds were considered in the study, because in Mexico these kinds of peppers are eaten with the seeds.

Extraction of Phenolic Compounds. The powdered dry hot peppers (20 g) were mixed with 125 mL of 80% methanol, stirred, and sonicated for 30 min in the dark. The extract was centrifuged (2000*g*) for 5 min, and the supernatant was collected. The residues were re-extracted under the same conditions, and both supernatants were combined. The solvent was partially removed under vacuum at 45 °C, and the concentrated slurries were freeze-dried for 72 h at −45 °C (Labconco 6 freezone, Labconco Corp.). Dried extracts (pepper crude extract, PCE) were stored at −20 °C under vacuum. The yield of each extract was determined for further analyses.

Determination of Total Phenolic Content. Total phenols were determined by mixing 0.5 mL of dissolved PCE (8 mg/mL in 80% methanol) with 2.5 mL of 10% Folin−Ciocalteu's reagent (v/v), incubated at room temperature for 2 min, before the addition of 2 mL of 7.5% sodium carbonate (w/v). The mixture was incubated at 50 °C for 15 min and cooled to room temperature, and the absorbance was read at 760 nm using a diode array spectrophotometer (8452A, Agilent Technologies Canada Inc., Mississauga, ON, Canada). Gallic acid was used as a standard, and results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of fresh or dry weight.

Determination of Total Flavonoid Content. Total flavonoids were determined according to the method of Menichini et al. (*10*), with slight modifications. The dissolved PCE (0.5 mL, 8 mg/mL in 80% methanol) was mixed with 2 mL of water and 150 μL of 5% $NaNO_2$. After 5 min, 150 μL of 10% $AlCl_3$ was added to the mixture, and then, after 3 min, 2 mL of 0.5 M NaOH was added and incubated at room temperature for 30 min; the absorbance was read at 510 nm using a diode array spectrophotometer (8452A, Agilent Technologies Canada Inc.). Catechin was used as a standard, and the results were expressed as milligrams of catechin equivalents (CE) per 100 g of fresh or dry weight.

Extraction and Quantification of Ascorbic Acid. Ascorbic acid was extracted as described by Gonzalez-Aguilar et al. (*11*) for green peppers, with slight modifications. The dried hot peppers (0.2 g) were sonicated in the dark for 20 min with 2 mL of 5% (w/v) metaphosphoric acid. The mixture was centrifuged at 2000*g* for 5 min and the supernatant collected. Quantification was carried out immediately after the extraction. Ascorbic acid was determined according to the method of Oboh et al. (*12*, *13*). Three hundred microliters of supernatant was mixed with 200 μL of 6.65% trichloroacetic acid (TCA) and 75 μL of DNPH (2 g of dinitrophenylhydrazine, 230 mg of thiourea, and 270 mg of $CuSO_4 \cdot 5H_2O$ in 100 mL of 5 M H_2SO_4). The mixture was incubated for 3 h at 37 °C, before the addition of 0.5 mL of 65% H_2SO_4. Absorbance was read at 520 nm, using ascorbic acid as a standard. Results were reported as milligrams of ascorbic acid (AA) per 100 g of fresh or dry weight.

Extraction and Quantification of Capsaicinoids. Capsaicinoids were extracted and quantified according to the methodology proposed by Ornelas-Paz et al. (*9*) with slight modifications. The dried hot peppers (0.5 g) were mixed with 10 mL of methanol and sonicated in the dark for 20 min and then centrifuged at 2000*g* for 5 min, and the supernatant was collected. The extraction was repeated, and both supernatants were combined. Extracts were kept at −20 °C until analysis (next day). Identification and quantification of hot pepper capsaicinoids were carried out by HPLC-MS, using an Agilent 1100 HPLC system (Agilent Technologies Canada Inc.) with a UV diode array detector (UV-DAD). Separation was achieved using a Supercosil LC-18 reverse-phase column (5 μm particle size, 25 cm × 4.6 mm i.d., Sigma-Aldrich Canada Ltd.). Extracts were filtered through a 0.45 nm polypropylene membrane filter (Whatman Canada Ltd., Toronto, ON, Canada), and 25 μL was automatically injected into the system and eluted using an isocratic mobile phase (50:50, v/v, acetonitrile/1% acetic acid in water) at a 1 mL/min flow rate. LC flow was analyzed online by a mass spectrometric detector system (LC-MSD-Trap-SL, Agilent) with an electrospray ionization (*10*), operated in negative ion mode. The operating conditions used were dry gas temperature of 350 °C, a capillary voltage of 106 V, N_2 as sheath gas at a flow rate of 10 mL/min, a nebulizer pressure of 70 psi, and a scan range from m/z 100 to 800. Capsaicinoids were detected at 280 nm; capsaicin (rt 11 min) and dihydrocapsaicin (rt 15.1) were quantified using standard compounds (78−500 μg/mL). Nordyhidrocapsaicin (rt 10.3) was identified by its MS spectrum and quantified using the capsaicin standard curve.

Chlorophyll Removal. *Liquid−Liquid Extraction.* The crude phenolic extracts (1.5 g) were dissolved in 50 mL of 80% methanol and poured into an extraction funnel. Twenty-five milliliters of CH_2Cl_2 was added; the extraction funnel was shaken and allowed to stand for phase separation. The organic phase was removed, and extraction was repeated one more time. Methanol was partially removed under vacuum at 45 °C, and the concentrated slurries were freeze-dried for 72 h at −45 °C (Labconco 6 freezone, Labconco Corp.). Dried extracts (L-L chlorophyll-free extracts) were stored at −20 °C under vacuum. The yield of each extract was determined for further analysis.

Column Chromatographic Extraction. The column (1.5 × 20 cm) packed with silicic acid gel was washed with 150 mL of methanol and then with 200 mL of *n*-hexane. An extract (200 mg) suspended in 5 mL of *n*-hexane was applied on the column. Chlorophylls were removed from the column using *n*-hexane (sufficient volume to remove a green band). The polar phenolic compounds were washed from the column using 200 mL of methanol. The methanolic fraction was concentrated using a rotary evaporator (temperature of 40 °C) and then freeze-dried for 72 h at −45 °C (Labconco 6 freezone, Labconco Corp.). Dried extracts (c-c chlorophyll-free extracts) were stored at −20 °C under vacuum.

Antioxidant Activity. *Oxygen Radical Absorbance Capacity* (*ORAC*). The ORAC assay was performed according to the method of Madhujith and Shahidi (*14*), using a FLUOstar OPTIMA microplate reader (BMG Labtechnologies GmbH, Offenberg, Germany) equipped with FLUOstar OPTIMA evaluation software version 1.30-0 and black polystyrene, nontreated 96-well microplates (Costar Corning Inc., Corning, NY). Only the internal wells of the microplate were used. Measuring solutions (by triplicate) were prepared directly in a microplate by mixing 20 μL of diluted PCE (diluted in 75 mM phosphate buffer, pH 7.4) or Trolox calibration standards (0−50 μM, dissolved in 75 mM phosphate buffer, pH 7.4) with 120 μL of fluoresceine (96 nM dissolved in 75 mM phosphate buffer, pH 7.4) and kept at 37 °C for 20 min. Then 60 μL of AAPH (12 mM final concentration) was automatically injected onto each well, and fluorescence was measured every 2 min for 120 min, with excitation and emission filters of 485/20 and 528/25, respectively. A gain adjustment was performed by pipetting 200 μL of fluorescein onto a designated well before starting the program to optimize signal amplification. Trolox (0−50 μM) was used as a standard. Values of antioxidant capacity were calculated from the differences in the area under the fluorescence decay curves between blank and samples and reported as millimoles of Trolox equivalents (TE) per 100 g of sample (fresh or dry weight).

Determination of Hydroxyl Radical Scavenging Activity. The hydroxyl radical scavenging activity determination was performed electron paramagnetic resonance (EPR spectroscopy) as described by Madhujith and Shahidi (*15*) with slight modifications. EPR spectra were recorded on a food analyzer Bruker E-scan (Bruker Biospin Co., Billercia, MA), using the following parameters: 5.02 × 102 receiver gain, 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100.00 G sweep width, 3495.258 G

center field, 5.12 ms time constant, 9.795 GHz microwave frequency, 86.00 kHz modulation frequency, and 1.86 G modulation amplitude. PCE was dissolved in deionized water and diluted to approximately 0.5 mg/mL. The diluted extract (100 μL) was mixed with 100 μL of 10 mM H_2O_2, 200 μL of DMPO, and 100 μL of 100 μM $FeSO_4$ (dissolved in deoxygenated water). After 1 min, EPR spectra were recorded, and the remaining radical scavenging capacity (RSC) was calculated using eq 1.

$$\% \text{ RSC} = 100 - \frac{(\text{EPR signal of the sample})}{(\text{EPR signal of the control})} \times 100 \quad (1)$$

Gallic acid (0.62−10 mM) was used as a standard, and the results were reported as millimoles of GAE per 100 g of sample (fresh or dry weight).

Determination of DPPH Radical Scavenging Activity. The DPPH radical scavenging activity assay was performed using EPR spectroscopy, according to the method of Madhujith and Shahidi (*15*) with slight modifications. EPR spectra were recorded under the same experimental conditions as described for the DPPH assay. Samples were prepared by mixing 2 mL of 190 μM DPPH (in methanol) with 500 μL of PCE (2 mg/mL in methanol). After 10 min, the samples were injected into the EPR spectrometer, the height of the second positive peak was recorded, and the percentage of the remaining RSC was calculated according to eq 1. Trolox (31−250 μM in methanol) was used as a standard, and the results were reported as micromoles of TE per 100 g of sample (fresh or dry weight).

Total Antioxidant Capacity by Trolox Equivalent Antioxidant Capacity (TEAC) Assay. TEAC assay was performed according to the method of Madhujith and Shahidi (*16*). $ABTS^{\bullet-}$ radical anion was prepared in 100 mM saline phosphate buffer (PBS, pH 7.4, 0.15 M NaCl) by mixing 100 mL of 2.5 mM AAPH (in PBS) with 100 mL of 2 mM $ABTS^{2-}$ (in PBS); this solution was covered from light and heated at 60 °C for 30 min and then cooled to room temperature. This solution was filtered several times during the experiment through a no. 1 filter. The diluted PCE (40 μL, 0.5 mg/mL in 80% methanol) or 80% methanol (control) was mixed with 1960 μL of $ABTS^{\bullet-}$ solution, kept protected from the light for 6 min, and then the absorbance at 734 nm was read. The remaining RSC was calculated using eq 2.

$$\% \text{ RSC} = 100 - \frac{(\text{Abs (734 nm)}_{\text{sample}})}{(\text{Abs (734 nm)}_{\text{control}})} \times 100 \quad (2)$$

Trolox (50−400 μM) was used as standard, and the results were expressed as micromoles of TE per 100 g of sample (fresh or dry weight).

Statistical Analysis. All analyses were carried out at least in triplicate. Values are presented as the mean ± SD of at least three replicates. One-way ANOVA and Tukey analyses ($P < 0.05$) were performed to determine differences between samples, using the commercial software PAWS 18 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

In this work, two of the most consumed hot peppers in Mexico, namely, Jalapeño and Serrano peppers, in both fresh and canned (smoked and pickled) forms, were studied. Because Chihuahua is the main producer of Jalapeño in Mexico with >38% of the Mexican production (*1*), in the case of fresh Jalapeño peppers, we also studied samples from three of the main production regions. The moisture content of fresh peppers varied from 91.6 for Flores Magón (FM) Jalapeño to 88.4% for Serrano (**Table 1**). FM showed statistically higher moisture values compared with the other two Jalapeño production regions (Ascencion, As; and Meoqui, Mq). Both pickled peppers presented lower moisture content compared to fresh peppers. Chipotle, which is a smoked and canned processed pepper, showed the lowest moisture content, with a value of 76.9%. In all cases, moisture values were in the range of previously reported values for green peppers (*4*, *17*).

Phytochemical Profile. Hot peppers are known to be a good source of phenolic compounds, including flavonoids and capsaicinoids, as well as ascorbic acid. These phytochemicals show high antioxidant activity, and their consumption has been linked to a decreased risk of developing chronic and degenerative diseases (*18*).

Table 1. Dry Matter and Phytochemical Content of Fresh and Processed Jalapeño and Serrano Peppers (Based on Dry Weight)[a]

variety/growth region	dry matter %	total phenols[b]	total flavonoids[c]	ascorbic acid[d]
Ascención	10.6	1028 ± 57a	332 ± 40 b	2153 ± 187 a
Flores Magón	8.4	745 ± 29 c	246 ± 18 cd	1185 ± 110 c
Meoqui	10.8	1010 ± 71 a	201 ± 22 d	1696 ± 122 b
chipotle	23.1	919 ± 18 b	284 ± 8 b	694 ± 136 d
pickled Jalapeño	11.5	802 ± 63 bc	389 ± 23 ab	794 ± 134 d
serrano	11.6	1032 ± 95 a	441 ± 13 a	1385 ± 100 bc
pickled Serrano	12.2	568 ± 44 d	307 ± 24 b	584 ± 20 d

[a] Values represent the mean of three or four measurements ± SD. Values in the same column with different letters are significantly different (Tukey test, $P < 0.05$). [b] mg GAE/100 g sample. [c] mg CE/100 g sample. [d] mg AA/100 g sample.

In the following section the results on total phenolic, total flavonoid, ascorbic acid, and capsaicinoid content will be discussed. Results are presented on a dry weight basis; however, comparisons against previously reported values will be done using both fresh (data not shown) and dry bases, using moisture content because previously published results are expressed indistinctly on both fresh and dry bases.

Total Phenolic Content. Different conditions have been used for the extraction of antioxidant compounds from peppers. After several trials, using acetone, methanol, and ethanol as solvent, different extraction times and extraction techniques (sonication, heating, and shaking), sonication with 80% methanol for 30 min, at room temperature, was chosen for the extraction of pepper antioxidant compounds, because these conditions showed the highest phenolic extraction. The phenolic contents of the PCE from the fresh and processed peppers are shown in **Table 1**. Phenolic concentration ranged from 568 mg GAE/100 g DW for pickled Serrano to 1032 mg GAE/100 g DW for fresh Serrano. These values are in the range (33−250 mg GAE/100 g FW or 400−1200 mg GAE/100 g DW) of those reported by several authors for different green sweet and hot peppers (*2*, *3*, *12*, *13*, *19*, *20*). A large variability in the content of phenolics of fresh Jalapeño peppers was observed; this can be explained in terms of differences in cultivar, soil and weather conditions, and maturity, as well as postharvest manipulation (*21*). Similar variability has been observed for Jalapeño peppers grown in different parts of the state of Chihuahua and in different years, using the same extraction conditions (63 mg GAE/100 g FW (*22*) to 161 mg GAE/100 g FW (*9*)). Fresh peppers (except FM sample) presented higher values compared to pickled peppers. These results can be explained by considering that during the pickling and canning process there is a loss of phenolic compounds and vitamin C due to lixiviation (*2*, *9*). Unexpectedly, chipotle showed high phenolic content compared to pickled peppers. This higher phenolic concentration could be due to a reduction of lixiviation because chipotle is first dried and smoked and then rehydrated and canned. During this drying process, the external membrane of the pepper hardens, partially preventing lixiviation. Hervert-Hernández et al. (*23*) observed that chipotle showed more free phenolics and less bound phenolics, compared to other fresh hot peppers. It is also possible that smoke may deliver other phenolics to the products and hence give rise to a higher phenolic content in these products.

Total Flavonoid Content. Total flavonoid content was determined by using the aluminum complexation method (*10*). All samples presented high variability, probably due to matrix interferences (even though blank samples were done for each pepper), which varied greatly among samples (**Table 1**). Both Serrano samples showed the highest flavonoid concentration, followed by As and processed Jalapeños. FM and Mq Jalapeño peppers showed the lowest flavonoid concentrations. These values are

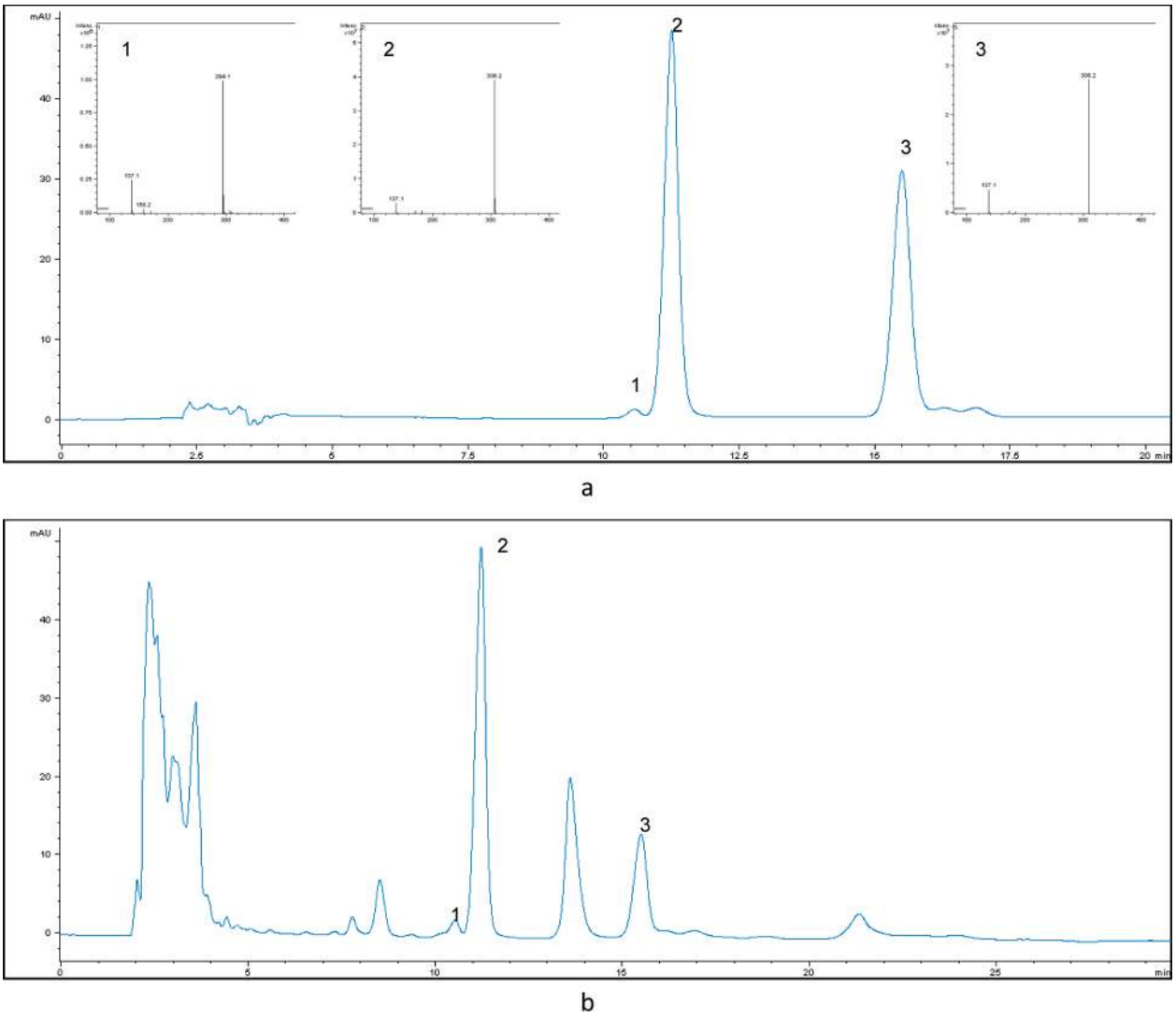

Figure 1. HPLC-DAD-MS-ESI (negative mode) chromatogram of (**a**) capsaicinoids standards (peaks: 1, nordihydrocapsaicin; 2, capsaicin; 3, dihydrocapsaicin) (inset, ESI negative ion mass spectra of capsaicinoids) and (**b**) fresh Serrano extract. UV detection at 280 nm.

similar to those reported for Jalapeño and Serrano peppers by HPLC (*24*) and for hot peppers by the aluminum complexation procedure (*10*, *19*), but much higher than those reported by other authors (*20*, *21*).

Ascorbic Acid Content. Peppers are the vegetables with highest ascorbic acid content. It has been reported that consumption of 100 g FW of peppers provides 100−200% of the RDA of ascorbic acid (AA) (*7*). AA content was determined by using the DNPH spectroscopic method, and values are presented in **Table 1**. AA content ranged from 584 mg AA/100 g DW for pickled Serrano to 2153 mg AA/100 g DW for As in Jalapeño. AA values shown in the present work are in the range of those reported for green hot and sweet peppers by HPLC and spectroscopic methods (*2*, *3*, *20*, *25*), as well as for Serrano and Jalapeño peppers (*24*). The high variability in AA content among Jalapeño samples could be due to the existing differences in growing conditions, maturity, and, especially, postharvest manipulations. It has been reported that high storage temperature can reduce AA content up to 88% in kale upon storage at 20° for 2 days (*7*). From the analysis of the values reported in **Table 1**, it is evident that heat processing of peppers had a drastic effect on the concentration of AA, due to heating degradation. Chuah et al. (*2*) observed a dramatic reduction in AA concentration of colored peppers when boiled in water for 5 or 30 min.

Capsaicinoid Content. Different extraction and chromatographic procedures for the determination and quantification of capsaicinoids have been published (*9*, *26*, *27*). In this work the method of Ornelaz-Paz et al. (*9*) was used, because they worked with Jalapeño peppers. **Figure 1a** shows the chromatogram of a mixture of the major capsaicinoids found in peppers. Capsaicin and dihydrocapsaicin were identified and quantified using standard compounds. Nordihydrocapsaicin was identified using its MS spectrum and quantified using capsaicin, because no commercial standard was available, and values were reported as capsaicin equivalents. The chromatogram of fresh Serrano peppers is presented in **Figure 1b**, where it was possible to identify the three capsaicinoids. Capsaicinoid contents of fresh and processed peppers are presented in **Table 2**. Total capsaicinoids content ranged from 525.7 μg capsaicinoids/g DW for chipotle to 3330.9 μg capsaicinoids/g DW for Serrano. These values are in the range of those reported by different authors for Serrano, Jalapeño, and other hot peppers (*C. annuum* L.) (*5*, *9*, *26*, *28*). Fresh peppers showed a higher capsaicinoid content compared to processed peppers, in agreement with Ornelas-Paz et al. (*9*), Harrison and Harris (*29*), and Schweiggert et al. (*27*), who observed a decrease in capsaicinoid content in processed peppers due to heat treatment. In contrast to these results, Kozukue et al. (*26*) observed an increase in total capsaicinoid content of pickled and chipotle Jalapeño peppers,

Table 2. Capsaicin, Dihydrocapsaicin, and Nordihydrocapsaicin (Micrograms per Gram of Dry Weight) and Their Proportion in Terms of Total Capsaicinoids Content of Fresh and Processed Jalapeño and Serrano Peppers[a]

variety/growth region	capsaicin	DHC	nor-DHC	total capsaicinoids
Ascención	346.2 ± 14.3 d 46.7%	395.8 ± 15.8 d 53.3%	nd	742.0 ± 29.6 e
Flores Magón	2308.0 ± 36.5 a 72.2%	875.0 ± 6.1 b 27.4%	14.3 ± 0.2 c 0.4%	3197.3 ± 38.1 b
Meoqui	1318.6 ± 14.4 c 46.5%	1420.0 ± 58.5 a 50.1%	94.9 ± 11.3 b 3.4%	2833.5 ± 41.1 c
chipotle	259.2 ± 16.4 d 49.3%	266.5 ± 26.4 e 50.7%	nd	525.7 ± 10.0 e
pickled Jalapeño	323.9 ± 63.0 d 60.0%	215.6 ± 12.3 e 40.0%	nd	539.5 ± 50.7 e
Serrano	1606.1 ± 59.2 b 48.2%	1499.9 ± 49.0 a 45.0%	224.9 ± 18.1 a 6.8%	3330.9 ± 115 a
pickled Serrano	167.5 ± 10.4 b 65.7%	87.4 ± 7.0 c 34.3%	nd	254.9 ± 17.4 c

[a] Values represent the mean of two measurements ± SD. Values in the same column with different letters are significantly different (Tukey test, $P < 0.05$).

compared to fresh Jalapeño. Among fresh peppers, Serrano presented the highest levels of capsaicinoids. These results are in agreement with those obtained by Ornela-Paz et al. (*9*) and Kozukue et al. (*26*). Fresh Jalapeño peppers showed high variability in total capsaicinoids content depending on the growing location (742−3197 μg/g DW); similar results were observed by Rowland et al. (*30*) for different Jalapeño pepper cultivars (1100−7260 μg/g DW), showing once again the large variability in phytochemical content due to cultivar, maturity, and pre- and postharvest conditions. Capsaicin (46−72% of total capsaicinoids) and dihydrocapsaicin (27−53%) were the major capsaicinoids found in fresh and processed peppers. Interestingly, and in contrast with other studies, nordihydrocapsaicin was not detected in processed peppers (*26*, *29*).

Antioxidant Activity. Four different methods based on radical scavenging inhibition (ORAC, hydroxyl radical, DPPH, and TEAC assays) were used to evaluate the antioxidant activity of the fresh and processed Jalapeño and Serrano peppers, and results are presented in **Table 3**. To compare the results obtained by different methods, results were expressed in terms of micromoles of TE per 100 g of dry peppers, except in the case of hydroxyl radical, for which results were expressed as millimoles of GAE per 100 g of dry peppers, due to the low solubility of Trolox in water.

ORAC. The antioxidant activity of the fresh and processed peppers obtained by the ORAC method showed high variability, with values from 42.4 mmol TE/100 g DW for pickled Jalapeño pepper to 58.7 mmol TE/100 g DW for Mq Jalapeño (**Table 3**). These values were about 5 times higher than those reported for sweet peppers by different authors (*20*, *31*, *32*), but in the range of those reported for Jalapeño (*33*) and chili peppers (*19*). Due to the high variability among samples, no significant differences were observed among peppers.

Hydroxyl Radical Scavenging Activity. The hydroxyl radical was formed from hydrogen peroxide (H_2O_2) by an Fe(II)-catalyzed Fenton reaction. This short time active oxygen species ($HO^{\bullet}$) is trapped by DMPO, forming a [DMPO−OH]· adduct, which can be detected as a 1:2:2:1 quartet signal by EPR spectroscopy (**Figure 2a**). The addition of an antioxidant (gallic acid or crude phenol extract) scavenges the $HO^{\bullet}$, decreasing the concentration of [DMPO−OH]$^{\bullet}$ adduct. The reduction of the intensity of the EPR signals as the concentration of gallic acid increases can be seen in **Figure 2b−d**. From the reduction of the EPR signal, in the presence of gallic acid, the percentage inhibition can be calculated (eq 1), and a calibration curve of percent inhibition against gallic acid can be obtained (**Figure 2e**) to determine hydroxyl radical scavenging activity of peppers. **Figure 2f** depicts a typical EPR spectrum from Serrano pepper. Samples at a concentration of approximately 0.5 mg extract/mL showed between 20 and 52% inhibition (data not shown). Oboh et al. (*12*) observed a 25% inhibition for similar red pepper extract concentrations. Hydroxyl radical scavenging activity of Jalapeño and Serrano fresh and processed peppers is shown in **Table 3**. Fresh peppers presented higher antioxidant activity compared to pickled peppers. High variability in antioxidant activity was observed among fresh Jalapeño peppers.

Table 3. Antioxidant Activities of Fresh and Processed Jalapeño and Serrano Peppers, Expressed in Dry Weight[a]

variety/growth region	ORAC[b]	OH radical[c]	DPPH[d]	TEAC[d]
Ascención	44.6 ± 4.6	693.7 ± 30.4 a	4401 ± 486 a	5211 ± 403 a
Flores Magón	52.1 ± 2.7	546.2 ± 40.5 b	2728 ± 71 b	3513 ± 376 c
Meoqui	58.7 ± 4.9	640.2 ± 19.6 a	4125 ± 202 a	5541 ± 543 a
Chipotle	54.3 ± 9.7	426.2 ± 26.3 c	2337 ± 105 bc	2864 ± 286 c
pickled Jalapeño	42.4 ± 5.5	147.7 ± 13.0 d	2467 ± 33 bc	2776 ± 113 c
Serrano	58.6 ± 10	475.6 ± 21.9 bc	4103 ± 296 a	4787 ± 625 a
pickled Serrano	52.3 ± 2.6	97.6 ± 16.2 d	2018 ± 221 c	3988 ± 615 b

[a] Values represent the mean of three measurements ± SD. Values in the same column with different letters are significantly different (Tukey test, $P < 0.05$). [b] mmol TE/100 g sample. [c] mmol GA/100 g sample. [d] μmol TE/100 g sample.

DPPH Radical Scavenging Activity. Spectroscopic measurement of DPPH scavenging read at 515 nm has been widely used to determine the antioxidant activity of hot and sweet peppers (*2*, *19*, *20*, *34*−*36*). However, it has been reported that DPPH values measured at 515 nm can be underestimated due to sample interferences (*37*). In the present research, the DPPH radical scavenging activity of fresh and processed Jalapeño and Serrano peppers was measured using EPR spectroscopy. An advantage of EPR spectroscopy is that there is no matrix interference during the measurement of DPPH radical scavenging, because the technique measures directly the scavenging of the DPPH radical.

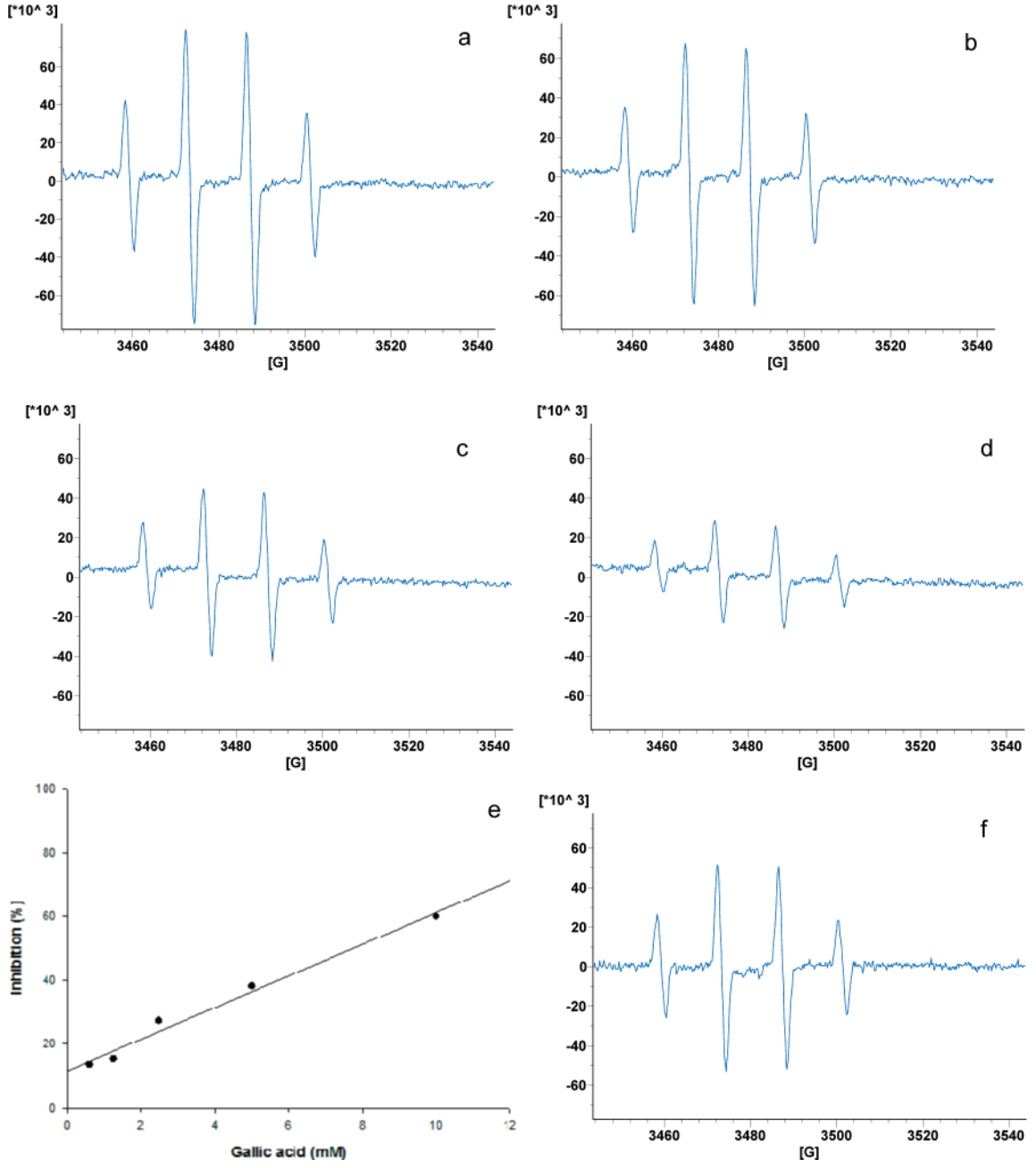

Figure 2. EPR resonance spectra obtained for hydroxyl radical−DMPO adduct in the presence of different concentrations of gallic acid: (**a**) 0.0, (**b**) 0.6, (**c**) 5.0, and (**d**) 0−10 mM; calibration curve of hydroxyl radical−DMPO inhibition versus GA concentration (**e**) and fresh Serrano extract (**f**, 2 mg/mL).

DPPH values varied from 2018 μmol TE/100 g DW for pickled Serrano to 4125 μmol TE/100 g DW for Mq Jalapeño (**Table 3**). These values are in the range of those reported for hot and sweet peppers (*2*, *35*). As for hydroxyl radical scavenging activity, fresh peppers had higher DPPH scavenging activity compared to processed peppers.

Total Antioxidant Capacity by TEAC Assay. The TEAC assay measures the scavenging ability of fruit and vegetable extracts against $ABTS^{\bullet-}$. TEAC antioxidant activity values are reported in **Table 3**. Values ranged from 2776 μmol TE/100 g DW for pickled Jalapeño to 5541 μmol TE/100 g DW for Mq Jalapeño. Fresh peppers showed higher TEAC values than processed peppers. TEAC values reported elsewhere for chipotle (*23*) and hot chili pepper (*38*) were approximately 30% higher than those reported in the present work.

The high variability of antioxidant activity among fresh Jalapeño peppers (As ≈ Mq > FM) is in agreement with the results on phytochemical profile and can be explained in terms of differences in cultivar, soil and weather conditions, and maturity, as well as postharvest manipulations (*6*). The higher antioxidant capacity of fresh peppers, compared to processed peppers, can be explained in terms of a decrease of phenolic compounds and ascorbic acid concentration due to processing. Jímenez-Monreal et al. (*39*) observed a reduction in hydroxyl radical scavenging activity of up to 72% when fresh peppers were submitted to pressure cooking (similar conditions to pickling processing). These authors explained the reduction of antioxidant activity to be due to lixiviation of phenolic compounds and decrease of ascorbic acid concentration after boiling and pressure cooking. Similarly, Chuah et al. (*2*) reported a reduction of up to 36% of DPPH activity after boiling green and red peppers for 30 min due to lixiviation of both phenolic compounds and ascorbic acid. The higher antioxidant activity of chipotle (compared to pickled peppers) could be due to a reduction on lixiviation (**Table 1**), formation of Maillard

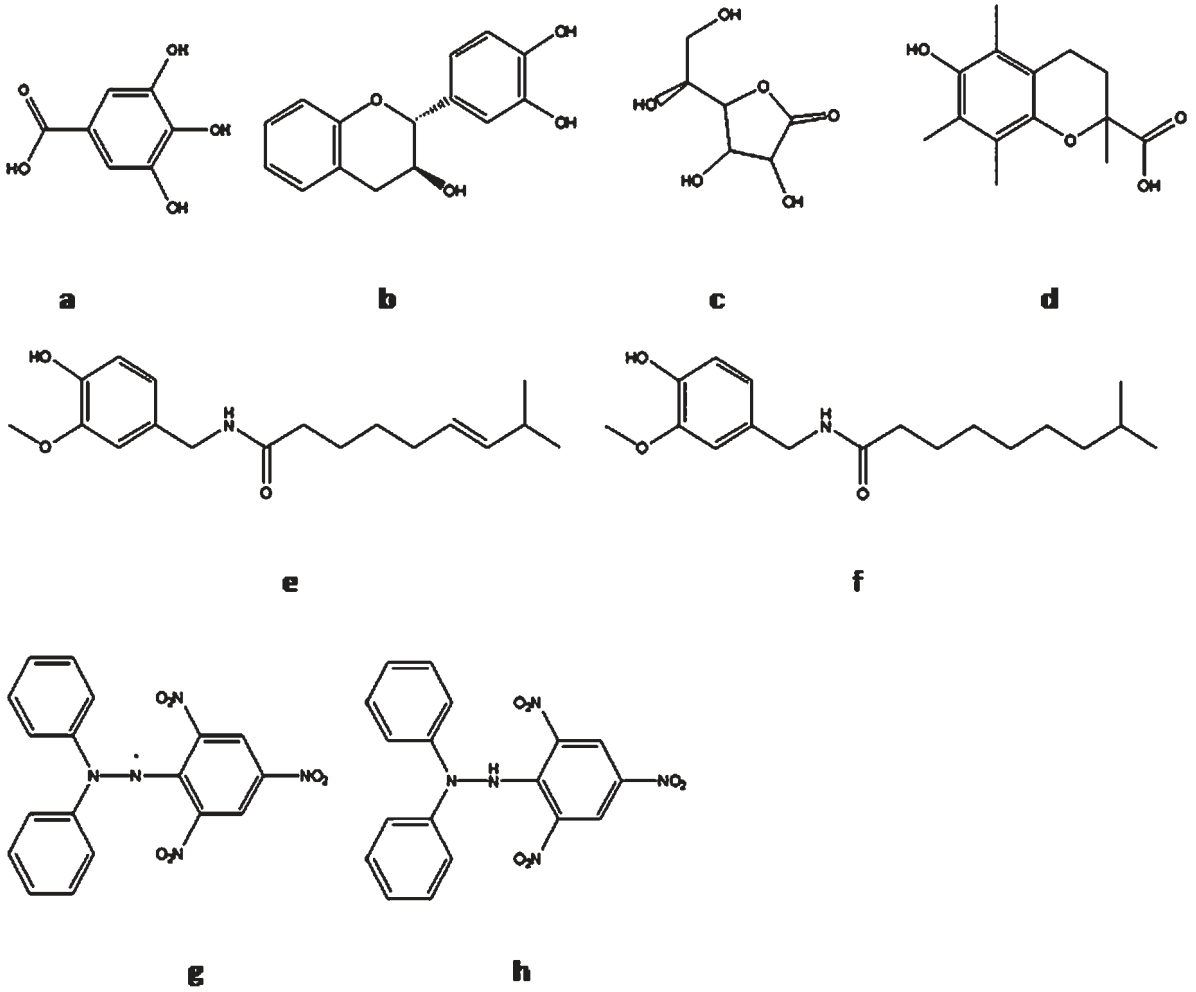

Figure 3. Chemical structures of (**a**) gallic acid, (**b**) (+)-catechin, (**c**) ascorbic acid, (**d**) Trolox, (**e**) capsaicin, (**f**) dihydrocapsaicin, (**g**) DPPH$^{\bullet}$, and (**h**) DPPH−H.

reaction products, and the smoking process, which may deposit phenolics from the smoke on the product (*39*). Vega-Galvez et al. (*36*) reported an increase in antioxidant capacity of air-dried peppers due to the accumulation of Maillard-derived melanoidins with high antioxidant activities.

To define the influence of the main phytochemicals found in Jalapeño and Serrano peppers on the antioxidant activity of the crude phenolic extract, the antioxidant activity, measured with the DPPH assay, of equimolar concentrations (25 μM) of gallic acid, (+)-catechin, ascorbic acid, Trolox, capsaicin, and dihydrocapsaicin (**Figure 3**) was measured, and results are presented in **Figure 4**. Gallic acid and (+)-catechin were used because total phenolics and flavonoids were reported as gallic acid and (+)-catechin equivalents, respectively. In this figure, it is possible to observe that the antioxidant activity decreases in the order gallic acid $\gg$ (+)-catechin > Trolox > ascorbic acid > capsaicin $\approx$ dihydrocapsaicin. These results are in agreement with those previously published (*40*−*42*). In agreement with our results, Materska and Perucka (*4*) observed low DPPH scavenging with both capsaicin and dihydrocapsaicin. It has been reported that the antioxidant activity of polyphenols depends on the number and position of hydroxyl groups, the number of rings present in the structure, and the number and position of conjugated double bonds (*40*, *43*, *44*). Kinetics and mechanistic studies on the interaction of DPPH$^{\bullet}$ with different antioxidants have been carried out, using NMR (*43*), UV (*42*, *45*), and fluorescence spectroscopy (*46*), mass spectrometry (*45*), and amperometry (*41*). These studies suggest that the DPPH$^{\bullet}$−antioxidant interaction follows a complex mechanism, in which several stepwise reactions are involved. Equations 3−6 show some of the steps involved in the reaction sequence.

$$\mathrm{DPPH}^{\bullet} + \mathrm{AH} \rightleftarrows \mathrm{DPPH{-}H} + \mathrm{A}^{\bullet} \quad (3)$$

$$\mathrm{DPPH}^{\bullet} + \mathrm{A}^{\bullet} \rightleftarrows \mathrm{DPPH{-}H} + \text{oxidative compounds} \quad (4)$$

$$\mathrm{DPPH}^{\bullet} + \mathrm{A}^{\bullet} \rightleftarrows \mathrm{DPPH{-}A} \quad (5)$$

$$\mathrm{DPPH}^{\bullet} + \mathrm{DPPH}^{\bullet} \rightleftarrows \mathrm{DPPH{-}DPPH} \quad (6)$$

All of the antioxidants follow at least the first reaction (*3*), and depending on their structure different numbers of subsequent reactions may be present. The most common reported stoichiometry is 2:1 DPPH/antioxidant (catechin, Trolox, ascorbic acid, capsaicin) (*40*, *43*, *45*, *46*), although higher stoichiometries (6:1) for gallic acid have been reported (*40*, *42*). Sawai and Sakata (*43*) determined that epigallocatechin, which has three hydroxyl groups in the phenolic moiety, showed higher antioxidant activity than catechin. They concluded that the antioxidant activity increases as the number of hydroxyl groups increases.

Kogure et al. (*45*) determined by NMR that capsaicin reacts with DPPH$^{\bullet}$ through the C−N bond and not by the hydroxyl group. This different mechanism of reaction may be the reason for the low antioxidant activity of both capsaicinoids compared

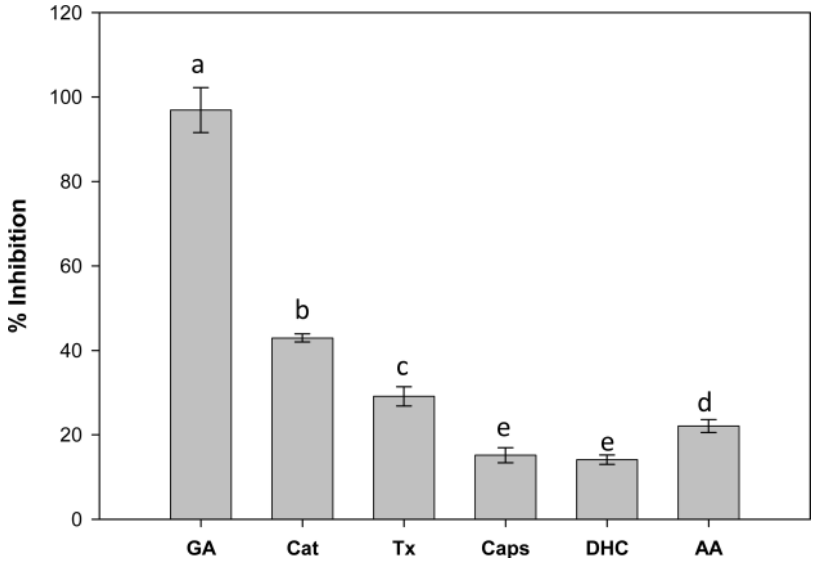

Figure 4. Antioxidant activity, estimated by the DPPH scavenging assay measured by EPR spectroscopy (expressed as percent of DPPH radical inhibition), of equimolar solutions of the phytochemicals found in peppers (plus Trolox). Values are the mean ± SD from four estimations. Different letters in the bars indicate statistically significant differences (Tukey test, $P < 0.05$).

to the rest of the tested compounds. In agreement with our results, Materska and Perucka (*4*) observed low antioxidant activity (approximately 10%) for both capsaicin and dihydrocapsaicin.

Considering that total phenols and ascorbic acid were the main phytochemicals found in fresh and processed Jalapeño and Serrano peppers and that gallic acid showed higher antioxidant activity than ascorbic acid, it may be inferred that total phenols are the main contributors to their antioxidant activity. To corroborate this idea, correlation analysis between total phenols and ascorbic acid with the four antioxidant activity assays were carried out, and the results are shown in **Table 4**. Statistically significant correlations between phenolics and DPPH and hydroxyl radical scavenging assays were observed on both fresh and dry bases. ORAC and TEAC showed some correlation only on fresh basis. Similar trends have been observed for sweet and hot red, green, or yellow peppers, using FRAP (*23*), TEAC (*17*, *23*, *25*), β-carotene bleaching (*24*), and DPPH (*2*, *4*, *35*) assays. High correlations between ascorbic acid and DPPH and hydroxyl assays and, to a lesser extent, with TEAC were observed. Serrano et al. (*25*) observed high correlation between ascorbic acid and TEAC in ripe and unripe sweet peppers. Hanson et al. (*17*) observed high correlations between ascorbic acid and inhibition of lipid peroxidation. In agreement with Cho et al. (*31*) no correlation existed between ORAC and ascorbic acid content. Good correlations were observed between the different antioxidant assays, except for ORAC. Contrary to the findings of Matreska and Perucka (*4*), who observed high correlation with total capsaicinoids, especially for red peppers, in our case no correlation existed between capsaicinoids and antioxidant activity. This lack of correlation could be due to two reasons: first, the low antioxidant activity of both capsaicin and dihydrocapsaicin and, second, the low capsaicinoid content (approximately 3−10 times less than the phenolic content). As a consequence of these two factors, the effect of capsaicinoids on antioxidant activity can be neglected. These results suggest that both total phenols and ascorbic acid play an important role in the antioxidant activity of fresh and processed Jalapeño and Serrano peppers.

Depending on the experimental conditions, chlorophylls may act as prooxidant or antioxidant compounds in food lipids. In the presence of light, chlorophylls can act as a photosensitizer to produce $^1O_2$, which reacts with the double bonds, forming hydroperoxides, and hence promoting the autoxidation of food lipids (*47*, *48*). However, in the dark, chlorophylls behave as antioxidants by donation of a hydrogen atom (*8*, *49*, *50*). It has been demonstrated that chlorophylls and some derivatives present antiradical activity against DPPH$^\bullet$ and $ABTS^{\cdot+}$ (*50*−*52*). To study the possible effects of chlorophylls on the antioxidant activity of Jalapeño and Serrano pepper extracts, chlorophylls were removed from the extracts through liquid−liquid extraction and column chromatography. Liquid−liquid extraction resulted in the total removal of chlorophylls, capsaicinoids, and carotenoids. However, it was not possible to use liquid−liquid extraction of either pickled pepper because they formed stable emulsions. Column chromatography specifically removed chlorophylls without removing capsaicinoids and carotenoids.

Table 4. Correlation Coefficients (*r*) in Fresh and Dry Basis (Probability) of Total Phenols and Ascorbic Acid with Antioxidant Activity and between Antioxidant Activity Assays

	dry weight[a]	fresh weight[a]
total phenols		
DPPH	0.844* (0.017)	0.832* (0.020)
hydroxyl	0.755* (0.049)	0.788* (0.035)
TEAC	0.533 (0.218)	0.732 (0.061)
ORAC	0.222 (0.633)	0.904* (0.005)
ascorbic acid		
DPPH	0.938* (0.002)	0.828* (0.021)
hydroxyl	0.857* (0.014)	0.790* (0.034)
TEAC	0.818* (0.025)	0.663 (0.104)
DPPH−hydroxyl	0.795* (0.033)	0.861* (0.013)
DPPH−TEAC	0.859* (0.013)	0.874* (0.010)
hydroxyl−TEAC	0.633 (0.127)	0.722 (0.067)

[a] *, presents significant correlation ($P < 0.05$).

The DPPH$^\bullet$ scavenging activity of fresh and processed Jalapeño and Serrano peppers, expressed as micromoles TE per gram of extract, is presented in **Figure 5**. For all peppers, except chipotle, chromatographic samples (chlorophyll free) showed statistically significant lower DPPH values, compared to crude extracts. Considering that no differences in total phenols or capsaicinoids

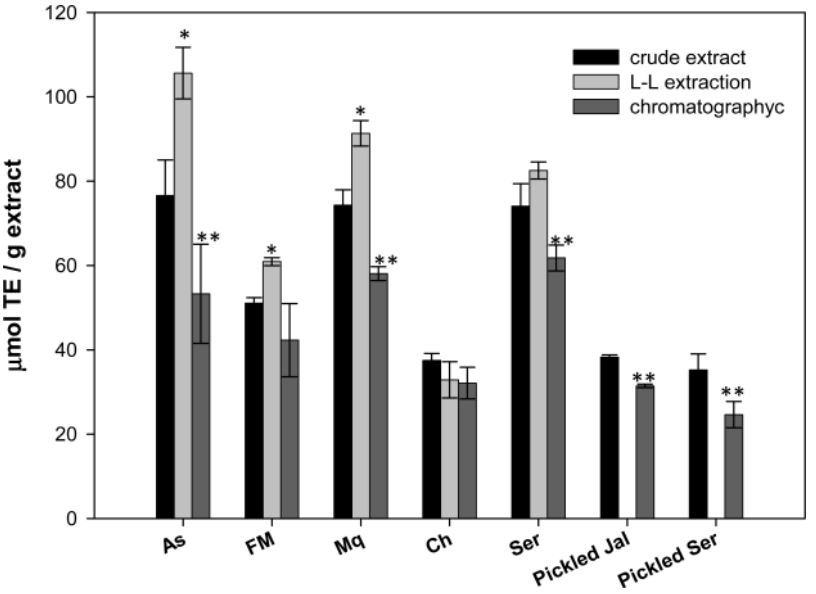

Figure 5. Antioxidant activity, estimated by the DPPH scavenging assay measured by EPR spectroscopy (expressed as μmol of TE/g extract) of fresh and processed Jalapeño and Serrano crude extracts, liquid−liquid extract, and chromatographic extract. Abbreviations: As, Ascencion Jalapeño pepper; FM, Flores Magon Jalapeño pepper; Mq, Meoqui Jalapeño pepper; Ch, chipotle; Ser, Serrano pepper. Values are the mean ± SD from three estimations. * indicates statistically significant differences (Tukey test, $P < 0.05$) between crude extract and L-L extraction; ** indicates statistically significant differences (Tukey test, $P < 0.05$) between crude extract and chromatographic extraction.

were observed, the reduction in the antioxidant activity of the chlorophyll-free samples indicates that for all fresh and processed peppers, except chipotle, their chlorophyll contents contributed to their ability to scavenge DPPH$^{\bullet}$ (*50*−*52*). No statistical differences were observed in the case of chipotle. This can be justified because, during chipotle processing, chlorophylls decompose, and this may also explain why chipotle DPPH activity was lower compared to that of other peppers. The increase of the antioxidant activity of the liquid−liquid extracted samples could be because during the extraction chlorophyll capsaicinoids and carotenoids were also removed, retaining only phenolic compounds with higher DPPH$^{\bullet}$ scavenging activity.

In summary, the results of this work indicate that fresh and processed Jalapeño and Serrano peppers are good sources of phenolics and ascorbic acid, as well as capsaicinoids, and present high antioxidant activity. During pepper processing, a decrease in ascorbic acid and antioxidant activity is observed, probably due to lixiviation and oxidation processes (chipotle pepper). Nevertheless, processed peppers represent an important source of antioxidants in the Mexican diet. DPPH scavenging results showed that gallic acid and catechin had the highest activity, whereas ascorbic acid and capsaicinoids gave low values. DPPH results also confirmed the antioxidant activity of chlorophylls in Jalapeño and Serrano peppers.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate); GAE, gallic acid equivalents; TE, Trolox equivalents; PCE, phenolic crude extract; FW, fresh weight; DW, dry weight; TAC, total antioxidant capacity; ORAC, oxygen radical absorbance capacity; RSC, radical scavenging capacity; EPR, electron paramagnetic resonance; PBS, phosphate buffer saline solution; As, Ascencion Jalapeño pepper; FM, Flores Magon Jalapeño pepper; Mq, Meoqui Jalapeño pepper.

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