JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 3399–3406 3399
DOI:10.1021/jf904220w

Bioactive Compounds of Four Hot Pepper Varieties (*Capsicum annuum* L.), Antioxidant Capacity, and Intestinal Bioaccessibility

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Pepper fruits (*Capsicum annuum*) contain a wide array of phytochemicals with well-known antioxidant properties. Since bioactive compounds depend on their bioavailability to exert beneficial effects, it was crucial to estimate the extent of release from the food matrix and thus their bioaccessibility. Accordingly, we determined the individual carotenoid and phenolic content as well as the antioxidant properties of four red hot dried cultivars (*Capsicum annuum* L.) of high consumption in Mexico and estimated the extent of intestinal bioaccessibility of carotenoids with significance in human health, β-carotene, β-cryptoxanthin, and zeaxanthin, using an in vitro gastrointestinal model. Hot dried peppers at ripe stage had a high content of bioactive compounds that exhibited significant antioxidant properties (26−80 μmol trolox equivalents/g of dry matter), such as polyphenols (>2000 mg/100 g of dry matter) and carotenoids (95−437 mg/100 g of dry matter), which were partially bioaccessible. The amount released from the food matrix by the action of digestive enzymes was about 75% for total polyphenols, up to 49% for both β-carotene and zeaxanthin, and up to 41% for β-cryptoxanthin. The results suggest that from 50 to 80% of these carotenoids could reach the colon to be potentially fermented or could remain unavailable.

KEYWORDS: ***Capsicum annuum*; β-carotene; β-cryptoxanthin; zeaxanthin; bioavailability; polyphenols; carotenoids; antioxidant capacity**

INTRODUCTION

Pepper fruits (*Capsicum annuum*) are important vegetables usually consumed as food and as spice. Many studies have demonstrated that peppers contain a wide array of phytochemicals mainly vitamins C, A, and E, as well as phenolic and carotenoids compounds with well-known antioxidant properties (*1*−*3*). Hot cultivars are rich in capsaicinoids which are responsible for the specific taste of pepper fruits (*4*). Peppers are a good source of provitamin A carotenoids (β-carotene, α-carotene, and β-cryptoxanthin) and oxygenated carotenoids or xanthophylls that have been shown to be effective free radical scavengers and may be significant in preventing common degenerative conditions (*3*, *5*). Carotenoids are among the active components of plant foods with potential health effects, and enhancement of carotenoids levels might thus be desirable.

Peppers are consumed worldwide either raw or cooked. In Mexico, a wide variety of colored pepper fruits (sweet, semihot, and hot varieties) are consumed at different stages of maturity in fresh and canned forms, or air-dried and sun-dried. The intake of peppers in the Mexican diet was estimated to be 9 to 15 kg/person/year in recent years (*6*), exceeding the intake of rice and potatoes. The dehydrated fruits in the dark red ripe stage of hot peppers are the most popular commercialized form in Mexico (*7*), with Arbol, Chipotle, Guajillo and Morita varieties being among the most widely consumed. There is a considerable increase in carotenoid content during the course of ripening (*1*, *2*), and the majority of carotenoids present in peppers are esterified with fatty acids at the mature stage (*8*).

This work was focused on the major carotenoid constituents with significance in human health, β-carotene and β-cryptoxanthin with pro-vitamin A activity, and zeaxanthin due to its role in the prevention of age-related macular degeneration and cataracts (*2*). Since all bioactive compounds depend on their bioavailability to exert beneficial effects, it is crucial to estimate the extent of release from the food matrix and its bioaccessibility in the small intestine, which may be an indicator of the potential to be further absorbed through the intestinal barrier.

The objectives of this work were to determine the individual carotenoid and phenolic content as well as the antioxidant properties of four *Capsicum annum* L. varieties of high consumption in Mexico, in order to compare their bioactive profile and to estimate the extent of intestinal bioaccessibility of β-carotene, β-cryptoxanthin, and zeaxanthin present in red hot dried cultivars studied using an in vitro gastrointestinal model.

MATERIALS AND METHODS

Plant Material and Sample Preparation. Commercial products packed in polypropylene bags (*Don Zabor* brand, Mexico) of four hot varieties from dehydrated whole fruit of *Capsicum annuum* L. (Arbol,

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Published on Web 03/01/2010

Chipotle, Guajillo and Morita) were acquired in a local supermarket (Acapulco, Mexico). Fruits of all varieties were fully red ripe before dehydratation. Arbol fruit is 7−11 cm in length and 1 cm wide, with a mean weight of 1.0−1.5 g and pungency values from 5000 to 30000 Scoville units. Chipotle comes from the dehydratation of smoked fruits of a red Jalapeño cultivar; its dimensions range from 2.5 to more than 8 cm in length and 2.5 cm wide, with a mean weight of 4.3 g and pungency values from 5500 to 40000 Scoville units. Guajillo fruit is 10−14 cm in length and 2.5−3.0 cm wide, with a mean weight of 5.0−9.0 g and pungency values from 3000 to 5000 Scoville units. Morita results from the dehydratation of smoked fruits of a short Jalapeño cultivar; its dimensions are length, 3 cm, and width, 2 cm, with a mean weight of 1.1 g and a pungency of 10000 Scoville units.

Samples of dehydrated whole fruits (200 g) were slightly washed with distilled water, and the peduncles were eliminated. Samples were dried in a vacuum oven at 40 °C for 1 h. Then, the dried material was ground to fine powder (sieve ring 0.5 mm) and stored at −20 °C until use.

Proximate Analysis. Samples were analyzed using the following AOAC methods (*9*): protein (Method 950.48), fat (Method 983.23), ash (Method 940.26), and moisture (Method 925.09). Protein content was evaluated using a Nitrogen Determinator LECOFP-2000 (Leco Corporation, St. Joseph, MI, USA).

Indigestible Fraction. Indigestible fraction is defined as the part of plants foods that is not digested or absorbed in the small intestine and reaches the colon, where it serves as a substrate for fermentative microflora (*10*). The indigestible fraction is composed of mainly dietary fiber and other minor compounds resistant to the action of digestive enzymes such as indigestible proteins, resistant starch, and lignin. Thus, in this work dietary fiber was determined as an indigestible fraction following the procedure described by Saura-Calixto et al. (*10*). Briefly, the sample (300 mg) was incubated with pepsin (0.2 mL of a 300 mg/mL solution in 0.2 M HCl-KCl buffer, pH 1.5, 40 °C, 1 h; Merck 7190), pancreatin (1 mL of a 5 mg/mL solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h; Sigma P1750), lipase (2 mL of a 7 mg/mL solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h; Sigma L-3126), and porcine bile extract (2 mL of a 7 mg/mL solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h; Sigma B-8631) in subsequent steps. Then samples were centrifuged (15 min, 3000*g*) and supernatants removed and combined. Residues from centrifugation were freeze-dried and then quantified gravimetrically. This value was the insoluble indigestible fraction. Supernatants were dialyzed against water for 48 h at 25 °C to eliminate all compounds susceptible to be absorbed through the intestinal barrier. Dialysis retentates were then submitted to acid hydrolysis with 1 M sulfuric acid, and nonstarch polysaccharides were measured spectrophotometrically by the dinitrosalysilic acid method (*11*). This value was the soluble indigestible fraction. The total dietary fiber is the sum of the soluble indigestible fraction (SIF) and the insoluble indigestible fraction (IIF). Representative samples of retentates (SIF) and freeze-dried residues (IIF) were stored at −20 °C for further carotenoid determination.

Extractable Polyphenols. Dried samples (0.5 g) were extracted by constant shaking at room temperature with methanol−water (50:50 v/v, 50 mL/g of sample, 60 min) and acetone−water (70:30 v/v, 50 mL/g of sample, 60 min). After each extraction step, samples were centrifuged (15 min, 25 °C, 3000*g*), and supernatants were collected. At the end of the extraction process, methanol−water and acetone−water supernatants were combined. Total polyphenols in supernatants and dialysis retentates were determined by the Folin−Ciocalteau procedure (*12*).

Nonextractable Polyphenols. Proanthocyanidins (condensed tannins) and hydrolyzable polyphenols were determined in the residues from the methanol−water and acetone−water extraction.

Proanthocyanidins. The residues were treated with 5 mL/L HCl-butanol (3 h, 100 °C) (*13*) for proanthocyanidin hydrolysis. Proanthocyanidins were calculated from the absorbance at 550 nm using as standard the Mediterranean carob pod (*Ceratoniasiliqua L.*) supplied by Nestle S.A.

Hydrolyzable Polyhenols. Hydrolyzable polyphenols were determined by methanol/H_2SO_4 90:10 (v/v) hydrolysis at 85 °C for 20 h (*14*) of the residues from the methanol−water and acetone−water extraction. Samples were centrifuged (15 min, 25 °C, 3000*g*), and the hydrolyzable polyphenols were determined in supernatants by the Folin−Ciocalteau procedure (*12*), using gallic acid as the standard.

Antioxidant Capacity Assay. FRAP and ABTS assays were used to estimate the antioxidant capacity of supernatants extracted.

Ferric Reducing Ability Assay (*FRAP*). The method was described by Benzie and Strain (*15*). Briefly, the FRAP reagent, containing 2,4,6-tri(2-pyridyl)-*s*-triazine(TPTZ) (Fluka Chemicals, Madrid, Spain), $FeCl_3$, and acetate buffer, was mixed with 90 μL of distilled water and 30 μL of the sample or the blank (solvents used for extraction). Absorbance values at 595 nm were taken every 15 s at 37 °C, using a UV-1800 UV−vis spectrophotometer (Shimadzu Europe GmbH, Duisburg, Germany). The readings at 30 min were selected for the calculations of FRAP values. A standard curve of trolox was used to estimate the antioxidant capacity of samples, and it was expressed as trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble analogue of vitamin E) equivalents.

Free Radical Scavenging Assay (*ABTS Method*). The antioxidant capacity was estimated in terms of radical scavenging activity following the procedure described elsewhere (*16*) with some modifications (*17*). Briefly, the ABTS [2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulfate in the dark at room temperature for 12−16 h before use. The $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 730 nm. After the addition of 0.1 mL of sample to 3.9 mL of diluted $ABTS^{\bullet+}$ solution, absorbance readings were taken every 20 s using a UV-1800 UV−vis spectrophotometer (Shimadzu Europe GmbH, Duisburg, Germany). The reaction was monitored for 6 min. Inhibition of absorbance versus time was plotted, and the area below the curve (0−6 min) was calculated. Solutions of known trolox concentration were used as antioxidant capacity equivalents.

Extraction and Analysis of Carotenoids. Samples were extracted and separated using the method of Mínguez-Mosquera and Hornero-Méndez (*8*) with slight modifications. Dried sample (0.05−0.1 g) or dialysis retentates (5 mL) were extracted with acetone (5 mL) by constant shaking (45 min) using an orbital shaker at room temperature. This treatment was repeated until no more color was extracted. After each extraction step, samples were centrifuged (10 min, 25 °C, 3000*g*), and supernatants were collected and combined. The extracts were treated with diethyl ether (5−10 mL) and 10% (w/v) NaCl solution (1−2 mL). Then, the ether phase was separated after centrifugation (10 min, 25 °C, 3000*g*) and washed with a 2% Na_2SO_4 solution. The ether phase was saponified with 20% KOH−MeOH (5−10 mL) at room temperature and left for 1 h with periodic shaking. The aqueous phase was removed, while the organic phase was washed with distilled water until neutral and then washed with a 2% Na_2SO_4 solution. The organic phase was separated after centrifugation (10 min, 25 °C, 3000*g*) and evaporated to dryness in a rotary evaporator at a temperature lower than 40 °C. The pigments were dissolved in acetone (5−10 mL) and kept at −20 °C until HPLC analysis.

Carotenoids were separated using an HPLC system consisting of a Hewlett-Packard System Series model 1100 with a photodiode array detector. The column was a 4.6 mm × 250 mm C-18 i.d., 5 μm Nucelosil 100 (Teknokroma, Barcelona, Spain). A guard column (4 mm × 23 mm) packed with the same material was installed ahead to protect the carotenoid column. Carotenoid separation was achieved using a gradient program previously described (*8*) with some modifications: the flow rate was reduced to 1.0 mL/min, the injection volume was 5−50 μL, the column was maintained at 25 °C, and the detection was carried out at 450 and 470 nm. β-Carotene (≥95.0%, Sigma-Aldrich CAS 7235-40-7), β-cryptoxanthin (Sigma-Aldrich C6368), and zeaxanthin (≥95.0%, Sigma-Aldrich CAS 144-68-3) were quantified by using calibration curves prepared with pure standards in acetone, in the range of 2.5−20 μg/mL.

To estimate total carotenoid content, peak areas of unknown compounds measured at 450 nm were summed for each sample and quantified as β-carotene equivalents because of the lack of standards for positive identification. Finally, the content of β-carotene, β-cryptoxanthin, and zeaxanthin plus β-carotene equivalents were added up to obtain total carotenoid content.

Estimation of Bioaccessibility. Bioaccessible carotenoids and polyphenols in the small intestine were determined as the difference of carotenoid and polyphenol content in the original sample and carotenoid and polyphenol content in both indigestible fractions, soluble and insoluble (**Figure 1**). Carotenoid and polyphenol bioaccessibility was expressed as a percentage.

Statistical Analysis. All data were expressed as mean ± standard error of the mean (SEM). Determinations were performed in triplicate in

two independent experimental assays. Significant differences between mean values were determined by performing a one-way ANOVA F test and Fisher's least significant differences ($p < 0.05$). ANOVA test and simple analysis regression were performed using STATGRAPHICS Plus 5.1 (Rockville, MD, USA).

RESULTS AND DISCUSSION

In this work, we studied the main varieties most consumed of the dried red hot peppers in the Mexican diet: Arbol, Chipotle, Guajillo, and Morita. The proximate composition (**Table 1**) was in accordance with data previously reported for other varieties (Pasilla and Ancho) of *Capsicum annuum* that also are air-dried or sun-dried (*18*). It is important to note the high content of total dietary fiber, especially the insoluble fraction. This is interesting because of the considerable intake of hot peppers in Mexico and other Latin America countries, which results in a significant contribution of dietary fiber to the diet of these countries. The indigestible fraction determined in this work consists of dietary fiber (nonstarch polysaccharides and lignin) and also other indigestible food constituents such as resistant starch, oligosaccharides, resistant protein, polyphenols, and other minor associated compounds. All of these compounds may reach the colon and be used as the substrate for colonic fermentation (*19*).

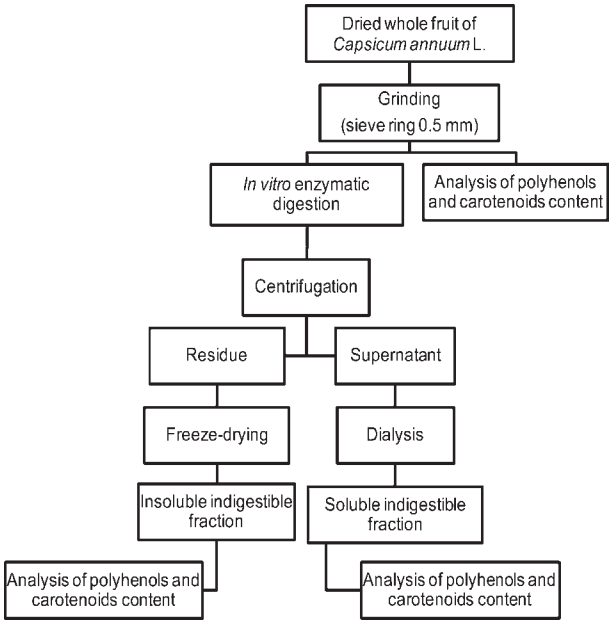

Figure 1. Methodology to estimate carotenoid and polyphenol bioaccessibility in the small intestine.

The results in **Table 2** show the polyphenolic and total carotenoid content. Extractable polyphenols (low and intermediate molecular mass) correspond to most the of data referenced in the literature as total phenolic content which are estimated from aqueous−organic extracts. However, a significant amount of high molecular mass polyphenols (hydrolyzable polyphenols) remain in the residues of extraction. This could be one reason why our results do not agree with some of the published data. Until now, this is the first report of polyphenol content for the hot pepper varieties studied. Arbol, Chipotle, Guajillo, and Morita varieties contain both extractable polyphenols and hydrolyzable polyphenols; however, proanthocyanidins were not identified.

There are data of polyphenol content reported in the literature for other *Capsicum* varieties that are similar (*1*), higher, or lower than our results (*2*, *3*). These differences could be explained by variations in sample preparation, extraction, and quantification methods, chemical forms of compounds analyzed, diversity of varieties and genotypes (as sweet or hot peppers), maturity stage, and the use of fresh or dehydrated fruits (*1*, *2*).

Regarding hydrolyzable polyphenols, its content was higher than extractable polyphenols in all of the varieties tested except Chipotle. Hydrolyzable polyphenols constituted 59, 58, and 55% of total phenolic content in Arbol, Guajillo, and Morita, respectively, and 41% in the case of Chipotle. In general, total phenolic content (extractable + nonextractable polyphenols) was the highest in Arbol and Morita, followed by Chipotle and Guajillo, and these results were similar or even higher than the phenolic content of foods considered rich in polyphenols such as cranberry, grape, banana, passion fruit, peanuts, almonds, walnuts, plum, strawberry, and blueberry (*20*−*22*).

The total carotenoid content of red hot peppers studied (**Table 2**), as in the case of extractable polyphenols content, was

Table 1. Proximate Composition of Dried Hot Pepper Varieties (*Capsicum annuum* L.)[a]

	g/100 g of dry matter			
	Arbol	Chipotle	Guajillo	Morita
protein	15.5 ± 0.2 a	13.4 ± 0.1 b	15.5 ± 0.2 a	15.5 ± 0.1 a
fat	13.2 ± 0.0 a	7.4 ± 0.1 b	11.3 ± 0.0 c	10.7 ± 0.2 d
ash	5.6 ± 0.1 ab	5.3 ± 0.1 a	5.5 ± 0.2 ab	5.9 ± 0.2 b
total dietary fiber[b]	41.7 ± 1.0 a	27.9 ± 0.2 b	31.3 ± 0.5 a	38.4 ± 0.2 d
soluble indigestible fraction	2.9 ± 0.2 a	5.3 ± 0.2 b	2.8 ± 0.1 a	5.2 ± 0.5 b
insoluble indigestible fraction	38.6 ± 0.1 a	22.5 ± 0.1 b	28.5 ± 0.1 c	33.2 ± 0.0 d

[a] Mean ± SEM. [b] It was determined as the sum of soluble and insoluble indigestible fractions (*10*). Values in a row not sharing the same lower case letter are significantly different ($p < 0.05$).

Table 2. Bioactive Compounds of Dried Hot Pepper Varieties (*Capsicum annuum* L.)[a]

	mg/100 g of dry matter			
	Arbol	Chipotle	Guajillo	Morita
extractable polyphenols	1173.9 ± 29.8 a	1414.4 ± 44.8 b	972.7 ± 10.7 c	1192.1 ± 32.3 a
hydrolyzable polyphenols	1669.6 ± 82.5 a	966.6 ± 21.2 b	1352.7 ± 31.1 c	1442.7 ± 31.0 c
proanthocyanidins	0	0	0	0
total polyphenols[b]	2843.5 ± 87.7 a	2381.0 ± 49.6 b	2325.4 ± 32.9 b	2634.8 ± 44.8 c
total carotenoids	365.8 ± 22.4 a	191.4 ± 29.9 b	87.6 ± 7.2 c	373.3 ± 14.8 a

[a] Mean ± SEM. [b] It was determined as the sum of extractable and hydrolyzable polyphenols values. Values in a row not sharing the same lower case letter are significantly different ($p < 0.05$).

significantly the highest for Arbol and Morita varieties, followed by Chipotle and Guajillo. It is interesting that Guajillo had a 4-fold variation in carotenoid content when compared with Arbol or Morita, which highlights the increased bioactive content and the nutritional importance of the latter varieties. Particularly, total carotenoid content data in the literature for red peppers are below our range determined. Collera-Zúñiga et al. (*7*) studied the carotenoid composition of three Mexican varieties of dried hot peppers (*Capsicum annuum* L.) popularly consumed in Mexico. The authors found a mean carotenoid content of 6.76 mg/100 g of dry weight for Guajillo and a range of 7.0−7.5 mg/100 g of dry weight for Ancho and Mulato varieties. These values are considerably low in comparison to our results. This could be explained by the influence of genotypes and maturity stages. Another explanation could be the differences in sample preparation since we used the whole fruit including seeds differing from the mentioned study. Seeds from various plant sources have been shown to contribute significantly toward high total phenolic content (*1*), and it was initially supposed that this could be extrapolated to carotenoid content. Mínguez-Mosquera et al. (*23*) reported the carotenoid composition of pepper seed (*Capsicum annuum* L.), and they found that β-carotene and zeaxanthin were the major pigments, followed by capsanthin and β-cryptoxanthin; however, their contribution to total carotenoid content is very low (0.85−1.45 ppm) to be significant. Otherwise, our results of total carotenoid content (**Table 2**) were in accordance with those found in other varieties such as red sweet pepper Anupam (*1*), peppers of *Capsicum annuum* var. *longum* (*24*), and ripe yellow pepper (*25*), and slightly lower than dried red peppers *Bola* and *Agridulce* varieties (*26*) and five red *Capsicum* fruits (*27*).

Several studies have been conducted to investigate the antioxidant properties of peppers, mainly in its fresh form (*3*, *5*). It has been reported that red hot peppers (*Capsicum annuum* Tepin and *Capsicum chinese* Habanero) prevent Fe^{2+}-induced lipid peroxidation probably due to its higher total phenol content and Fe^{2+} chelating ability (*3*). Additionally, it has been proved that colored peppers (*Capsicum annuum* L.) exhibit radical-scavenging activity (*5*).

We measured the antioxidant capacity in aqueous−organic extracts containing extractable polyphenols and hydrolyzable polyphenols in terms of ferric reducing antioxidant power (FRAP) and free radical-scavenging activity (ABTS). All varieties of red hot dried peppers, both extractable polyphenols and hydrolyzable polyphenols extracts, showed a high antioxidant capacity per g of dry matter by both methods (**Table 3**). Arbol and Chipotle varieties presented the highest values of antioxidant activity, followed by Morita and Guajillo. The antioxidant capacity of extractable polyphenols was similar to that reported for other common foods in diets such as peanuts, almonds, and walnuts, and higher than that corresponding to the most consumed fruits such as oranges, grapes, apples, and strawberries. In the case of hydrolyzable polyphenols, its antioxidant capacity was similar to that of legumes (beans, lentils, and chickpeas) and vegetables (tomato, onion, and garlic) (*20*). Notably, a positive correlation was observed between the extractable polyphenol content and the antioxidant capacity measured by ABTS (r = 0.980; r^2 = 0.961) and FRAP (r = 0.998; r^2 = 0.996). The same trend was observed in the case of hydrolyzable polyphenols: ABTS (r = 0.835; r^2 = 0.697) and FRAP (r = 0.921; r^2 = 0.848). In support of this, a high correlation was previously found between the content of phenolic compounds and the antioxidant activity of pepper fruits (*Capsicum annuum*) (*2*, *28*, *29*). It is worth noting that extractable polyphenols exhibited a higher antioxidant capacity (from 59 to 83% of total antioxidant capacity) measured by both methods in comparison with that of hydolyzable polyphenols (from 17 to 40% of total antioxidant capacity). An interesting finding was that for Arbol, Guajillo, and Morita, the antioxidant activity of extractable polyphenols measured by ABTS accounts for exactly 70% of the total antioxidant capacity, suggesting a relationship in the phenolic profile of these varieties, irrespective of the concentration present in each variety. The exception to this trend was Chipotle in which extractable polyphenols represented up to 82% of the total antioxidant capacity. The variations observed in antioxidant capacity between extractable polyphenols and hydrolyzable polyphenols may be attributed to differences in chemical structure. Pepper fruits contain a wide array of complex phenolic compounds, which are usually bound with sugars as glycosides. Some of the major phenolics identified are flavanoids, derivatives of cinnamic acid and capsaicinoids (*4*). In our study, hydrolyzable polyphenols were determined after a treatment of acidic hydrolysis. In this regard, after acidic hydrolysis of flavanoid extracts from pepper fruits, luteolin and quercetin have been identified (*2*), which suggests that a similar behavior could be observed in the hydrolyzable polyphenol fraction. Otherwise, some authors have suggested that the antioxidant activity of phenolics is related to the number and positions of hydroxyl groups in the aromatic rings, esterification or free form of compounds analyzed, and the methoxy substituents in the ortho position to the OH (*4*). This statement highlights the influence of the phenolic profile in antioxidant activity.

Table 3. Antioxidant Capacity of Polyphenol Extracts of Dried Hot Pepper Varieties (*Capsicum annuum* L.)[a]

	μmol trolox equivalents/g of dry matter			
	Arbol	Chipotle	Guajillo	Morita
	extractable polyphenols			
FRAP	49.3 ± 1.0 a	66.5 ± 1.1 b	37.5 ± 0.5 c	52.1 ± 0.9 d
ABTS	27.5 ± 0.3 a	36.4 ± 0.6 b	18.6 ± 1.0 c	24.9 ± 0.5 d
	hydrolyzable polyphenols			
FRAP	33.0 ± 0.9 a	14.1 ± 0.5 b	26.4 ± 0.7 c	21.5 ± 0.3 d
ABTS	11.0 ± 0.3 a	8.0 ± 0.1 b	8.0 ± 0.3 b	10.1 ± 0.2 c
	total antioxidant capacity[b]			
FRAP	82.3 ± 1.3 a	80.6 ± 1.2 b	63.9 ± 0.9 c	73.9 ± 0.9 d
ABTS	38.5 ± 0.4 a	44.4 ± 0.6 b	26.6 ± 1.0 c	35.0 ± 0.5 d

[a] Mean ± SEM. [b] It was determined as the sum of antioxidant capacity values of extractable and hydrolyzable polyphenols. Values in a row not sharing the same lower case letter are significantly different ($p < 0.05$).

Moreover, the antioxidant activity of pepper fruits may be attributed also to ascorbic acid, carotenoids, and capsaicinoids. Hence, the significance of an appropriate correlation of the antioxidant activity values with pepper constituents because of the influence of other soluble compounds, besides polyphenols, present in extracts which could affect the total antioxidant capacity. An additional consideration is that lipophilic compounds with antioxidant properties, as the majority of carotenoids, are usually ignored in these assays. Therefore, it would be important to determine the contribution of carotenoids to the total antioxidant capacity of red dried hot peppers studied in our work. However, a main limitation is that few methods allow for the successful measurement of antioxidant activity in lipophilic fractions (*1*). Additionally, several studies support that the majority of antioxidant activity in peppers is given by polyphenols rather than vitamins C, E, and β-carotene (*3*, *4*, *22*, *30*).

In addition to the carotenoid composition of red dried hot peppers, diverse authors have identified capsanthin as the main carotenoid in several varieties of red peppers (*7*, *8*, *23*, *25*). Pérez-López et al. (*31*) reported capasanthin as 43.6% of the total

Table 4. Carotenoid Content in Original Samples and Associated with Indigestible Fractions of Dried Hot Pepper Varieties (*Capsicum annuum* L.)[a]

	mg/100 g of dry original sample			
	Arbol	Chipotle	Guajillo	Morita
original sample				
β-carotene	85.7 ± 1.2 a	14.6 ± 1.7 b	7.3 ± 0.3 c	22.0 ± 0.9 d
β-cryptoxanthin	10.6 ± 0.1 a	2.9 ± 0.3 b	2.0 ± 0.2 c	3.8 ± 0.2 d
zeaxanthin	10.3 ± 1.2 a	4.1 ± 0.4 b	0.9 ± 0.1 c	5.5 ± 0.3 b
insoluble indigestible fraction				
β-carotene	14.3 ± 0.9 a	4.0 ± 0.1 b	2.1 ± 0.0 c	5.6 ± 0.2 d
β-cryptoxanthin	2.0 ± 0.0 a	0.8 ± 0.0 b	0.4 ± 0.0 c	1.0 ± 0.0 d
zeaxanthin	2.6 ± 0.8 a	1.6 ± 0.0 a	0.4 ± 0.1 b	2.1 ± 0.1 a
soluble indigestible fraction				
β-carotene	29.8 ± 2.6 a	4.4 ± 0.0 b	traces	9.2 ± 0.0 b
β-cryptoxanthin	4.3 ± 0.0 a	1.6 ± 0.0 b	traces	1.9 ± 0.1 c
zeaxanthin	5.2 ± 0.9 a	1.1 ± 0.0 b	not detected	0.7 ± 0.3 b
total indigestible fraction				
β-carotene	44.0 ± 2.8 a	8.4 ± 0.1 b		14.8 ± 0.2 d
β-cryptoxanthin	6.3 ± 0.0 a	2.4 ± 0.0 b		2.9 ± 0.1 d
zeaxanthin	7.7 ± 1.3 a	2.7 ± 0.0 b	0.4 ± 0.1 c	2.8 ± 0.3 b

[a] Mean ± SEM. Values in a row not sharing the same lower case letter are significantly different ($p < 0.05$).

carotenoids in red Almuden peppers. Capsanthin had similar percentages in red pepper fruits, 37.8% (*32*) and 43.6% (*29*) of the total carotenoid concentration. In accordance, we detected a main peak in all red hot pepper varieties (data not shown) which represented 44.9% (Arbol), 53% (Chipotle), 40.1% (Guajillo), and 49.4% (Morita) of the total carotenoid content. Thus, on the basis of these values, the order of elution, and the retention time, it can be suggested that the most abundant carotenoid determined in Mexican varieties could be also capsanthin.

Even though capsanthin seems to be the most abundant carotenoid in peppers, other authors determined that it has a very low bioavailability (*33*). It is interesting to note that from the major carotenoids present in paprika, only zeaxanthin, β-carotene, and β-cryptoxanthin were detected in human chylomicrometers in unesterified form (*33*). In agreement, we selected the same carotenoids to be investigated.

The individual carotenoid contents in the original samples and those associated with the corresponding indigestible fractions are shown in **Table 4**. Our results of β-carotene, β-cryptoxanthin, and zeaxanthin content in original samples were consistent with other studies performed in peppers (*8*, *23*, *30*, *31*). By contrast, the results of the three carotenoids studied were appreciably above other data of pepper carotenoid composition reported in the literature (*1*, *2*, *7*). This may also be explained by the variations in plant material and the methodology used, as it was previously discussed. We found that β-carotene was the major carotenoid in all varieties, accounting for 15.4% (Arbol), 6.9% (Chipotle), 7.6% (Guajillo), and 5.4% (Morita) of total carotenoid content. β-Cryptoxanthin contributions were 1.9% (Arbol), 1.4% (Chipotle), 2.0% (Guajillo), and 0.9% (Morita) to the total carotenoid content. Finally, zeaxanthin content ranged from 1.8% (Arbol), 1.9% (Chipotle), 0.9% (Guajillo), and 1.3% (Morita) of the total carotenoid content.

Comparing among varieties, we found that the content of β-carotene and β-cryptoxanthin was significantly different ($P < 0.05$) among all peppers cultivars studied. In addition to zeaxanthin content, there were no significant differences between Chipotle and Morita, whereas Guajillo contained the lowest concentration. In summary, Arbol had the highest content of β-carotene, β-cryptoxanthin, and zeaxanthin, followed by Morita and Chipotle, and Guajillo had the lowest content of bioactive carotenoids.

This work was focused on the carotenoid constituents with biological activity and nutritional importance. In general, in vivo studies of bioavailability are more limited than those in vitro due mainly to their demand for resources and more time. Also, a limitation is the lack of validated biomarkers and the low availability of study subjects. Hence, the significance of in vitro bioaccessibility studies of carotenoids, whose results may be subsequently used for the design of in vivo studies (*34*).

Carotenoids follow the same absorption mechanism as dietary triacilglycerides. After ingestion, carotenoids are incorporated into micelles formed from lipids and bile acids and subsequently transferred into intestinal mucosa cells, where a part of the carotenoids and retinyl esters is assembled into chylomicrometers and transported to the blood via the lymphatic system (*33*). For xanthophylls, ester hydrolysis by lipases may be required and some authors have suggested that cholesterol esterase could be responsible for the generation of free carotenoids in the gut (*35*).

The method used allowed us to estimate the content of carotenoids released from the food matrix by the action of the digestive enzymes and the amount of carotenoids remaining in the food matrix which is not available. In order to estimate the bioaccessibility of carotenoids in the small intestine, we quantified the individual carotenoid content in indigestible fractions isolated from samples of the four pepper cultivars. We found significant amounts of β-carotene, β-cryptoxanthin, and zeaxanthin associated with indigestible fractions of samples, being predominant in the soluble indigestible fraction (**Table 4**). The Guajillo variety was excluded from the bioaccessibility discussion because the carotenoid amounts detected in the soluble indigestible fraction were below our detection limit, and the estimation of bioaccessibility could be overestimated.

The bioaccessibility in the small intestine of individual carotenoids is shown in **Figure 2**. The bioaccessibility values were similar among carotenoids. A range from 33 to 49% for β-carotene, from 20 to 41% for β-cryptoxanthin, and from 25 to 49% in the case of zeaxanthin was estimated in Arbol, Chipotle, and Morita varieties. β-Carotene seemed to be the most bioaccessible. Our results were in accordance with the bioaccessibility reported for carotenoids present in diverse fruits and vegetables, including a red pepper (*36*).

The bioaccessibility of carotenoids depends on several factors: physical properties of the food matrix, genotype of the plant material, chemical form of carotenoids and polarity, solubility of carotenoids in digesta, potential susceptibility of carotenoids to be released from the food matrix induced by food processing, quantity of fat required for absorption, and methodology used for bioaccessibility assessment (*37*, *38*). The chemical structure of compounds affects the rate and extent of intestinal absorption. In paprika, it has been observed that β-carotene cannot occur in the esterified form, β-cryptoxanthin can occur in the free or in the monoesterified forms, and zeaxanthin can occur in the free, mono, and diesterified forms. Capsanthin usually occurs in the diesterified form (*26*). The fact that β-carotene is found only in the free form in paprika may be related to its greater bioaccessibility in all of the varieties studied. Thus, it can be suggested that esterification may affect bioaccessibility and may be considered in in vitro bioaccessibility studies. Accordingly, carotenoid extracts were saponified prior to HPLC analysis in order to hydrolyze carotenol esters and avoid an underestimation of carotenoid content.

In addition to differences among red hot cultivars, the bioaccessibility of β-carotene and β-cryptoxanthin in Arbol was significantly higher in comparison with that in the rest of varieties. This is important because Arbol had the greatest content of provitamin A carotenoids and, besides, is the most bioaccessible.

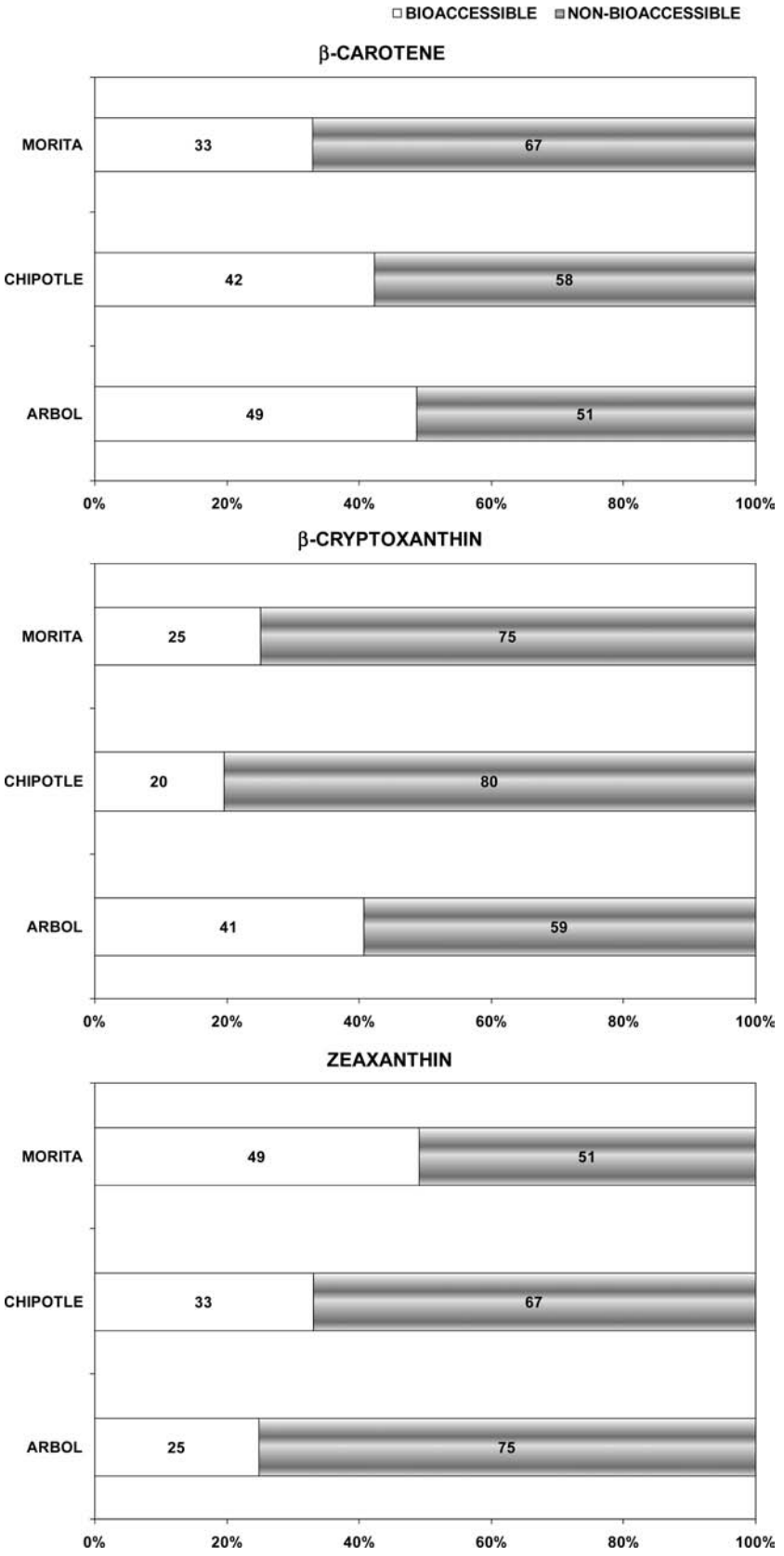

Figure 2. Bioaccessibility in the small intestine of individual carotenoids of dried hot pepper varieties (*Capsicum annuum* L.).

Exceptionally, zeaxanthin was the carotenoid least bioaccessible in the Arbol cultivar, which probably may be related to the extent of zeaxanthin esterification or structural differences of the food matrix such as dietary fiber since Arbol had the highest content of insoluble and total dietary fiber. Serrano et al. (*39*) determined that Klason lignin and nonstarch polysaccharides may directly reduce the availability of carotenoids from green leafy vegetables in the small intestine.

Regarding the bioaccessibility in the small intestine of phenolic compounds, the majority of total polyphenols were bioaccessible

in the small intestine for Arbol (72%), Chipotle (77%), Guajillo (76%), and Morita (74%) varieties, whereas about 25% of the total polyphenols remained nonbioaccessible which could reach the colon to be potentially fermented. A range from 77 to 86% for extractable polyphenols and from 62 to 76% in the case of hydrolyzable polyphenols was released from the food matrix of the four pepper fruit varieties studied. Extractable polyphenols were the most bioaccessible due to mainly their low molecular mass. Our results showed that polyphenols from red pepper fruits exhibited a greater bioaccessibility in comparison with the polyphenols present in diverse fruits and vegetables (*10*). This finding may be explained partially by the fact that proanthocyanidins, polyphenols resistant to the action of digestive enzymes, are not present pepper fruits.

In conclusion, our results highlight that the four varieties of red hot peppers studied can be considered a good source of antioxidant bioactive compounds which are intestinally bioaccessible, particularly extractable polyphenols β-carotene and zeaxanthin. Further research is required to assess the in vivo bioavailability of bioactive compounds from pepper fruits.

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Received for review December 1, 2009. Revised manuscript received February 9, 2010. Accepted February 20, 2010. This research was supported by the Spanish Ministerio de Ciencia e Innovación (project AGL2008-01633). D.H.-H. acknowledges a predoctoral fellowship from Mexican Consejo Nacional de Ciencia y Tecnología (CONACyT). S.G.S.-A. acknowledges Dirección General de Eduación Superior Tecnológica (DGEST) in México for a scholarship (PROMEP, 022005009P).