DOI:10.1021/jf100413m

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Bioaccessibility, Uptake, and Transport of Carotenoids from Peppers (*Capsicum* Spp.) Using the Coupled in Vitro Digestion and Human Intestinal Caco-2 Cell Model

Laurie O'Sullivan, Marvin A. Jiwan, Trevor Daly, Nora M. O'Brien, and S. Aisling Aherne*

School of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland

Spanish bell peppers (*Capsicum annuum* L.) and chili peppers sourced from Kenya and Turkey were analyzed for their carotenoid content, bioaccessibility, and bioavailability. The order of total carotenoid content in peppers and their respective micelles was red > green > yellow. In terms of cellular carotenoid transport as a percentage of original food and micelle content, the order was yellow peppers > green > red; however, the opposite trend was seen for the actual amount of total carotenoids transported by Caco-2 cells. Although lutein was generally the most abundant carotenoid in the micelles (496.3−1565.7 μg 100 g^{-1}), cellular uptake and transport of β-carotene were the highest, 8.3−31.6 and 16.8−42.7%, respectively. Hence, the actual amount of carotenoids present in the original food and respective micelles seems to reflect the amount transported by Caco-2 cells. Therefore, color influenced the carotenoid profile, bioaccessibility, and bioavailability of carotenoids rather than pepper type.

KEYWORDS: Bioaccessibility; bioavailability; *Capsicum*; Caco-2 cells; carotenoids; peppers

INTRODUCTION

Peppers (*Capsicum annuum* L.) are commonly consumed vegetables because they provide a variety of color and flavor to food products and meals. According to Sun et al. (*1*), the color of sweet bell peppers is the major factor associated with consumer purchasing decisions. Depending on flavor intensity and texture, their culinary use changes from that as a vegetable (bell pepper) to a spice (chili pepper) or a colorant (paprika). Peppers have been reported to possess certain biological properties (*1*, *2*) that may have positive effects on human health and chronic disease. For instance, reduced risks of prostrate cancer have been associated with increased intake of certain vegetables including bell peppers (*3*). In addition, peppers have been shown to possess radical scavenging activity (*1*,*2*,*4*) as well as prevent the oxidation of cholesterol and docosahexaenoic acid (DHA) during heating (*1*). Peppers are one of the top 10 contributors to the dietary intake of the carotenoids lutein and zeaxanthin in both the Republic of Ireland and Spain (*5*, *6*). These plant foods are a rich source of bioactive phytochemicals including capsaicinoids and carotenoids (*7*).

Numerous epidemiological studies have shown an association between the consumption of carotenoid-rich foods and a reduction in the risk of several human chronic diseases (*8*,*9*). The major interest in studying carotenoids is because of their bioactive effects, which include provitamin A activity, antioxidant actions, immune modulation, and involvement in cell signaling (*6*, *10*). Bioavailability is a critical feature in the assessment of the role of dietary components, plant food consumption, and human health (*11*). Therefore, to enhance our knowledge about the amounts of carotenoids that are potentially available for absorption from commonly consumed carotenoid-rich foods such as peppers, carotenoid bioavailability from these foods should be investigated. In addition, factors that influence the content and bioavailability of carotenoids from peppers should be identified and established to evaluate the actual health effects obtained from consuming such foods. Consequently, in the present study, we determined the content, bioaccessibility, and bioavailability of carotenoids from bell peppers (red, green, and yellow) and chili peppers (red and green) that were available to the Irish consumer at the time of testing, by using the in vitro digestion model coupled with differentiated human intestinal Caco-2 cells. Furthermore, we investigated if carotenoid content and bioaccessibility varied between chili peppers sourced from Kenya and from Turkey.

MATERIALS AND METHODS

Material. All reagents including DMEM growth media, nonessential amino acids, Hank's balanced salts solution (HBSS), β-carotene, zeaxanthin (Fluka), lutein (Fluka), and cholesterol esterase (Fluka) were purchased from Sigma-Aldrich Chemical Co. (Dublin, Ireland). β-Cryptoxanthin (> 95% purity) was purchased from LGC Prochem (Middlesex, U.K.). Fetal bovine serum was sourced from Bio-Sciences (Co. Dublin, Ireland). All solvents employed were of HPLC grade.

Sample Preparation and in Vitro Digestion. During one season, uniformly ripe healthy peppers were purchased from a well-known supermarket chain. The red, green, and yellow bell peppers originated from Spain. Chili peppers (red and green) were from Turkey and Kenya. The peppers were not identical in genotype (i.e., seven different varieties).

*Corresponding author (telephone +353 21 4902496; fax +353 21 4270244; e-mail a.aherne@ucc.ie).

 Published on Web 03/23/2010 © 2010 American Chemical Society

All manipulations with the vegetables were performed under subdued (yellow) light to minimize photodecomposition of the carotenoids. Vegetables were stored at 4 °C overnight. They were then washed in distilled deionized H_2O and cut longitudinally. The dimensions for cutting the peppers were standardized to ensure replication of conditions.

In Vitro Digestion. Samples were accurately weighed (~2 g) and homogenized (Janke and Kunkel, Ultra-Turrax T25; IKA-Labortechnik, Staufen, Germany) in 5 mL of HBSS. The in vitro digestion procedure was performed according to the method of Garrett et al. (*12*) with minor modifications as previously described by Aherne et al. (*13*) and O'Sullivan et al. (*14*). Briefly, digestion was carried out by acidifying the homogenized samples to pH 2 using porcine pepsin (0.04 g mL^{-1} 0.1 mmol L^{-1} HCl) followed by incubation at 37 °C in a Grant OLS 200 orbital shaking water bath (Grant Instruments, Cambridge, U.K.) for 1 h. After simulated gastric digestion, the pH was increased to 5.3 followed by the addition of glycodeoxycholate (0.8 mmol L^{-1}), taurodeoxycholate (0.45 mmol L^{-1}), taurocholate (0.75 mmol L^{-1}), porcine pancreatin (0.08 g mL^{-1}), and cholesterol esterase (1 U mL^{-1}). Granado-Lorencio et al. (*11*) first introduced the use of cholesterol esterase in the in vitro digestion model because the use of human pancreatic lipase in this model did not hydrolyze carotenoid esters or cleave different xanthophyll esters. Then the pH of each sample was increased to 7.4. The final volume of the digesta was approximately 20 mL. Samples were incubated for 2.5 h at 37 °C in an orbital shaking water bath to complete the intestinal phase of the in vitro digestion process. Aliquots of the digested samples (5 mL) were frozen at −80 °C after overlaying the headspace with a layer of nitrogen gas. The remainders of the samples were ultracentrifuged at 194270*g* (Beckman L7-65 ultracentrifuge, Palo Alto, CA) for 95 min to isolate the micelle (aqueous) fraction. The resulting supernatant was collected with a syringe and filter-sterilized using a surfactant-free cellulose acetate filter (0.2 μm; Millipore, Bedford, MA) to remove any microcrystalline aggregates. Samples were stored at −80 °C for 1 day, after overlaying the headspace with nitrogen gas.

Uptake and Transport of Carotenoids by Caco-2 Cells. Human colon adenocarcinoma Caco-2 cells (European Collection of Animal Cell Cultures, Wilts, U.K.) were maintained as previously described (*15*). For experiments, cells were seeded at a density of 5×10^4 cells cm^{-2} on transwell plates (0.4 μm pore size membrane) and were grown for 21−25 days to obtain a differentiated intestinal cell monolayer. Transepithelial electrical resistance (TEERS) measurements were taken twice weekly by a TEERS voltohmmeter to ensure the monolayer was intact. At the beginning of each experiment, the apical chamber of the transwell plate received 2 mL of micelle-enriched medium. The basolateral chamber received 2 mL of serum-free medium. The incubation time for all experiments was 4 h, after which time the micelle-enriched medium was removed. Preliminary work showed that the micelle-enriched medium was not toxic to the cells (data not shown). Medium (2 mL) containing 0.5 mmol L^{-1} taurocholate, 1.6 mmol L^{-1} oleic acid, and 45 mmol L^{-1} glycerol was added to the apical chamber and incubated for 16 h for the stimulation and secretion of chylomicrometers as previously described (*15*). After incubation, media from each side of the membrane were removed, the monolayer was washed, and cells were scraped in HBSS. Samples were sonicated for 30 s on ice and were stored at −80 °C for 1 day, after overlaying the headspace with nitrogen gas.

Carotenoid Extraction and Saponification. Carotenoids were extracted from samples of homogenized peppers, digesta, micelles, cells, and media from apical and basolateral compartments according to the method of Olives-Barba et al. (*16*), as previously reported (*13*, *14*). Briefly, samples were extracted twice with hexane/ethanol/acetone (50:25:25, v/v/v) and centrifuged (Sorvall TC6; DuPont Instruments, Herts, U.K.) at 1499*g* for 5 min. The resulting supernatants were removed, pooled, and dried down using a solvent evaporation system (miVac; Genevac Ltd., Suffolk, U.K.). To enable better chromatographic separation and detection of the carotenoids, the food-related samples were saponified using a simple procedure (*17*), as previously described by O'Sullivan et al. (*18*). Granado et al. (*17*) established this saponification method because they stated that saponification is a necessary step for the determination of carotenoid content in foods of plant origin. It is considered the best means of removing chlorophylls, "unwanted" lipids, and other interfering substances and of hydrolyzing carotenoid esters (*17*). The inclusion of this step does not result in any loss of carotenoid content (*18*). The resultant supernatants were removed. and the solvent was dried down. Samples were frozen at −80 °C for 1 day, after overlaying the headspace with nitrogen gas.

HPLC Analysis. The residues were reconstituted in 200 μL of mobile phase, and the carotenoid content of the samples was analyzed by reverse phase HPLC as previously described (*15*). The HPLC system (Finnigan SpectraSYSTEM; Thermo Scientific, Philadelphia, PA) consisted of a P2000 pump connected to an AS3000 autoinjector and a UV6000LP photodiode array (PDA) detector. The column system consisted of a Spherisorb ODS-2 C18 5 μm PEEK guard column (Alltech Associate Applied Science Ltd., Lancs, U.K.) connected to a Vydac 201TP54 (250 × 4.6 mm) reverse phase C18 column (Alltech Associate Applied Science Ltd.). Column temperature was maintained at 28 °C by an internal column oven. The injection volume was 50 μL; samples were eluted using an isocratic mobile phase composed of acetonitrile/methanol/dichloromethane (75:20:5, v/v/v) containing 10 mmol L^{-1} ammonium acetate, 4.5 mmol L^{-1} butylated hydroxytoluene, and 3.6 mmol L^{-1} triethylamine at a flow rate of 1.5 mL min^{-1}. Carotenoids were detected at 450 nm. The mobile phase was filtered through a 0.5 μm organic filter and degassed using ultrasonic agitation. Results were collected and analyzed using ChromQuest software (version 4.2, Thermo Fisher Scientific, PA). Lutein, β-cryptoxanthin, β-carotene, and zeaxanthin levels in the samples were calculated by comparing retention times and areas under the curves (AUC) with those of authentic carotenoid standards, after correction for extraction efficiency by using β-apo-8′-carotenal as an internal standard. The limits of detection for this method were as follows: lutein, 0.01 μg mL^{-1}; zeaxanthin, 0.015 μg mL^{-1}; β-cryptoxanthin, 0.025 μg mL^{-1}; and β-carotene, 0.045 μg mL^{-1}.

Data Analysis. Results are expressed as the mean ± SEM of four or five independent experiments, with each experiment containing two or three replicates of each sample. Data were analyzed by ANOVA and, when appropriate, Tukey's multiple-comparison test (Prism 4.03; GraphPad Inc., San Diego, CA). Carotenoid bioaccessibility is defined as the amount of ingested carotenoid(s) available for absorption in the gut after digestion. Therefore, bioaccessibility (%) is defined as the proportion of carotenoids present in the micelles compared with that contained in the original food. Carotenoid transport was determined by expressing carotenoid uptake plus secretion by the Caco-2 cells as a percentage of the initial amount added to the cells.

RESULTS AND DISCUSSION

Carotenoid Content of Bell and Chili Peppers. Variety is an important factor affecting both the composition and content of plant pigments (*13*, *19*) such as carotenoids, which are responsible for the coloration of many fruits and vegetables (*19*) including peppers (*20*, *21*). The carotenoid contents of the peppers analyzed in the present study are in line with those in the literature (*20*−*27*). It should be noted that there is large variability in carotenoid content between and within bell and chili pepper varieties. For instance, red bell peppers have been reported to contain β-carotene at levels ranging between 337 and 9951 μg 100 g^{-1}, β-cryptoxanthin at 131−7672 μg 100 g^{-1}, lutein at 57−8506 μg 100 g^{-1}, and zeaxanthin at 148−9996 μg 100 g^{-1} (*4*, *16*, *20*−*27*). This is due to the fact that the physicochemical properties of peppers, including their carotenoid synthesis and, hence, content, are influenced by several factors, namely, (i) endogenous (genotype, plant growth, maturity), (ii) ecological (geographical origin, climate, cultivation conditions), and/or (iii) technological (processing, storage) attributes (*7*, *10*, *20*, *24*, *27*).

Color changes during the ripening of peppers are due to alterations in carotenoid composition (*24*, *28*−*30*) and, hence, the different colors of peppers are due to the different levels of these compounds (*1*, *24*, *29*). Of the red, green, and yellow Spanish bell peppers, the red variety contained significantly ($P < 0.05$) greater amounts of β-carotene, β-cryptoxanthin, and zeaxanthin (**Table 1**). The yellow Spanish bell peppers contained the lowest amounts of β-carotene, β-cryptoxanthin, lutein, and zeaxanthin (**Table 1**). These findings are in line with the

Table 1. Carotenoid Content of Bell and Chili Peppers[a]

	carotenoid content (μg 100 g^{-1})			
	provitamin A carotenoids		non-provitamin A carotenoids	
	β-carotene	β-cryptoxanthin	lutein	zeaxanthin
bell peppers				
a. red	5634.8 ± 277.2 bc	2484.0 ± 272.4 bc	1411.5 ± 256.1	741.7 ± 130.3 c
b. green	1467.2 ± 146.5 a	185.0 ± 19.7 a	2211.1 ± 183.2 c	322.9 ± 47.9
c. yellow	833.7 ± 146.9 a	114.7 ± 18.7 a	753.4 ± 119.3 b	282.1 ± 73.4 a
chili peppers				
d. red (Kenya)	34137.3 ± 2572.6 efg	3327.0 ± 299.9 fg	2178.5 ± 273.2 fg	4888.2 ± 510.0 efg
e. red (Turkey)	5241.6 ± 538.0 d	3338.4 ± 221.6 fg	1297.9 ± 153.7 fg	664.8 ± 113.8 d
f. green (Kenya)	1527.7 ± 161.4 d	246.1 ± 23.5 de	3185.0 ± 215.5 deg	308.3 ± 26.4 d
g. green (Turkey)	3279.2 ± 235.4 d	110.6 ± 18.5 de	4332.0 ± 175.6 def	86.0 ± 7.1 d

[a] Analysis of carotenoid content in the peppers is described under Materials and Methods. Data are the mean and SE of three or four independent experiments. Statistical analysis of each carotenoid within the same variety (bell peppers; chili peppers) was by ANOVA and Tukey's multiple-comparison test ($P < 0.05$): letters denote a significant difference and correspond to the letter that appears next to each sample.

literature (*22*, *23*, *25*, *31*, *32*). For instance, Aizawa and Inakuma (*32*) reported relatively large variations in carotenoid contents between color varieties of bell peppers, the red variety containing greater amounts of β-carotene, β-cryptoxanthin, and zeaxanthin compared with green and yellow bell peppers. Similarly, Sun et al. (*1*) reported that yellow peppers had the least amount of β-carotene (0.2 μg g^{-1}) compared with red (5.4 μg g^{-1}), green (5.8 μg g^{-1}), and orange (2.9 μg g^{-1}) bell peppers.

The green color of green peppers is principally due to the presence of chlorophyll and to carotenoids typical of the chloroplast such as lutein (*23*). Likewise, in the present study, the green bell variety had more lutein than the yellow ($P < 0.05$) and red bell peppers (**Table 1**). In addition, the lutein content of the green chili peppers was greater ($P < 0.05$) than that of the red chilies. Other studies have shown that lutein dominates over β-carotene in green peppers (*25*, *32*). Granado et al. (*25*) stated that red-orange pigmented vegetables have a more complicated carotenoid profile than green vegetables, with other carotenoids being more abundant than lutein. β-Cryptoxanthin was found in greater ($P < 0.05$) quantities in the red chilies compared with their green counterparts (**Table 1**). Furthermore, red chili peppers sourced from Kenya contained the highest levels of β-cryptoxanthin, β-carotene ($P < 0.05$), and zeaxanthin ($P < 0.05$) when compared with the other three types of chilies tested. Of the four carotenoids analyzed, β-carotene and lutein were generally predominant in the bell and chili peppers compared with β-cryptoxanthin and zeaxanthin.

We purchased chili peppers that were sourced from two different countries, namely, Turkey and Kenya, and it is important to bear in mind that their genotypes were not identical. On comparison, red chili peppers sourced from Kenya were superior in β-carotene and zeaxanthin content than those originating from Turkey (**Table 1**). In terms of green chilies, those located from Turkey had significantly ($P < 0.05$) greater amounts of lutein than their Kenyan counterparts. In general, color seemed to have a greater impact on the carotenoid profile of peppers than type (i.e., bell vs chili pepper).

Micelle Content and Bioaccessibility of Carotenoids from Peppers. Granado-Lorencio et al. (*33*) reported that carotenoid bioaccessibility from red peppers ranged between 48 and 97%. They stated that carotenoid bioaccessibility varied widely for different carotenoids in a given vegetable as well as for a given carotenoid in different vegetables. In the present study, the bioaccessibility of carotenoids from red peppers varied from 6.2 to 87.6% depending on the carotenoid (**Table 2**). β-Carotene, β-cryptoxanthin, and lutein ($P < 0.05$) were more bioaccessible from red Kenyan chilies than the other three chili varieties

Table 2. Bioaccessibility of Carotenoids from Bell and Chili Peppers[a]

	carotenoid bioaccessibility (%)			
	provitamin A carotenoids		non-provitamin A carotenoids	
	β-carotene	β-cryptoxanthin	lutein	zeaxanthin
bell peppers				
a. red	6.2 ± 0.5 bc	33.1 ± 2.6 bc	54.3 ± 4.2	87.6 ± 17.9
b. green	13.4 ± 1.7 a	74.7 ± 5.3 ac	45.9 ± 4.5 c	66.2 ± 10.3
c. yellow	12.7 ± 1.8 a	112.8 ± 14.9 ab	63.7 ± 4.8 b	76.5 ± 9.6
chili peppers				
d. red (Kenya)	16.9 ± 2.7 g	78.3 ± 9.0 ef	106.2 ± 6.6 efg	64.2 ± 4.2 f
e. red (Turkey)	11.6 ± 1.6	30.3 ± 3.0 dg	67.0 ± 5.0 dfg	86.1 ± 13.1 g
f. green (Kenya)	15.1 ± 2.1	34.7 ± 4.1 d	40.0 ± 4.0 de	106.9 ± 10.6 dg
g. green (Turkey)	8.2 ± 1.4 d	59.1 ± 5.4 e	36.3 ± 3.3 de	34.1 ± 4.7 ef

[a] Determination of carotenoid bioaccessibility is described under Materials and Methods. Bioaccessibility (%) is defined as the proportion of carotenoids present in the micelles compared with that contained in the original food. Data are the mean and SE of three or four independent experiments. Statistical analysis of each carotenoid within the same variety (bell peppers; chili peppers) was by ANOVA and Tukey's multiple-comparison test ($P < 0.05$): letters denote a significant difference and correspond to the letter that appears next to each sample.

(**Table 2**). Carotenoid bioaccessibility from the red bell peppers was zeaxanthin (87.6%) > lutein (54.3%) > β-cryptoxanthin (33.1%) > β-carotene (6.2%), which is in agreement with other studies (*34*−*38*). For instance, O'Connell et al. (*35*) and Ryan et al. (*38*) showed that the micellarization of lutein (81.3−97.7%) from red bell peppers was greater than that of zeaxanthin (77.1%), β-cryptoxanthin (29.7−65.7%), and β-carotene (13.3-21.2%). This trend could be due to the lower lipophilicity of lutein, which leads to a higher solubility of this carotenoid in the micelles (*36*, *39*) and/or carotenoid−carotenoid interactions. On the other hand, zeaxanthin bioaccessibility was greater from green Kenyan chili peppers (106.9%) compared with red Kenyan (64.2%; $P < 0.05$), green Turkish (34.1%; $P < 0.05$), and red Turkish (86.1%) chilies.

Failla et al. (*40*) investigated the bioaccessibility of carotenoids from different varieties of sweet potatoes. The efficiency of β-carotene micellarization varied between 0.6 and 3%; however, the actual amounts present in the micelle fractions from the different sweet potatoes were similar. Therefore, information on carotenoid content in the micelles is more important than just determining the percentage bioaccessibility in view of the fact that, although carotenoid bioaccessibility (%) may be low, actual carotenoid content in the micelles may be high. For instance, even though lutein was more bioaccessible from yellow bell peppers (63.7%) than from the green ($P < 0.05$; 45.9%) or red (54.3%) varieties (**Table 2**), micelles derived from the yellow bell peppers contained the lowest amounts of lutein (496.3 μg 100 g^{-1} vs 858.7

Table 3. Carotenoid Content of Micelles Obtained from Bell and Chili Peppers[a]

	carotenoid content (μg 100 g^{-1})			
	provitamin A carotenoids		non-provitamin A carotenoids	
	β-carotene	β-cryptoxanthin	lutein	zeaxanthin
bell peppers				
a. red	349.4 ± 34.4 bc	816.1 ± 106.6 bc	858.7 ± 34.3	515.0 ± 94.5 bc
b. green	191.8 ± 32.3 a	135.4 ± 10.1 a	1004.5 ± 124.7 c	180.9 ± 38.7 a
c. yellow	102.1 ± 11.6 a	118.3 ± 14.6 a	496.3 ± 133.5 b	178.5 ± 62.7 a
chili peppers				
d. red (Kenya)	5668.8 ± 651.6 efg	2549.8 ± 307.6 efg	2584 ± 31.7 efg	3119.1 ± 272.4 efg
e. red (Turkey)	591.0 ± 50.6 d	1010.0 ± 95.2 dfg	906.9 ± 69.3 d	577.0 ± 42.7 d
f. green (Kenya)	228.7 ± 44.3 d	85.2 ± 10.9 de	1258.4 ± 114.0 d	324.5 ± 33.5 d
g. green (Turkey)	267.1 ± 42.3 d	63.7 ± 4.6 de	1565.7 ± 162.4 d	32.9 ± 3.1 d

[a] Determination of carotenoid content of micelles is described under Materials and Methods. Data are the mean and SE of three or four independent experiments. Statistical analysis of each carotenoid within the same variety (bell peppers; chili peppers) was by ANOVA and Tukey's multiple-comparison test ($P < 0.05$): letters denote a significant difference and correspond to the letter that appears next to each sample.

Table 4. Carotenoid Uptake and Overall Transport by Caco-2 Cells[a]

	orovitamin A carotenoids				non-provitamin A carotenoids			
	β-carotene		β-cryptoxanthin		lutein		zeaxanthin	
	%U	%T[b]	%U	%T	%U	%T	%U	%T
Spanish bell peppers								
a. red	8.3 ± 1.6 c	17.6 ± 3.8 cd	6.2 ± 1.1 bce	10.1 ± 1.8 bcde	3.4 ± 0.6 b	5.4 ± 1.0 b	4.7 ± 0.6	7.2 ± 1.3
b. green	13.3 ± 1.6 cd	16.8 ± 1.7 cd	0.0 ae	0.0 ae	7.5 ± 1.1 ad	11.6 ± 1.7 ade	7.8 ± 1.9 d	13.6 ± 3.9 d
c. yellow	31.6 ± 3.0 abde	42.7 ± 6.8 abde	0.0 ae	0.0 ae	4.8 ± 0.6 d	7.0 ± 0.6 d	7.8 ± 0.4 d	14.2 ± 0.7 d
Kenyan chili peppers								
d. red	1.3 ± 0.3 bce	2.4 ± 0.6 abc	3.6 ± 1.6 e	3.7 ± 0.5 ae	1.4 ± 0.1 bce	2.3 ± 0.3 bce	1.2 ± 0.2 bce	2.2 ± 0.4 bce
e. green	10.0 ± 3.4 cd	14.3 ± 3.2 c	26.0 ± 2.1 abcd	27.6 ± 1.7 abcd	5.3 ± 0.7 d	6.8 ± 1.1 bd	7.2 ± 1.3 d	11.1 ± 2.6 d

[a] Differentiated Caco-2 cells were supplemented with micelles obtained from different types of peppers for 4 h followed by incubation with chylomicron-stimulating compounds for 16 h. Determination of percentage uptake (%U) and overall transport (%T) is described under Material and Methods. Data are expressed as the mean ± SE of four independent experiments. $P < 0.05$: different letters denote significant difference and correspond to the letter that appears next to each sample (ANOVA and Tukey's multiple-comparison test). [b] Percentage transport is the sum of carotenoid uptake and secretion by Caco-2 cells.

and 1004.5 μg 100 g^{-1} respectively; **Table 3**). β-Carotene, β-cryptoxanthin, and zeaxanthin were more abundant ($P < 0.05$) in micelles from red bell peppers than their green and yellow counterparts. The β-carotene content of micelles derived from red peppers has been reported to range between 139.7 and 434 μg 100 g^{-1} (*33*, *34*), which is in agreement with the present study. Micelle fractions derived from green bell peppers contained the most lutein, and this trend was also seen in the chili peppers (**Table 3**). Of the four carotenoids tested, lutein was generally the predominant carotenoid in the micelles, which is in agreement with the literature (*36*−*39*).

Cellular Uptake and Transport of Carotenoids from Digested Peppers. There are limited data in the literature in relation to cellular uptake and transport of carotenoids from peppers. The differentiated Caco-2 human intestinal cell model was used to assess carotenoid uptake and transport. In a comparison of all pepper varieties, β-cryptoxanthin uptake and overall transport by Caco-2 cells was greatest ($P < 0.05$) from green chili-derived micelles (**Table 4**). Furthermore, β-cryptoxanthin uptake and transport were greatest from the chili peppers compared with the three other carotenoids analyzed.

Large differences in the uptake and transport of carotenoids by Caco-2 cells have been reported in the literature (*41*, *42*). Lutein has been reported to be absorbed to a greater extent than β-carotene (*12*, *36*, *37*, *39*); however, the evidence is conflicting in view of the fact that β-carotene accumulates to the greatest extent in Caco-2 cells when compared with β-cryptoxanthin (*41*) or lutein (*42*, *43*). Furthermore, data from a human study reported that β-carotene was absorbed to a greater extent than lutein (*44*). With the exception of the red Kenyan chilies, lutein was the most abundant carotenoid in the micelles derived from the peppers analyzed (**Table 3**), but β-carotene was taken up and transported to a greater extent by the Caco-2 cells (**Table 4**). A recent study in our laboratory reported that carotenoid−carotenoid interactions occurred when equimolar concentrations of lutein and β-carotene were present in the growth media (*45*). However, no interactions were seen when the two carotenoids were added at different concentrations. Similarly, During et al. (*46*) reported that higher concentrations of lutein did not impair the uptake, secretion, and, hence, overall transport of β-carotene. Garrett et al. (*12*, *47*) suggested that the greater accumulation of β-carotene than lutein by Caco-2 cells suggests differences in uptake, metabolism, and/or efflux of the hydrocarbon carotenes and lutein.

Zeaxanthin uptake and overall transport were similar from micelles of green and yellow peppers (both bell and chili), whereas the red varieties provided the least amount of bioavailable zeaxanthin (4.7% uptake and 7.2% transport) (**Table 4**). The extent of zeaxanthin uptake and transport by Caco-2 cells is similar to those reported in the literature (*48*). For instance, Chitchumroonchokchai and Failla (*48*) reported that cellular uptake of zeaxanthin from digested red peppers was in the range of 11−14% and that zeaxanthin transport by Caco-2 cells was 4.5%. In a human study, it was estimated that 3.3% of zeaxanthin (5 mg) was absorbed by human subjects (*49*). Although carotenoid uptake and transport may reach greater extents from certain peppers, again it is important to consider the original carotenoid content of their appropriate micelles. Therefore, it would be beneficial to look at not only overall carotenoid transport but also the amount of total carotenoids that have been transported by Caco-2 cells.

Table 5. Total Carotenoid Content and Cellular Transport[a]

	total carotenoid				
	content (μg 100 g^{-1})		cellular transport		
pepper	food	micelle	%[b]	%[c]	μg 100 g^{-1}[d]
red bell	10272	2539	10	40	1015
green bell	4186	1513	15	42	635
yellow bell	1727	1152	55	82	945
red chili	44531	13922	3.4	11	1530
green chili	5267	1897	22	60	1138

[a] Total carotenoid refers to the sum of the four carotenoids analyzed in this study, namely, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin. Bell peppers were sourced from Spain and chili peppers from Kenya. [b] Amount of total carotenoids transported by Caco-2 cells expressed as a percentage of the carotenoid content in the original food. [c] Amount of total carotenoids transported by Caco-2 cells expressed as a percentage of the carotenoid content in the micelle fractions. [d] Values are the mean concentration (μg 100 g^{-1}) of total carotenoids transported by Caco-2 cells.

In terms of percentage cellular carotenoid transport from the original food and micelles, the decreasing order was yellow peppers > green > red. Although the percentage transport of total carotenoids was greatest from the yellow bell peppers (55%) and their respective micelles (82%), the actual amount of total carotenoids transported by the cells was the second lowest (945 μg 100 g^{-1}; **Table 5**). On the other hand, only 4 and 11% of total carotenoids were transported from red Kenyan chilies and their micelle fractions, respectively, but the greatest amount of total cellular carotenoid transport was from these peppers (1530 μg 100 g^{-1}). This was due to the very high carotenoid content in the original food and their respective micelles (**Table 5**). The general decreasing order of carotenoid content in the peppers was red > green > yellow. Likewise, Ha et al. (*29*) reported that red peppers contained higher levels of total carotenoids than yellow, orange, or white peppers. Chuah et al. (*4*) also found that total carotenoid content was greater in red bell peppers than their green counterparts. This trend was also seen in the pepper-derived micelle fractions and in the actual amount of total carotenoids transported by Caco-2 cells (**Table 5**). Therefore, the actual amounts of carotenoids present in the original food and respective micelles seem to reflect the amount transported by the cells.

The beneficial bioactive effects of carotenoids make their presence in the diet, and their bioavailability, of considerable interest. More information about the bioavailability of carotenoids from foods is required as a means of improving public health through the development of carotenoid-enhanced foods and dietary programs (*50*). In light of environmental and geographical variation, this research provides valuable information on the content, bioaccessibility, and bioavailability of carotenoids from peppers that are commercially available to consumers. In the present study we have shown that color affected carotenoid content in and bioaccessibility from the bell peppers more so than variety. We also report that both geographical source and color influenced the profile and bioavailability of carotenoids from chili peppers. Additionally, we have shown that although the percentage cellular transport of carotenoids was greatest from yellow bell pepper-derived micelles, the actual amount of total carotenoids transported from these peppers into intestinal cells was greatest from red peppers. Future work is warranted to address year-to-year variability and reproducibility.

ACKNOWLEDGMENT

We thank Dr. Karen Galvin for technical assistance.

LITERATURE CITED

(1) Sun, T.; Xu, Z.; Wu, C. T.; Janes, M.; Prinyawiwatkul, W.; No, H. K. Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). *J. Food Sci.* **2007**, *72*, S98–102.

(2) Materska, M.; Perucka, I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L). *J. Agric. Food Chem.* **2005**, *9*, 1750–1756.

(3) Ambrosini, G. L.; de Klerk, N. H.; Fritschi, L.; Mackerras, D.; Musk, B. Fruit, vegetable, vitamin A intakes, and prostate cancer risk. *Prostate Cancer Prostatic Dis.* **2008**, *11*, 61–66.

(4) Chuah, A. M.; Lee, Y. C.; Yamaguchi, T.; takamura, H.; Yin, L. J.; Matoba, T. Effect of cooking on the antioxidant properties of coloured peppers. *Food Chem.* **2008**, *111*, 20–28.

(5) Granado, F.; Olmedilla, B.; Blanco, I. Nutritional and clinical relevance of lutein in human health. *Br. J. Nutr.* **2003**, *90*, 487–502.

(6) Maiani, G.; Periago Caston, M. J.; Catasta, G.; Toti, E.; Cambrodon, I. G.; Bysted, A.; Granado-Lorencio, F.; Olmedilla-Alonso, B.; Knuthsen, P.; Valoti, M.; Bohm, V.; Mayer-Miebach, E.; Behsnilian, D.; Schlemmer, U. Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Mol. Nutr. Food Res.* **2009**, *53*, S194–S218.

(7) Topuz, A.; Ozdemir, F. Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.) grown in Turkey. *J. Food Compos. Anal.* **2007**, *20*, 596–602.

(8) Cooper, D. A. Carotenoids in health and disease: recent scientific evaluations, research recommendations and the consumer. *J. Nutr.* **2004**, *134*, 221S–224S.

(9) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55*, 207–216.

(10) de Quiros, A. R.; Costa, H. S. Analysis of carotenoids in vegetable and plasma samples: a review. *J. Food Compos. Anal.* **2006**, *19*, 97–111.

(11) Granado-Lorencio, F.; Olmedilla-Alonso, B.; Herrero-Barbudo, C.; Blanco-Navarro, I.; Perez-Sacristan, B.; Blazquez-Garcia, S. In vitro bioaccessibility of carotenoids and tocopherols from fruits and vegetables. *Food Chem.* **2007**, *102* (3), 641–648.

(12) Garrett, D. A.; Failla, M. L.; Sarama, R. J. Development of an in vitro digestion method to assess carotenoid bioavailability from meals. *J. Agric. Food Chem.* **1999**, *47*, 4301–4309.

(13) Aherne, S. A.; Jiwan, M. A.; Daly, T.; O'Brien, N. M. Geographical location has greater impact on carotenoid content and bioaccessibility from tomatoes than variety. *Plant Foods Hum Nutr* **2009**, *4*, 250–256.

(14) O'Sullivan, L.; Galvin, K.; Aherne, S. A.; O'Brien, N. M. Effects of cooking on the profile and micellarization of 9-*cis*-, 13-*cis*- and all-*trans*-β-carotene in green vegetables. *Food Res. Int.* **2010**, doi:10.1016/j.foodres.2010.02.012.

(15) O'Sullivan, L.; Ryan, L.; Aherne, S. A.; O'Brien, N. M. Cellular transport of lutein is greater from uncooked rather than cooked spinach irrespective of whether it is fresh, frozen, or canned. *Nutr. Res. (N.Y.)* **2008**, *28*, 532–538.

(16) Olives-Barba, A. I.; Camara-Hurtado, M.; Sanchez Mata, M. C.; Fernandez Ruiz, V.; Lopez Saenz de Tejada, M. Application of a UV−vis detection HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food Chem.* **2006**, *95*, 328–336.

(17) Granado, F.; Olmedilla, B.; Gil-Martinez, E.; Blanco, I. A fast, reliable and low-cost saponification protocol for analysis of carotenoids in vegetables. *J. Food Compos. Anal.* **2001**, *14*, 479–489.

(18) O'Sullivan, L.; Galvin, K.; Jiwan, M. A.; Aherne, S. A.; O'Brien, N. M. Content and bioaccessibility of carotenoids from green vegetables is not affected by saponification. In *Proceedings of the 38th Annual Conference on Food, Nutrition and Consumer Sciences*, Sept 11, 2008; University College Cork: Ireland, **2008**.

(19) Guil-Guerrero, J. L.; Martinez-Guirado, C.; del Mar Rebolloso-Fuentes, M.; Carrique-Perez, A. Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annuum*) varieties. *Eur. Food Res. Technol.* **2006**, *224*, 1–9.

(20) De Masi, L.; Siviero, P.; Castaldo, D.; Cautela, D.; Esposito, C.; Laratta, B. Agronomic, chemical and genetic profiles of hot peppers (*Capsicum annuum* ssp.). *Mol. Nutr. Food Res.* **2007**, *51*, 1053–1062.
(21) Garcia, M. I.; Lozano, M.; deEspinosa, V. M.; Ayuso, M. C.; Bernalte, M. J.; Vidal-Aragon, M. C.; Perez, M. M. Agronomic characteristics and carotenoid content of five Bola-type paprika red pepper (*Capsicum annuum* L.) cultivars. *Sci. Hortic.* **2007**, *113*, 202–207.
(22) Minguez-Mosquera, M. I.; Hornero-Mendez, D. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika and oleoresin by reversed-phase HPLC. *J. Agric. Food Chem.* **1993**, *41*, 1616–1620.
(23) Marin, A.; Ferreres, F.; Tomas-Barberan, F. A.; Gil, M. I. Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuu*m L.). *J. Agric. Food Chem.* **2004**, *52*, 3861–3869.
(24) Howard, L. R.; Talcott, S. T.; Brenes, C. H.; Villalon, B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J. Agric. Food Chem.* **2000**, *48*, 1713–1720.
(25) Granado, F.; Olmedilla, B.; Blanco, I.; Rojas-Hidalgo, E. Carotenoid composition in raw and cooked spanish vegetables. *J. Agric. Food Chem.* **1992**, *40*, 2135–2140.
(26) Bernhardt, S.; Schlich, E. Impact of different cooking methods on food quality: retention of lipophilic vitamins in fresh and frozen vegetables. *J. Food Eng.* **2006**, *77*, 327–333.
(27) Kopsell, D. A.; Kopsell, D. E. Accumulation and bioavailability of dietary carotenoids in vegetable crops. *Trends Plant Sci.* **2006**, *11*, 499–507.
(28) Hornero-Mendez, D.; Minguez-Mosquera, M. I. Xanthophyll esterification accompanying carotenoid overaccumulation in chromoplast of *Capsicum annuum* ripening fruits is a constitutive process and useful for ripeness index. *J. Agric. Food Chem.* **2000**, *48*, 1617–1622.
(29) Ha, S. H.; Kim, J. B.; Park, J. S.; Lee, S. W.; Cho, K. J. A comparison of the carotenoid accumulation in *Capsicum* varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *J. Exp. Bot.* **2007**, *58*, 3135–3144.
(30) Lee, J. J.; Crosby, K. M.; Pike, L. M.; Yoo, K. S.; Leskovar, D. I. Impact of genetic and environmental variation on development of flavonoids and carotenoids in pepper (*Capsicum* spp.). *Sci. Hortic.* **2005**, *106*, 341–352.
(31) Marin, A.; Gil, M. I.; Flores, P.; Hellin, P.; Selma, M. V. Microbial quality and bioactive constituents of sweet peppers from sustainable production systems. *J. Agric. Food Chem.* **2008**, *56*, 11334–11341.
(32) Aizawa, K.; Inakuma, T. Quantitation of carotenoids in commonly consumed vegetables in Japan. *Food Sci. Technol. Res.* **2007**, *13* (3), 247–252.
(33) Granado-Lorencio, F.; Olmedilla-Alonso, B.; Herrero-Barbudo, C.; Perez-Sacristan, B.; Blanco-Navarro, I.; Blazquez-Garcia, S. Comparative in vitro bioaccessibility of carotenoids from relevant contributors to carotenoid intake. *J. Agric. Food Chem.* **2007**, *55*, 6387–6394.
(34) O'Connell, O.; Ryan, L.; O'Brien, N. M. Xanthophyll carotenoids are more bioaccessible from fruits than dark green vegetables. *Nutr. Res.* (*N.Y.*) **2007**, *27*, 258–264.
(35) O'Connell, O.; Ryan, L.; O'Sullivan, L.; Aherne, S. A.; O'Brien, N. M. Carotenoid micellarisation varies greatly between individual and mixed vegetables with or without the addition of fat or fibre. *Int. J. Vitamin Nutr. Res.* **2008**, *4*−*5*, 238–246.
(36) Reboul, E.; Richelle, M.; Perrot, E.; Desmoulins-Malezet, C.; Pirisi, V.; Borel, P. Bioaccessibility of carotenoids and vitamin E from their main dietary sources. *J. Agric. Food Chem.* **2006**, *54*, 8749–8755.
(37) Tyssandier, V.; Reboul, E.; Dumas, J. F.; Bouteloup-Demange, C.; Armand, M.; Marcand, J.; Sallas, M.; Borel, P. Processing of vegetable-borne carotenoids in the human stomach and duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2003**, *284*, G913–G923.
(38) Ryan, L.; O'Connell, O.; O'Sullivan, L.; Aherne, S. A.; O'Brien, N. M. Micellarisation of carotenoids from raw and cooked vegetables. *Plant Foods Hum. Nutr.* **2008**, *63* (3), 127–133.
(39) van Het Hof, K. H.; West, C. E.; Weststrate, J. A.; Hautvast, J. G. Dietary factors that affect the bioavailability of carotenoids. *J. Nutr.* **2000**, *130*, 503–506.
(40) Failla, M. L.; Thakkar, S. K.; Kim, J. Y. In vitro bioaccessibility of β-carotene in orange fleshed sweet potato (*Ipomoea batatas*, Lam.). *J. Agric. Food Chem.* **2009**, *57* (22), 10922–10927.
(41) Dhuique-Mayer, C.; Borel, P.; Reboul, E.; Caporiccio, B.; Besancon, P.; Amiot, M. J. β-Cryptoxanthin from citrus juices: assessment of bioaccessibility using an in vitro digestion/Caco-2 cell culture model. *Br. J. Nutr.* **2007**, *97*, 883–890.
(42) During, A.; Dawson, H. D.; Harrison, E. H. Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in Caco-2 cells treated with ezetimibe. *J. Nutr.* **2005**, *135*, 2305–2312.
(43) Liu, C. S.; Glahn, R. P.; Liu, R. H. Assessment of carotenoid bioavailability of whole foods using a Caco-2 cell culture model coupled with an in vitro digestion. *J. Agric. Food Chem.* **2004**, *52*, 4330–4337.
(44) O'Neill, M. E.; Thurnham, D. I. Intestinal absorption of β-carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol-rich lipoprotein fraction. *Br. J. Nutr.* **1998**, *79*, 149–159.
(45) O'Sullivan, L.; Aherne, S. A.; O'Brien, N. M. Investigation of β-carotene and lutein transport in Caco-2 cells: carotenoid-carotenoid interactions and transport inhibition by ezetimibe. *Int. J. Vitam. Nutr. Res.* **2009**, in press.
(46) During, A.; Hussain, M. M.; Morel, D. W.; Harrison, E. H. Carotenoid uptake and secretion by CaCo-2 cells: β-carotene isomer selectivity and carotenoid interactions. *J. Lipid Res.* **2002**, *43*, 1086–1095.
(47) Garrett, D. A.; Failla, M. L.; Sarama, R. J. Estimation of carotenoid bioavailability from fresh stir-fried vegetables using an in vitro digestion/Caco-2 cell culture model. *J. Nutr. Biochem.* **2000**, *11*, 574–580.
(48) Chitchumroonchokchai, C.; Failla, M. L. Hydrolysis of zeaxanthin esters by carboxyl ester lipase during digestion facilitates micellarization and uptake of the xanthophyll by Caco-2 human intestinal cells. *J. Nutr.* **2006**, *136*, 588–594.
(49) Breithaupt, D. E.; Weller, P.; Wolters, M.; Hahn, A. Comparison of plasma responses in human subjects after the ingestion of 3R,3R′-zeaxanthin dipalmitate from wolfberry (*Lycium barbarum*) and non-esterified 3*R*,3*R*′-zeaxanthin using chiral high-performance liquid chromatography. *Br. J. Nutr.* **2004**, *91*, 707–713.
(50) van den Berg, H.; Faulks, R.; Granado, H. F.; Hirschberg, J.; Olmedilla, B.; sandmann, G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J. Sci. Food Agric.* **2000**, *80*, 880–912.

Received for review February 1, 2010. Revised manuscript received March 1, 2010. Accepted March 15, 2010. This research was funded by Science Foundation Ireland under PI Award 04/IN3/B509.