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Effect of Heat Processing on the Profile of Pigments and Antioxidant Capacity of Green and Red Jalapeño Peppers

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ABSTRACT: Raw and heat-processed jalapeño peppers (green and red) were evaluated for their pigment profile and antioxidant capacity. Sixty-seven pigments were separated and characterized by HPLC-DAD-MS, including carotenoids (isomers and esters), chlorophylls, and pheophytins. The distinctive characteristics of this pepper genotype were the presence of antheraxanthin monoesters, zeaxanthin monoesters, mutatoxanthin diesters, and a higher content of free capsanthin relative to the mono- and diesterified forms. Chlorophyll *a* and free *all-trans*-lutein were the major pigments in raw green peppers, whereas free *all-trans*-capsanthin was the most abundant pigment in raw red peppers. Twelve compounds were generated by the heat treatments, mainly pheophytins and *cis* isomers of carotenoids. Heat treatments affected differentially the concentration of individual pigments. Red peppers showed a higher antioxidant capacity than green fruits. Heating caused minor changes in the antioxidant capacity of peppers.

KEYWORDS: *Capsicum annuum, carotenoids speciation, cooking, pigment thermostability, antioxidant capacity*

INTRODUCTION

Jalapeño peppers have been regarded as a good source of carotenoids and chlorophylls, pigments that are able to exert several health-promoting activities, presumably as a consequence of their antioxidant activity.[1−3] However, the content of these pigments is highly variable, depending mainly on the cultivar, ripening stage, and type of processing. Levy et al. found variations of up to 6 times in the quantity of total and individual carotenoids in different pepper genotypes.[4] The concentrations of β-carotene, β-cryptoxanthin, lutein, and zeaxanthin are highly variable (up to 7 times) between fruits of pungent and sweet genotypes at the same ripening stage.[5] Similar variations in the content of total carotenoids and β-carotene were found for several Mexican pepper genotypes.[6] The qualitative content of carotenoids also seems to be cultivar-dependent in peppers. Howard et al. analyzed the carotenoid content in seven pepper cultivars and reported the presence of β-cryptoxanthin and α-carotene in the majority of the cultivars, except in Francisca and Mesilla cultivars.[2] Guil-Guerrero et al. identified 22 carotenoids in the fruit of 10 pepper cultivars and observed variations in the qualitative composition of carotenoids between cultivars.[7] The genotype affects only the concentration of chlorophylls in green peppers.[8] To date, the whole profile of pigments in jalapeño peppers remains unknown. On the other hand, the composition of pigments in peppers is also modulated by ripening, when chloroplasts are transformed into chromoplasts, accompanied by a reduction in the content of some carotenoids (lutein and neoxanthin) and chlorophylls, whereas other carotenoids (mainly capsanthin and capsorubin) are biosynthesized and then esterified with fatty acids.[8] The effect of the ripening process on the whole profile of carotenoids and chlorophylls in jalapeño peppers also remains unknown. Heat processing also alters the pigment composition in peppers. Carotenoids are highly unsaturated and, therefore, highly prone to isomerization, oxidation, and degradation under light and heat conditions, leading to the formation of epoxides, *cis* isomers, and apocarotenoids, among others.[1,9−11] The exposure of chlorophylls to severe heat and/or acidic conditions results in loss of the central Mg^{2+} metal, generating pheophytin and pyropheophytin pigments.[12] To date, the study of the pigments of jalapeño peppers has been limited to a few carotenoids (α-carotene, β-carotene, lutein, violaxanthin, and zeaxanthin), especially as a function of the industrial processing conditions and ripening.[1,6] The objective of this work was to determine the effect of boiling and grilling, two common processing methods

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Table 1. Physicochemical Attributes of Raw and Heat-Treated Jalapeño Peppers at Two Stages of Ripening[a]

	green			red		
physicochemical attribute	raw	boiled	grilled	raw	boiled	grilled
weight (g)	40.71 ± 1.53 a	35.07 ± 1.45 b	28.03 ± 1.57 c	43.21 ± 1.88 a	45.22 ± 1.96 a	36.23 ± 1.66 b
length (cm)	8.39 ± 0.12 a	7.64 ± 0.11 b	6.85 ± 0.10 c	8.64 ± 0.20 a	8.10 ± 0.18 a	8.17 ± 0.22 a
major diameter (cm)	3.57 ± 0.05 a	3.15 ± 0.05 b	2.84 ± 0.05 c	3.76 ± 0.11 a	3.38 ± 0.05 b	3.19 ± 0.08 b
dry matter (%)	9.22 ± 0.21 b	9.66 ± 0.38 ab	10.37 ± 0.30 a	12.44 ± 0.63 ab	12.12 ± 0.16 b	13.87 ± 0.52 a
firmness (N)	38.85 ± 0.91 a	11.01 ± 0.46 b	6.52 ± 0.60 c	34.81 ± 0.54 a	13.45 ± 0.27 b	4.77 ± 0.27 c
tristimulus color						
L^*	55.83 ± 1.58 a	48.87 ± 0.56 b	41.17 ± 0.87 c	36.27 ± 0.38 b	42.08 ± 0.81 a	35.07 ± 0.92 b
a^*	−16.03 ± 0.27 c	−6.22 ± 0.13 b	−2.29 ± 0.17 a	32.62 ± 0.35 a	34.37 ± 0.70 a	25.35 ± 1.14 b
b^*	30.32 ± 0.60 b	33.96 ± 0.30 a	23.79 ± 0.50 c	28.12 ± 0.51 b	36.94 ± 1.27 a	25.17 ± 1.38 b

[a]Values represent the mean of several individual measurements (n = 10−90) ± the standard error. Values in the same row for each ripening stage with different letters are significantly different ($p < 0.05$).

of jalapeño peppers,[6] on the physical attributes, profile of pigments (carotenoids and chlorophylls), and antioxidant capacity of green and red jalapeño peppers.

MATERIALS AND METHODS

Chemicals and Solvents. All reagents and solvents were of analytical or HPLC grade and were purchased from J. T. Baker (Baker-Mallinckrodt Inc., Mexico). *all-trans-β*-Cryptoxanthin (purity ≥ 97%), *all-trans-α-* and *all-trans-β*-carotene from carrots (purity ≥ 95%), *all-trans*-lutein (purity ≥ 70%), and chlorophylls *a* and *b* (purity ≈ 95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). *all-trans*-Violaxanthin (purity ≥ 95%) and *all-trans*-zeaxanthin (purity ≥ 97%) were supplied by CaroteNature GmbH (Lupsingen, Switzerland), and *all-trans*-capsanthin (purity ≈ 96%) was from Southcot Inc. (Chapel Hill, NC, USA).

Plant Material. Fresh jalapeño peppers (cv. Marajá) free of blemishes and defects were harvested from a commercial orchard in Chihuahua, Mexico. All fruits were physiologically ripe, and only green and red peppers were included in the experiment. The peppers were washed with tap water, dried using paper towels, and immediately subjected to heat treatments. The effect of these treatments on some physical (weight loss, biometrical characteristics, tristimulus color, and firmness), chemical (dry matter content and profile of carotenoids and chlorophylls), and bioactive (antioxidant capacity) attributes of jalapeño peppers was investigated.

Heat Treatments. Peppers of each ripening stage were divided into three samples of 100 fruits each. One sample of each ripening stage was boiled (94 °C/12.48 ± 1.48 min) in a covered pan, and another one was grilled (210 °C/13.23 ± 0.78 min) on a hot plate according to methodology described by Ornelas-Paz et al.[6] The remaining samples were used as nonheated control groups. Twenty fruits from each sample were individually weighed before and after the heat treatments to determine gravimetrically the weight loss during the treatment.

Biometrical Characteristics. Twenty-five fruits were used for this measurement. The peduncle of the fruit was removed, and then the fruit was weighed and sized (length and major diameter) using a vernier caliper.

Tristimulus Color. Five fruits of each sample were homogenized to puree using a kitchen blender. Triplicate samples of puree were immediately evaluated for tristimulus color using a CR-300 model Minolta colorimeter (Minolta Co. Ltd., Osaka, Japan) on the basis of the CIELAB color system (L^*, a^*, and b^*). The colorimeter was dotted by a xenon arc (PXA) lamp, and the viewing angle was 0° (specular component included).

Firmness. Ten peppers from each sample were longitudinally cut in three equal parts. The firmness was determined in the pericarp of the fruit using a TA-XT2i texture analyzer (Stable Micro Systems Ltd., Godalming, UK). This apparatus was equipped with a 6 mm diameter stainless steel striker pin, puncturing a half of the pericarp thickness at a rate of 10 mm/s. The maximum force (in newtons) that was needed to puncture the fruit was recorded.

Dry Matter Content. The dry matter content was individually determined in 10 peppers of each sample. Each fruit was longitudinally cut in halves, recording the initial weight of the halves. The halves were maintained in an oven at 110 °C until constant weigh. The percent dry matter was gravimetrically determined considering the initial and final weights of the halves.

Pigment Extraction and HPLC-DAD-MS Analysis. The procedure for the extraction of pigments was based on the method described by Ornelas-Paz et al. but including one additional extraction step with a mixture (75 mL) of acetone and hexane (1:1, v/v), containing 0.1% of BHT as antioxidant, until complete extraction of pigments from peppers (4 g).[13] Carotenoid esters were identified by comparing the chromatographic pattern of the saponified and crude extracts. For saponification, the residue was dissolved in 30 mL of diethyl ether and mixed with 0.3 mL of 40% methanolic KOH, keeping the mixture for 16 h at room temperature. Saponified and crude extracts were automatically injected (20 μL) into an HP 1100 series HPLC system (Hewlett-Packard GmbH, Waldbronn, Germany) equipped with a diode array detector (DAD). UV−vis spectra for all peaks were recorded between 200 and 600 nm (each 1 nm). The purity of peaks was determined automatically by the DAD. The HPLC system was equipped with a C_{30} reversed-phase column (4.6 × 150 mm, 3 μm) (YMC Inc., Milford, MA, USA). The column was operated at 15 °C. The mobile phase consisted of water as eluent A, methanol as eluent B, and methyl *tert*-butyl ether (MTBE) as eluent C. Flow rate was fixed at 0.75 mL/min with the following gradient program: 4% A/94.5% B/1.5% C at 0 min; 4% A/68% B/28% C at 31 min; 4% A/30% B/66% C at 83 min; and 4% A/0% B/96% C, at 85−90 min. The mass spectra of pepper carotenoids and chlorophylls were obtained using the chromatographic system described above, coupled to a 6210 time-of-flight (TOF) mass spectrometer (Agilent, Palo Alto, CA, USA) equipped with an atmospheric pressure chemical ionization ($APCI^+$) interface and Mass Hunter manager software (A.02.01). The operation conditions for the MS system were described previously.[13] The carotenoids and chlorophylls were identified in pepper samples by analyzing their chromatographic pattern, UV−vis features (λ_{max}, %III/II, and peak purity), and MS data (m/z 100−1200). These data were also compared with those of reference compounds or reported in the literature. Quantitative data for carotenoids and chlorophylls were obtained by calibration curves constructed with pure compounds with a minimum of five concentration levels. Derivatives of chlorophylls and *cis* isomers of carotenoids were quantified as their precursors and *all-trans* isomers, respectively.

Antioxidant Capacity. DPPH and FRAP assays were performed as reported by Corral-Aguayo et al. with slight modifications.[14] Aliquots (280 μL) of 100 μM DPPH/methanol or FRAP solutions were mixed with 20 μL of crude extracts of green and red peppers that had been diluted 41 and 101 times, respectively. These dilution factors were considered in the calculation of antioxidant capacity. The reactions were carried out in 90-well plates during 30 min in the dark,

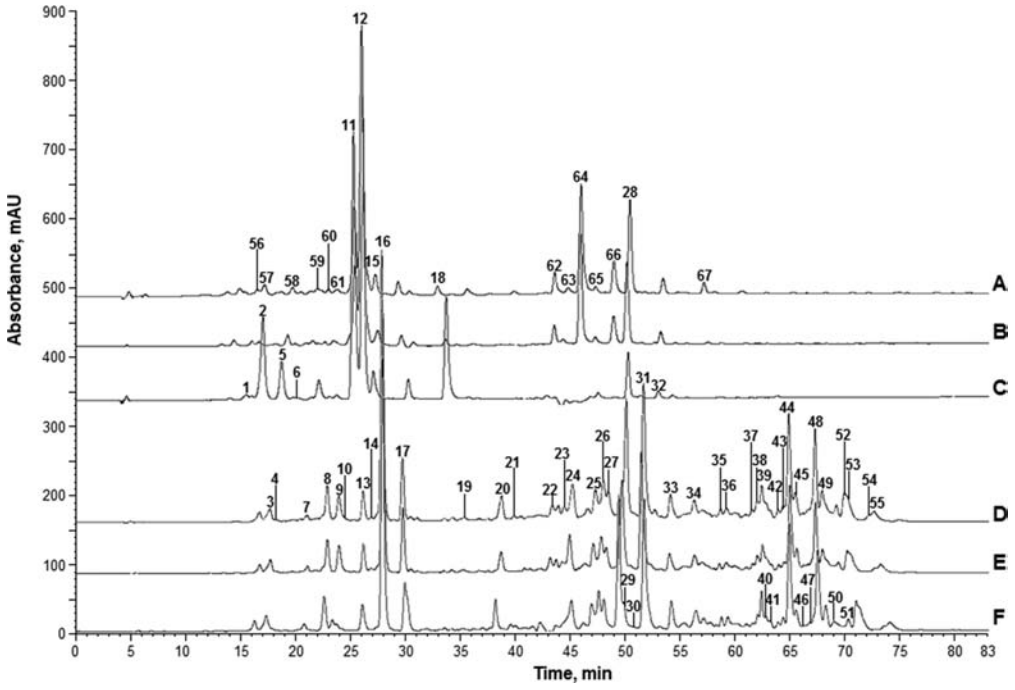

Figure 1. Typical chromatograms (λ = 452 nm) of crude extracts from raw and heat-treated green and red jalapeño peppers: (A) grilled green jalapeño peppers; (B) boiled green jalapeño peppers; (C) raw green jalapeño peppers; (D) grilled red jalapeño peppers; (E) boiled red jalapeño peppers; (F) raw red jalapeño peppers.

and measurements were taken at λ = 517 nm (DPPH assay) or λ = 630 nm (FRAP assay) in a MRX microplate reader (Dynex Technology, Chantilly, VA, USA). Calibration curves were prepared using Trolox as a standard. The results were expressed as micromoles of Trolox equivalents per g of fresh weight (μmol TE/g FW).

Statistical Analysis. The data were analyzed using a completely randomized design. The statistical significance of the differences between treatments was determined using an ANOVA followed by the Tukey–Kramer post hoc test; 0.05 was the significance limit. Data analysis was performed using JMP statistical software (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Changes in the Biometrical Characteristics. The weight and size (length and major diameter) were similar for raw green and red peppers (Table 1). However, grilling significantly reduced the weight (31.1%), length (18.4%), and major diameter (20.4%) of green peppers, whereas the impact of boiling on these characteristics was smaller (13.9, 8.9, and 11.8%, respectively) (Table 1). Grilling reduced only the weight (16.2%) and major diameter (15.2%) of red peppers. Boiling reduced the major diameter (10.1%) of red peppers. Weight losses by grilling have been explained in terms of the absence of water in the heating medium during this type of processing, whereas weight losses by boiling could occur during the cooling at room temperature. The weight losses during heating have been also explained in terms of the outflow and leaching out from the food and several characteristics of the fruit (initial water content, surface area, surface morphology, and the differential affectation of fruit cuticles by heating in peppers at different ripening stages).[6]

Changes in the Content of Dry Matter. Dry matter content tended to be higher in red peppers than in green peppers (Table 1), indicating an effect of the ripening stage on this variable. The difference in dry matter content between green and red peppers in the raw form was 3.2%. Similar differences in dry matter content (1.7%) have been previously reported for peppers at two stages of ripening.[15] Heat treatments also affected dry matter content. This variable was similar for raw and boiled peppers at the two stages of ripening, but grilling increased dry matter content in green (1.2%) and red peppers (1.4%). Increases of 2.5% in dry matter content of jalapeño peppers after heating have been reported in the literature.[16]

Changes of Tristimulus Color. For green peppers, the variables L^* and a^* were sequentially diminished (up to 26.2%) and increased (up to 85.6%), respectively, by both heat treatments, whereas b^* values were differentially affected by boiling and grilling (Table 1). These changes can be related to the degradation of chlorophylls and carotenoids, especially in grilled peppers, probably as a consequence of the high temperatures used in these treatments.[17] Diminishing L^* and increasing b^* values by boiling in green peppers can also be attributed to the gain of water by the fruit and to the formation of some brown pigments related to nonenzymatic reactions.[18] Boiling caused an increase of the L^* (16%) and b^* (31%) values in red peppers, whereas a^* was not significantly affected by this heat treatment, indicating the development of a brownish color.[19] Similar effects of boiling and grilling on the tristimulus color of green and red jalapeño peppers have been reported previously.[6]

Firmness Changes. The firmness of raw peppers depended on the ripening stage, the green peppers being 10.4% firmer than the red fruits (Table 1). A slight decrease (3.5 N) in the firmness of some pepper genotypes during ripening has been observed by others.[20] In our study, the firmness of green peppers was reduced 71.7 and 83.2% by boiling and grilling (Table 1). The reduction of firmness in red peppers by these heat treatments was 61.4 and 86.3%, respectively (Table 1). Gu et al. reported similar decreases (61–78%) in the firmness of jalapeño peppers after heat processing in water (75 °C for 10

Table 2. Retention Time (t_R), UV–Vis Data (λ_{max}, %III/II, and % Purity), MS Data (m/z and Relative Abundance of the Main Ion Fragments), and Tentative Identification of Pigments from Raw and Heat-Treated Jalapeño Peppers at Two Stages of Ripening

peak[a]	t_R (min)	(*cis* peak), λ_{max} (nm)	III/II[b] (%)	purity (%)	m/z (% relative abundance)[c]	compound
1	15.4	417, 441, 470	86	92.8	601(51)*, 583(25), 615(100), 547(39), 565(9)	*all-trans*-neoxanthin
2	16.9	414, 436, 466	90	98.9	601(82), 583(100)*, 565(50), 547(6), 534(17)	*cis*-violaxanthin
3	17.3	421, 444, 475	78	96.8	601(100)*, 603(35), 583(22), 453(3), 429(3)	NI[d]
4	18.2	398, 418, 443	ND[e]	ND	601(8)*, 583(6), 429(14), 554(75)), 716(100)	*cis*-luteoxanthin
5	18.4	417, 441, 470	93	91.8	601(100)*, 583(24), 565(3), 534(2), 429(3)	*all-trans*-violaxanthin
6	20.0	400, 422, 448	113	99.4	601(40)*, 583(18), 534(7), 615(100), 149(66)	*all-trans*-luteoxanthin
7	20.9	288, 471	ND	95.9	601(100)*, 583(77), 567(11), 543(5), 536(12)	capsanthin 5,6-epoxide
8	22.8	423, 448, 476	52	56.2	585(100)*, 567(11), 545(20), 493(4), 409(9)	*all-trans*-antheraxanthin
9	23.9	(330), 420, 443, 472	50	97.2	585(11)*, 567(100), 549(47), 487(8), 469(6)	*cis*-antheraxanthin
10	24.3	471	65	75.0	585(100)*, 567(100), 545(7), 493(3), 181(3)	*cis*-capsanthin
11	25.2	346, 469	ND	99.9	907(100)*, 601(71), 619(8), 339(30), 589(28)	chlorophyll *b*
12	26.0	422, 447, 474	66	92.4	569(5)*, 551(100), 589(1), 552(45), 533(3)	*all-trans*-lutein
13	26.2	407, 430, 456	62	98.2	585(100)*, 567(11), 549(2), 493(3), 477(2)	*all-trans*-mutatoxanthin
14	26.9	409, 430, 455	64	99.8	585(100)*, 567(11), 493(5), 271(3), 149(19)	*cis*-mutatoxanthin
15	27.2	346, 468	ND	93.3	907(78)*, 409(100), 617(11), 551(36), 395(18)	chlorophyll *b*′
16	28.0	292, 475	ND	100	585(100)*, 567(19), 493(5), 387(4), 109(2)	*all-trans*-capsanthin
17	30.0	429, 453, 478	29	97.5	569(100)*, 551(19), 477(4), 427(55), 397(42)	*all-trans*-zeaxanthin
18	33.6	341, 434	ND	99.1	893(100)*, 613(7), 575(21), 313(6), 593(1)	chlorophyll *a*
19	35.4	421, 445, 475	61	96.0	553(8)*, 663(100), 567(10), 425(5), 351(5)	*all-trans*-α-cryptoxanthin
20	38.2	335, 351, 371, 427, 451, 477	27	92.8	553(100)*, 543(26), 535(3), 473(4), 407(4)	*all-trans*-β-cryptoxanthin
21	39.8	423, 445, 472	97	70.1	811(48)*, 793(100), 583(18), 751(75), 565(29)	NI
22	43.5	(344), 403, 426, 451	ND	86.1	783(33)*, 583(100), 765(28), 565(9), 677(7)	NI
23	44.3	423, 445, 475	66	93.6	537(4)*, 879(100), 599(35), 613(3), 149(4)	*all-trans*-α-carotene
24	45.2	424, 448, 476	58	96.7	767(100)*, 567(10), 749(22), 549(3), 853(3)	antheraxanthin laurate
25	46.2	458, 462	ND	71.8	811(48)*, 583(100), 793(47), 565(9), 545(21)	NI
26	48.0	467	ND	79.7	767(100)*, 567(33), 749(23), 583(79), 795(93)	NI
27	48.3	291, 475	ND	92.3	767(100)*, 567(43), 749(26), 549(6), 768(53)	capsanthin laurate
28	50.0	427, 452, 478	25	93.4	537(100)*, 567(37), 461(12), 539(9), 391(6)	*all-trans*-β-carotene
29	50.2	424, 448, 476	60	67.5	795(100)*, 567(80), 777(25), 565(8), 549(18)	antheraxanthin myristate
30	50.8	429, 452, 477	ND	98.1	795(34)*, 567(16), 777(8), 909(100), 853(9)	NI
31	51.7	291, 475	ND	83.4	795(100)*, 567(62), 777(40), 549 (5), 797(19)	capsanthin myristate
32	53.0	423, 449, 474	29	98.1	537(traces)*, 855(100), 575(93), 599(32)	9-*cis*-β-carotene
33	54.2	428, 453, 478	ND	82.8	779(100)*, 551(66), 761(19), 533(3), 883(85)	zeaxanthin myristate
34	56.5	291, 475	ND	98.3	823(100)*, 567(56), 805(12), 549(12), 545(5)	capsanthin palmitate
35	58.6	427, 451, 478	ND	98.8	735(53)*, 535(15), 883(100), 749(11)	β-cryptoxanthin laurate
36	59.2	472	ND	95.4	993(35)*, 765(100), 793(81), 565(13), 749(41)	NI
37	61.4	474	ND	91.5	977(76)*, 749(100), 793(45), 777(88), 921(43)	NI
38	62.0	429, 455, 477	18	96.7	977(37)*, 793(100), 749(83), 777(20), 750(46)	NI
39	62.4	291, 474	ND	99.4	949(30)*, 749(100), 721(15), 549(2), 750(58)	capsanthin dilaurate
40	62.8	292, 474	ND	97.4	949(21)*, 749(100), 721(50), 549(5), 777(15)	capsanthin dilaurate
41	63.2	455, 481, 512	ND	93.0	993(44)*, 793(100), 765(97), 565(5), 575(17)	capsorubin laurate myristate
42	63.9	408, 431, 456	107	99.9	977(100)*, 777(28), 749(10), 549(6)	mutatoxanthin laurate myristate
43	64.2	429, 543, 457	ND	97.4	933(94)*, 733(100), 793(60), 777(39), 533(4)	zeaxanthin dilaurate
44	64.9	292, 475	ND	99.8	977(42)*, 749(100), 777(15), 549(5), 751(14)	capsanthin laurate myristate
45	65.5	454, 481, 510	ND	94.4	1021(12)*, 793(100), 565(3), 777(61), 749(35)	capsorubin dimyristate
46	66.2	407, 431, 457	107	97.5	1005(36)*, 749(100), 805(32), 549(4), 765 (29)	mutatoxanthin palmitate laurate
47	66.9	429, 453, 478	ND	99.6	961(100)*, 761(82), 733(80), 777(22), 533(24)	zeaxanthin laurate myristate
48	67.4	292, 475	ND	99.9	1005(15)*, 777(100), 549(6), 579(3), 779(26)	capsanthin dimyristate
49	67.8	292, 475	ND	99.8	1005(13)*, 749(100), 805(19), 777(6), 549(4)	capsanthin palmitate laurate
50	69.0	454, 482, 509	ND	99.9	1049(4)*, 821(100), 793(38), 749(35), 805(30)	capsorubin myristate palmitate
51	69.2	429, 453, 478	28	99.7	989(59)*, 761(100), 777(14), 805(16), 533(14)	zeaxanthin dimyristate
52	70.0	292, 475	ND	99.6	1033(10)*, 777(100), 805(10), 549(4), 779(17)	capsanthin palmitate myristate
53	70.3	290, 475	ND	99.8	1033(11)*, 805(100), 777(32), 549(5), 713(4)	capsanthin myristate palmitate
54	72.1	429, 453, 478	28	99.7	1017(100)*, 761(88), 789(32), 533(30), 805(43)	zeaxanthin myristate palmitate
55	72.8	291, 475	ND	99.6	1061(6)*, 805(100), 549(3), 749(2), 475(3)	capsanthin dipalmitate
56	16.4	(299), 398, 420, 445	93	91.7	601(6)*, 583(17), 149(100), 565(10), 534(38)	*cis*-neochrome
57	17.1	401, 423, 449	93	99.1	601(19)*, 583(23), 149(100), 565(12), 534(17)	*all-trans*-neochrome
58	19.6	(335), 417, 441, 469	60	79.3	569(3)*, 551(25), 533(3), 391(6), 149(100)	9′-*cis*-lutein

Table 2. continued

peak[a]	t_R (min)	(*cis* peak), λ_{max} (nm)	III/II[b] (%)	purity (%)	*m/z* (% relative abundance)[c]	compound
59	22.1	418, 441, 469	58	78.8	569(3)*, 551(21), 567(10), 391(8), 149(100)	9-*cis*-lutein
60	23.0	383, 404, 429	98	ND	601(100)*, 583(22), 509(4), 391(5), 167(5)	auroxanthin
61	23.8	429, 453, 478	58	99.4	569(4)*, 551(18), 566(16), 397(9), 181(15)	*cis*-zeaxanthin
62	43.3	417, 438, 528	ND	83.2	885(100)*, 871(7), 853(44), 851(26), 793(7)	pheophytin *b*′
63	45.0	426, 452, 471	ND	92.4	844(100)*, 851(10), 877(8), 811(6), 767(13)	NI
64	46.1	417, 438, 528	ND	88.8	885(100)*, 879(9), 827(8), 599(3), 947(7)	pheophytin *b*
65	47.2	442, 472	ND	58.5	795(42)*, 879(100), 885(51), 749(10), 583(10)	NI
66	48.0	412, 505	ND	96.1	871(100)*, 583(54), 811(24), 567(10)	pheophytin *a*′
67	57.2	412, 505, 536	ND	99.1	871(100)*, 813(4), 881(4), 567(7), 461(3)	pheophytin *a*

[a]Numbering of peaks according to Figure 1. [b]Spectral fine structure. [c]An asterisk (*) indicates the quasimolecular ion ($[M + H]^+$). [d]NI, not identified. [e]ND, not detected (the inflection point could not be clearly identified or purity was not detected).

min), presumably as a consequence of the modification of the structure of the cell wall, including the breaking of heat-labile and covalent bonds between pectin molecules.[16]

Identification of Unesterified Pigments. Some typical chromatograms that were obtained from pigment extracts of green and red jalapeño peppers are shown in Figure 1. Sixty-seven peaks were separated in pepper samples (Figure 1), 12 of them were generated by the heat treatments (peaks 56−67). The UV−vis and MS data that were used for the identification of the chromatographic peaks are shown in Table 2. Twenty-three unesterified pigments were detected in raw pepper samples (peaks 1−20, 23, 28, and 32). Peak 1 was identified as *all-trans*-neoxanthin on the basis of its UV−vis and MS data, which were similar to those reported previously (λ_{max} = 416, 442, and 470 nm; %III/II = 85%; *m/z* 601) for *all-trans*-neoxanthin from tamarillo fruit.[21,22] Peaks 2 and 5 showed the same molecular ion and two main fragments (*m/z* 583 and 565). Both peaks showed similar UV−vis spectra and %III/II (Table 2); however, a hypsochromic shift of 3−5 nm was observed between the λ_{max} of peaks 5 and 2. Similar λ_{max}, %III/II, hypsochromic shifts, and fragmentation pattern have been reported previously for *cis* and *all-trans* isomers of violaxanthin.[13,23,24] The UV−vis and MS data of peak 5 were identical to those obtained using pure *all-trans*-violaxanthin. Therefore, peaks 2 and 5 were identified as *cis*-violaxanthin and *all-trans*-violaxanthin. Peak 3 could not be identified. Peaks 4 and 6 showed the same molecular ion and main fragment, suggesting that such peaks were isomers of the same carotenoid. The UV−vis spectra of these compounds coincided with those reported for *cis*- (λ_{max} = 396, 416, and 442 nm) and *all-trans*-luteoxanthin (λ_{max} 400, 423, and 448 nm).[22,23] The %III/II of peak 6 was identical to that reported (113%) for *all-trans*-luteoxanthin.[23] Thus, peaks 4 and 6 were identified as *cis* and *all-trans*-luteoxanthin. Peak 7 showed a molecular ion at *m/z* 601 and λ_{max} at 288 and 471 nm, which are distinctive of capsanthin 5,6-epoxide.[23] The λ_{max} of peaks 8 and 9 showed a hypsochromic shift of 3−5 nm between them; however, the UV−vis spectrum of peak 9 contained the so-called *cis* peak at λ = 330 nm, revealing that peak 9 was a *cis* isomer of carotenoid. The MS and UV−vis data of these peaks were virtually identical to those reported for *all-trans*-antheraxanthin and *cis*-antheraxanthin,[25] allowing the identification of these peaks. Peak 10 showed λ_{max} at 471 nm and a molecular ion at *m/z* 585, data that were used to identify peak 10 as *cis*-capsanthin according to the study of Rodríguez-Burruezo et al.[23] The UV−vis and MS data of peaks 11 and 18 were identical to those obtained using pure chlorophylls *b* and *a*. Peak 15 was identified as chlorophyll *b*′ because it showed the MS and UV−vis data of chlorophyll *b*.[26] The UV−vis and MS data of peaks 12 and 17 were identical to those obtained using pure *all-trans*-lutein and *all-trans*-zeaxanthin. Peaks 13 and 14 showed UV−vis data similar to those reported for *all-trans* (λ_{max} = 407, 430, and 458 nm; %III/II = 55) and *cis*-mutatoxanthin (λ_{max} = 405, 427, and 452 nm; %III/II = 78), respectively.[23] The mass spectrum (ions at *m/z* 585, 567, 549, and 493) was the same for these peaks and virtually identical to those reported for *all-trans* and *cis*-mutatoxanthin of cashew apple juice.[11] Thus, peaks 13 and 14 were identified as *all-trans* and *cis*-mutatoxanthin. Peak 16 was identified as *all-trans*-capsanthin by comparing its UV−vis and MS data in the pepper samples with those obtained with a commercial standard. Peaks 19 and 20 were identified as *all-trans*-α-cryptoxanthin and *all-trans*-β-cryptoxanthin, respectively. Peak 20 showed the same λ_{max} of pure *all-trans*-β-cryptoxanthin. These compounds also showed UV−vis data similar to those reported by Rodríguez-Burruezo et al. for *all-trans*-α-cryptoxanthin (λ_{max} = 421, 445, and 475 nm; III/II = 60%) and *all-trans*-β-cryptoxanthin (λ_{max} = 425, 451, and 477 nm; III/II = 25%).[23] Peaks 19 and 20 showed a molecular ion at *m/z* 553, which has been previously reported for *all-trans*-α-cryptoxanthin and *all-trans*-β-cryptoxanthin.[23] Interestingly, the UV−vis spectrum of peak 20 presented additional λ_{max} at 335, 351, and 371 nm, which have been reported for phytofluene.[27] The mass spectrum of peak 20 included one ion at *m/z* 543, the molecular ion of phytofluene.[27] This indicates that *all-trans*-β-cryptoxanthin and phytofluene were coeluting. The UV−vis and MS data of peaks 23 and 28 were identical to those obtained using pure *all-trans*-α-carotene and *all-trans*-β-carotene. Peak 32 showed almost the same MS spectrum of peak 28, but its UV−vis data showed a hypsochromic shift of 3−4 nm with respect to those of peak 28, suggesting that peak 32 was a *cis* isomer of peak 28. Ornelas-Paz et al. reported similar MS and UV−vis data for 9-*cis*-β-carotene.[13] Peak 32 was identified as 9-*cis*-β-carotene.

Twelve unesterified compounds (peaks 56−67) were detected in the peppers after heat treatments, mainly *cis* isomers of carotenoids and chlorophylls derivatives. Peaks 56 and 57 showed the same molecular ion and two main fragments. The UV−vis data for peaks 56 and 57 were similar to those reported by de Rosso and Mercadante for *cis*-neochrome (λ_{max} = 395, 419, and 440 nm) and *all-trans*-neochrome (λ_{max} = 398, 421, and 447 nm).[22] Therefore, these peaks were identified as *cis*- and *all-trans*-neochrome. Peaks 58 and 59 were identified as 9′-*cis*-lutein and 9-*cis*-lutein. These peaks showed virtually the same λ_{max} and molecular ion. Similar UV−vis data (λ_{max} = 418−420, 441, and 467−469 nm) have been previously reported by others for 9′-*cis* and 9-*cis* isomers

of lutein.[28] The mass spectra of these peaks also were similar to that obtained using pure *all-trans*-lutein. Peak 60 showed UV−vis data that were similar to those reported previously (λ_{max} = 382, 402, and 426 nm; III/II = 99%) for auroxanthin.[26] The mass spectrum of peak 60 was similar to that obtained using pure *all-trans*-violaxanthin. Kamffer et al. demonstrated that the fragmentation pattern of symmetrical epoxy xanthophylls is similar, including violaxanthin and auroxanthin, and hypothesized that auroxanthin is formed from violaxanthin as a consequence of heat treatments.[26] Peak 61 was identified as *cis*-zeaxanthin on the basis of similarities in the mass spectrum of this peak and that of the pure *all-trans*-zeaxanthin as well as considering the UV−vis data reported in the literature for *cis*-zeaxanthin.[29] Peaks 62, 64, 66, and 67 were identified as pheophytins *b*′, *b*, *a*′, and *a* according to their UV−vis and MS data, which have been previously reported in the literature.[26,30] Peaks 63 and 65 were not identified. In general, the unesterified pigments that were identified in this study have been already identified in other pepper genotypes, but only a few of them have been reported in jalapeño pepper.[8,23,31]

Identification of Carotenoid Monoesters. Green peppers did not contain carotenoid monoesters. Some studies have demonstrated that the carotenoid esterification starts during the ripening-related transformation of chloroplasts into chromoplasts, a physiological stage that is absent in green peppers.[8] Twelve carotenoid monoesters were detected in red peppers by comparing crude and saponified pigments extracts and analyzing their MS data, but only seven monoesters (peaks 24, 27, 29, 31, 33, 34, and 35) were identified. The fatty acids of these monoesters were identified on the basis of their MS data, specifically on the neutral losses of mass from the esterified carotenoids. The UV−vis spectra of peaks 24 and 29 were similar to that of free *all-trans*-antheraxanthin, and their mass spectra included a fragment ion at *m*/*z* 567, indicating the loss of molecules of lauric (200 Da) and myristic (228 Da) acids from the molecular ions. These peaks were identified as antheraxanthin laurate and antheraxanthin myristate. Peaks 27, 31, and 34 showed the same UV−vis spectra of pure *all-trans*-capsanthin, and their MS data contained one fragment at *m*/*z* 567 that revealed the loss of molecules of lauric, myristic, and palmitic (256 Da) acids. Peaks 27, 31, and 34 were identified as capsanthin laurate, capsanthin myristate, and capsanthin palmitate. The UV−vis spectra of peaks 33 and 35 were identical to those of pure *all-trans*-zeaxanthin and *all-trans*-*β*-cryptoxanthin, and the fragments at *m*/*z* 551 and 535 in their MS data indicated the loss of molecules of myristic and lauric acids. Peaks 33 and 35 were identified as zeaxanthin myristate and *β*-cryptoxanthin laurate. The presence of zeaxanthin monoesters seems to be cultivar dependent. Breithaupt and Schwack identified several zeaxanthin diesters but no zeaxanthin monoesters in fruits of *Capsicum annuum*.[32] In contrast, Schweiggert et al. detected two zeaxanthin monoesters (zeaxanthin myristate and zeaxanthin palmitate) in red peppers.[27] Thus, the presence of zeaxanthin monoesters also could be another distinctive characteristic of jalapeño peppers. The identification of peaks 21, 22, 25, 26, and 30 could not be completed on the basis of their UV−vis data. The mass spectra of peaks 21, 22, and 25 included a fragment ion at *m*/*z* 583, revealing the loss of molecules of myristic and lauric acids from carotenoids of 601 Da. Fragment ions at *m*/*z* 567 were observed in the mass spectra of peaks 26 and 30, indicating the liberation of lauric and myristic acids from carotenoids of 585 Da. Several laurates, myristates, and palmitates of some carotenoids (capsanthin, zeaxanthin, capsorubin, and *β*-cryptoxanthin) have been previously identified in other pepper genotypes.[27,32] However, no antheraxanthin monoester had been reported in peppers.

Identification of Carotenoid Diesters. Green peppers did not contain carotenoid diesters. Twenty carotenoid diesters were detected in red peppers. The fatty acids of diesters were also identified on the basis of the neutral losses of mass from the diesterified carotenoids. The UV−vis data of eight of these (peaks 39, 40, 44, 48, 49, 52, 53, and 55) were identical to those obtained using pure *all-trans*-capsanthin. Peaks 39 and 40 showed the same mass spectrum, exhibiting ion fragments at *m*/*z* 749 and 549 that revealed the sequential loss of two molecules of lauric acid from the molecular ion. These compounds were identified as capsanthin dilaurate. The mass spectrum of peak 44 exhibited abundant fragments at *m*/*z* 749 and 777, indicating the liberation of molecules of myristic and lauric acids. Peak 44 was identified as capsanthin laurate myristate. Peaks 48 and 49 showed the same molecular ion (*m*/*z* 1005). The MS data of peak 48 contained fragment ions at *m*/*z* 777 and 549 that indicated the sequential loss of two molecules of myristic acid. The fragments at *m*/*z* 749 and 805 in the mass spectrum of peak 49 indicated the loss of molecules of palmitic and lauric acids. Peaks 48 and 49 were identified as capsanthin dimyristate and capsanthin palmitate laurate, respectively. Peaks 52 and 53 showed the same molecular ion and fragments but at different abundances. These differences allowed the identification of peaks 52 and 53 as capsanthin palmitate myristate and capsanthin myristate palmitate. Similar identification of these regioisomers of capsanthin diesters has been reported.[27] The mass spectrum of peak 55 contained fragments at *m*/*z* 805 and 549, revealing the sequential loss of two molecules of palmitic acid. Peak 55 was identified as capsanthin dipalmitate. Several capsanthin diesters have been previously identified in fruits of other pepper genotypes.[27,32] However, no capsanthin diesters had been reported in jalapeño peppers.

Four zeaxanthin diesters (peaks 43, 47, 51, and 54) were also identified in red peppers. All of them showed the same UV−vis data as those of pure *all-trans*-zeaxanthin. Two losses of 200 Da (*m*/*z* 733 and 533) from the molecular ion allowed the identification of peak 43 as zeaxanthin dilaurate. The mass spectrum of peak 47 showed two fragments at *m*/*z* 761 and 733, which evidenced the loss of lauric and myristic acids. This peak was identified as zeaxanthin laurate myristate. Peaks 51 and 54 were identified as zeaxanthin dimyristate and zeaxanthin myristate palmitate on the basis of the loss of molecules of myristic and palmitic acids from the molecular ion. The zeaxanthin diesters in some pepper genotypes contain the same fatty acid (dilaurates, dimyristates, dipalmitates, and distearates), whereas in other pepper genotypes zeaxanthin diesters contain several combinations of lauric, myristic, and palmitic acids.[27,33] According to our results, jalapeño peppers belong to the second group of genotypes.

Peaks 41, 45, and 50 were identified as capsorubin diesters. Peak 41 was identified as capsorubin laurate myristate, because two fragments (*m*/*z* 793 and 765) evidenced the liberation of lauric and myristic acids from the molecular ion. The mass spectra of peaks 45 and 50 included some fragments (*m*/*z* 793 and 821) that indicated the liberation of molecules of myristic and palmitic acids. These peaks were identified as capsorubin dimyristate and capsorubin myristate palmitate. The presence

Table 3. Pigments Content (μg/g FW) in Raw and Heat-Treated Green Jalapeño Peppers[a]

peak[b]	compound	raw	boiled	grilled
1	*all-trans*-neoxanthin	0.08 ± 0.01	ND[c]	ND
2	*cis*-violaxanthin	3.19 ± 0.09 a	0.06 ± 0.00 b	0.17 ± 0.00 b
5	*all-trans*-violaxanthin	1.09 ± 0.04 a	ND	ND
6	*all-trans*-luteoxanthin	0.06 ± 0.00 b	0.05 ± 0.00 b	0.27 ± 0.01 a
8	*all-trans*-antheraxanthin	0.42 ± 0.02 a	0.23 ± 0.00 b	0.19 ± 0.00 b
11	chlorophyll *b*	16.16 ± 0.49 a	0.77 ± 0.04 c	3.49 ± 0.30 b
12	*all-trans*-lutein	4.39 ± 0.13 b	3.82 ± 0.05 c	4.89 ± 0.13 a
15	chlorophyll *b*′	0.18 ± 0.05 c	0.48 ± 0.03 b	1.06 ± 0.04 a
17	*all-trans*-zeaxanthin	0.45 ± 0.04 a	0.24 ± 0.01 b	0.39 ± 0.01 a
18	chlorophyll *a*	67.71 ± 1.50 a	2.65 ± 0.09 b	4.17 ± 0.02 b
20	*all-trans*-β-cryptoxanthin	ND	ND	0.11 ± 0.00
23	*all-trans*-α-carotene	0.13 ± 0.01	ND	ND
28	*all-trans*-β-carotene	0.46 ± 0.07 c	1.65 ± 0.04 b	2.42 ± 0.06 a
32	9-*cis*-β-carotene	0.12 ± 0.01 c	0.24 ± 0.01 b	0.41 ± 0.01 a
56	*cis*-neochrome	ND	0.22 ± 0.01 a	0.25 ± 0.00 a
57	*all-trans*-neochrome	ND	0.14 ± 0.01 a	0.14 ± 0.00 a
58	9′-*cis*-lutein	ND	0.08 ± 0.01 a	0.06 ± 0.00 a
59	9-*cis*-lutein	ND	0.13 ± 0.02 a	0.10 ± 0.00 a
60	auroxanthin	ND	0.05 ± 0.00 b	0.09 ± 0.00 a
61	*cis*-zeaxanthin	ND	0.06 ± 0.00 b	0.09 ± 0.00 a
62	pheophytin *b*′	ND	1.70 ± 0.03 b	2.38 ± 0.07 a
63	NI[d]	ND	ND	0.12 ± 0.01
64	pheophytin *b*	ND	8.59 ± 0.32 b	14.21 ± 0.18 a
65	NI	ND	ND	0.11 ± 0.01
66	pheophytin *a*′	ND	0.90 ± 0.03 b	8.98 ± 0.12 a
67	pheophytin *a*	ND	6.30 ± 0.07 a	5.83 ± 0.22 a

[a]Values represent the mean of four individual measurements ± the standard error. Values in the same row with different letters are significantly different ($p < 0.05$). [b]Numbering of peaks according to Figure 1 and Table 2. [c]ND, not detected. [d]NI, not identified.

of several capsorubin diesters has been demonstrated previously in other pepper genotypes, except in jalapeño peppers.[27,32]

Two new mutatoxanthin diesters (peaks 42 and 46) were tentatively identified in jalapeño peppers. The UV−vis data of these peaks were similar to those (λ_{max} = 407, 430, and 458 nm) reported for mutatoxanthin.[23] The mass spectrum of peak 42 showed product ions at m/z 777 and 749, which indicated the loss of lauric and myristic acids, respectively. Two fragments (m/z 749 and 805) revealed the loss of palmitic and lauric acids from the molecular ion of peak 46. These peaks were identified as mutatoxanthin laurate myristate and mutatoxanthin palmitate laurate, respectively, and they had not been identified in any pepper genotype, being distinctive of jalapeño peppers. Such carotenoid diesters have been identified in tamarillo fruit; however, their identification was somewhat unclear because they coeluted with other compounds.[21] Peaks 36, 37, and 38 could not be identified.

Effect of Heat Treatment on Pigment Concentration. The tendencies of the concentrations of individual pigments as a function of heating were similar on a fresh and dry weight bases. The pigment content in the green and red peppers (fresh weight basis) as a function of heat treatments is shown in Tables 3 and 4. Chlorophylls (*a* and *b*) and free *all-trans*-lutein were the major pigments in raw green peppers (67.7, 16.2, and 4.4 μg/g, respectively), being absent in red peppers. Similar results have been reported for Bola, Agridulce, Szentesi Kosszarvú, and other pepper genotypes at the green and other ripening stages.[8,31] The disappearance of chlorophylls and lutein during the ripening process of peppers has been explained in terms of the inhibition of their biosynthesis as a consequence of the transformation of chloroplasts into chromoplasts, the loss of their functionality as part of the light-harvesting system once photosynthesis is blocked, and their use as precursors of other compounds.[8] Heat treatments diminished the concentration of chlorophylls *a* (94−96%) and *b* (78−95%). This decrease was accompanied by the generation of pheophytins (peaks 62, 64, 66, and 67), as reported for heat-treated spinach leaves.[34] The free *all-trans*-lutein content decreased slightly by boiling (13%) but increased by grilling (11.4%), detecting two *cis* isomers (9-*cis* and 9′-*cis*) of lutein in heat-treated samples. This suggests the isomerization from *all-trans*-lutein to *cis*-lutein. This also suggests that *all-trans*-lutein might be degraded by heating; however, the reduction of the concentration of *all-trans*-lutein in grilled peppers was probably masked by a concentration phenomenon that derived from excessive weight losses (Table 1). Recently, Aparicio-Ruiz et al. demonstrated that the formation of these isomers of lutein is energetically favored by increasing processing temperature.[35]

Capsanthin was detected only in red peppers, as reported previously for other pepper genotypes.[32] The free *all-trans*-capsanthin was the most abundant carotenoid in raw red peppers (37.6 μg/g), tending to increase slightly as a consequence of boiling and grilling (41.7 and 38.1 μg/g, respectively). In addition, high levels of capsanthin myristate, capsanthin laurate myristate, and capsanthin dimyristate (13.1, 11.0, and 7.3 μg/g, respectively) were observed in these samples. Low to moderate concentrations (0.3−2.3 μg/g) of other capsanthin esters also were detected in raw red peppers. Some studies have demonstrated that the levels of mono-esterified or diesterified capsanthin are higher than those of the free form.[32,36] In our study, the concentration of free capsanthin was higher than that of monoesterified or

Table 4. Pigments Content (μg/g FW) in Raw and Heat-Treated Red Jalapeño Peppers[a]

peak[b]	compound	raw	boiled	grilled
2	*cis*-violaxanthin	1.03 ± 0.05 a	1.11 ± 0.02 a	0.83 ± 0.01 b
3	NI[c]	1.45 ± 0.06 b	1.72 ± 0.02 a	1.45 ± 0.03 b
4	*cis*-luteoxanthin	0.07 ± 0.01 b	0.12 ± 0.01 a	0.12 ± 0.01 a
5	*all-trans*-violaxanthin	0.10 ± 0.01 b	ND[d]	ND
6	*all-trans*-luteoxanthin	0.14 ± 0.01 a	0.06 ± 0.01 b	0.07 ± 0.00 b
7	capsanthin 5,6-epoxide	0.73 ± 0.06 a	0.87 ± 0.01 a	0.54 ± 0.02 b
8	*all-trans*-antheraxanthin	3.48 ± 0.09 a	3.42 ± 0.06 a	3.44 ± 0.07 a
9	*cis*-antheraxanthin	0.45 ± 0.01	ND	ND
10	*cis*-capsanthin	0.28 ± 0.02 c	4.86 ± 0.04 a	2.90 ± 0.04 b
13	*all-trans*-mutatoxanthin	1.37 ± 0.07 b	3.59 ± 0.03 a	3.53 ± 0.07 a
14	*cis*-mutatoxanthin	0.23 ± 0.00 a	0.17 ± 0.01 b	0.10 ± 0.01 c
16	*all-trans*-capsanthin	37.60 ± 0.78 b	41.68 ± 0.29 a	38.05 ± 0.38 b
17	*all-trans*-zeaxanthin	7.08 ± 0.03 a	7.00 ± 0.08 a	6.25 ± 0.09 b
18	chlorophyll *a*	2.22 ± 0.04	ND	ND
19	*all-trans*-α-cryptoxanthin	0.37 ± 0.01 a	0.15 ± 0.00 c	0.27 ± 0.01 b
20	*all-trans*-β-cryptoxanthin	2.56 ± 0.02 b	2.45 ± 0.06 b	2.89 ± 0.03 a
21	NI	0.14 ± 0.00 b	0.18 ± 0.01 a	0.19 ± 0.01 a
22	NI	0.39 ± 0.03 b	0.31 ± 0.08 b	0.81 ± 0.04 a
24	antheraxanthin laurate	1.92 ± 0.04 b	3.94 ± 0.07 a	4.06 ± 0.12 a
25	NI	2.40 ± 0.03 a	0.26 ± 0.02 c	0.58 ± 0.03 b
26	NI	2.25 ± 0.03 a	1.71 ± 0.02 b	1.28 ± 0.07 c
27	capsanthin laurate	0.64 ± 0.06 c	1.41 ± 0.01 a	1.11 ± 0.06 b
28	*all-trans*-β-carotene	6.08 ± 0.30 b	8.61 ± 0.18 a	8.21 ± 0.23 a
29	antheraxanthin myristate	1.59 ± 0.13 a	0.74 ± 0.02 c	1.21 ± 0.07 b
30	NI	1.17 ± 0.02	ND	ND
31	capsanthin myristate	13.11 ± 0.72 b	17.14 ± 0.66 a	18.53 ± 0.48 a
32	9-*cis*-β-carotene	0.19 ± 0.02 b	0.22 ± 0.02 b	0.44 ± 0.02 a
33	zeaxanthin myristate	2.77 ± 0.06 a	1.78 ± 0.06 b	1.99 ± 0.05 b
34	capsanthin palmitate	1.68 ± 0.11 b	2.23 ± 0.04 a	1.76 ± 0.02 b
35	β-cryptoxanthin laurate	0.59 ± 0.01 a	0.58 ± 0.02 a	0.55 ± 0.02 a
36	NI	0.37 ± 0.03 a	0.59 ± 0.01 a	0.66 ± 0.01 a
37	NI	0.33 ± 0.05 a	0.23 ± 0.00 a	0.28 ± 0.00 a
38	NI	0.90 ± 0.01 a	0.79 ± 0.01 a	0.59 ± 0.03 a
39	capsanthin dilaurate	2.25 ± 0.17 a	1.85 ± 0.02 a	1.27 ± 0.01 b
40	capsanthin dilaurate	0.38 ± 0.03 a	0.16 ± 0.01 b	0.16 ± 0.01 b
41	capsorubin laurate myristate	0.15 ± 0.02 a	0.17 ± 0.01 a	0.13 ± 0.01 a
42	mutatoxanthin laurate myristate	0.11 ± 0.01 c	0.42 ± 0.01 b	0.48 ± 0.01 a
43	zeaxanthin dilaurate	0.55 ± 0.02 a	0.29 ± 0.01 b	0.29 ± 0.01 b
44	capsanthin laurate myristate	10.97 ± 0.80 a	11.90 ± 0.39 a	9.88 ± 0.12 a
45	capsorubin dimyristate	0.72 ± 0.03 b	1.04 ± 0.03 a	0.98 ± 0.03 a
46	mutatoxanthin palmitate laurate	0.19 ± 0.01 a	0.19 ± 0.01 a	0.10 ± 0.00 b
47	zeaxanthin laurate myristate	0.35 ± 0.00 a	0.10 ± 0.00 c	0.27 ± 0.02 b
48	capsanthin dimyristate	7.32 ± 0.49 ab	8.25 ± 0.27 a	6.62 ± 0.15 b
49	capsanthin palmitate laurate	1.69 ± 0.09 a	1.53 ± 0.04 a	1.27 ± 0.04 b
50	capsorubin myristate palmitate	0.35 ± 0.04	ND	ND
51	zeaxanthin dimyristate	0.52 ± 0.02 b	0.60 ± 0.04 b	0.82 ± 0.02 a
52	capsanthin palmitate myristate	1.03 ± 0.05 a	1.07 ± 0.02 a	0.86 ± 0.02 b
53	capsanthin myristate palmitate	0.29 ± 0.02 a	0.32 ± 0.00 a	0.29 ± 0.01 a
54	zeaxanthin myristate palmitate	0.18 ± 0.01 a	0.16 ± 0.00 a	0.20 ± 0.02 a
55	capsanthin dipalmitate	1.04 ± 0.06 b	1.48 ± 0.04 a	0.84 ± 0.01 c

[a]Values represent the mean of four individual measurements ± the standard error. Values in the same row with different letters are significantly different ($p < 0.05$). [b]Numbering of peaks according to Figure 1 and Table 2. [c]NI, not identified. [d]ND, not detected.

diesterified capsanthin. This could be a distinctive characteristic of jalapeño peppers. The levels of capsanthin myristate tended to increase (30.7−41.3%) by boiling and grilling, whereas the concentrations of capsanthin laurate myristate and capsanthin dimyristate were not significantly affected by the heat treatments. Other capsanthin esters were differentially altered by the heat treatments. The structural diversity of carotenoid esters results in a wide variability in the susceptibility of these compounds to oxidation, being degraded in a nonuniform rate.[37] In general, the proportion of monoesterified capsanthin, relative to the total capsanthin content (sum of free and esterified capsanthin), was sequentially increased by boiling and grilling (3.6−6.8%), whereas the proportions of free and diesterified capsanthin were either slightly diminished or not

affected by heat treatments, indicating a higher thermostability of monoesterified capsanthin than that of other capsanthin forms. Schweiggert et al. demonstrated that carotenoid monoesters of chili powder were more thermostable than free and diesterified carotenoids; however, this seemed to be dependent on the heating conditions.[37]

The contents of free *all-trans*-violaxanthin and free *cis*-violaxanthin in raw green peppers were 1.1 and 3.2 μg/g, respectively, diminishing to 0.1 and 1.03 μg/g in raw red peppers. Some studies have hypothesized that violaxanthin serves as a precursor for the biosynthesis of capsanthin 5,6-epoxide (present only in red peppers) during the ripening process through a pinacol rearrangement,[31] explaining the diminishing of violaxanthin in peppers from the green to the red stages. The *all-trans*-violaxanthin disappeared completely in heat-treated peppers, whereas the content of *cis*-violaxanthin decreased in these samples. Lee and Coates demonstrated that under heating conditions violaxanthin undergoes transformations in the end-groups from 5,6-epoxy to 5,8-furanoid, leading to the formation of luteoxanthin and auroxanthin,[24] which were also found in our study in small concentrations in heat-treated peppers. Our results suggest that auroxanthin was generated by heat treatments. Similar effects of heat treatments on the stability of violaxanthin have been reported previously in other food samples.[11]

The free *all-trans*-neoxanthin, which is also synthesized from violaxanthin, was detected only in raw green peppers at low concentration (0.1 μg/g). Deli et al. also found that neoxanthin is detected only in Kosszarvú peppers at early stages of ripening.[31] In our work, *all-trans*-neoxanthin was completely degraded by heat treatments. Interestingly, *all-trans*-neochrome (0.14 μg/g) and *cis*-neochrome (0.22−0.25 μg/g), absent in raw peppers, were detected in green heat-treated peppers, suggesting that these carotenoids derived from *all-trans*-neoxanthin. Kamffer et al. demonstrated that neoxanthin from grapes is converted into mutatoxanthin and then to neochrome under low pH conditions.[26] Mertz et al. also observed the generation of neochrome and auroxanthin from neoxanthin in heat-treated tamarillo fruits.[21]

Low concentrations of free *all-trans*-antheraxanthin (0.4 μg/g) were observed in raw green peppers; however, the content of this carotenoid in raw red pepper was 7.3 times higher than that in green peppers. The levels of free *all-trans*-antheraxanthin in green peppers were similar to those of the literature for peppers at the same stage of ripening (0.5 μg/g), but our concentrations for red peppers were considerably lower than those previously reported (35−635 μg/g) for other pepper genotypes.[8] Boiling and grilling significantly reduced the content of free *all-trans*-antheraxanthin in green peppers; however, the levels of this carotenoid were not changed by heat treatments in red peppers, indicating an effect of the food matrix on the stability of this carotenoid. Similarly, Schweiggert et al. demonstrated that the thermostability of free carotenoids is completely different in chilli and paprika.[37] Antheraxanthin laurate and antheraxanthin myristate were also present in raw red peppers (1.9 and 1.6 μg/g, respectively). The levels of antheraxanthin laurate were increased by heat treatments. In contrast, the levels of antheraxanthin myristate were considerably decreased (23.9−53.5%) by heating. These findings suggest an effect of the fatty acid moiety on the thermostability of monoesterified antheraxanthin. Mínguez-Mosquera and Hornero-Méndez also suggested that fatty acids modulate the thermostability of carotenoid esters.[38] Interestingly, the proportions of mono-esterified and free antheraxanthin, relative to the total antheraxanthin content (sum of free and esterified antheraxanthin), were increased and reduced, respectively, to the same extent (7.6−10.3%) by boiling and grilling, suggesting that the esterification of this carotenoid occurred during heating. Although this phenomenon is difficult to explain, previous studies have suggested that the de novo synthesis, transformation, and esterification of carotenoids can occur during heat processing of peppers.[38,39]

The free *all-trans*-mutatoxanthin, free *cis*-mutatoxanthin, mutatoxanthin laurate myristate, and mutatoxanthin palmitate laurate were detected in raw red peppers at low to moderate concentrations (1.4, 0.2, 0.1, and 0.2 μg/g, respectively). Both heat treatments increased the concentrations of free *all-trans*-mutatoxanthin (3.5−3.6 μg/g) and mutatoxanthin laurate myristate (0.42−0.48 μg/g), whereas the levels of *cis*-mutatoxanthin and mutatoxanthin laurate palmitate were diminished (0.1−0.2 μg/g for both compounds). Mutatoxanthin diesters were differentially affected by heat treatments, indicating again an effect of the fatty acid moiety on the thermostability of individual carotenoid diesters. Mínguez-Mosquera and Pérez-Gálvez suggested that minimal variations of the chain length of the fatty acids alter the stability of carotenoid esters.[40] On the other hand, the thermostability of fatty acids in carotenoid esters is also highly variable.[39] However, the proportions of diesterified and free mutatoxanthin, relative to the total mutatoxanthin content (sum of free and esterified mutatoxanthin), were diminished and increased, respectively, to the same extent (3.3−3.7%) by heat treatments, demonstrating the occurrence of the heat-induced de-esterification of this carotenoid rather than its degradation. De-esterification of carotenoid esters by heating has been suggested previously, probably as a consequence of the degradation of the fatty acids.[39,40]

The free *all-trans*-zeaxanthin was observed at low concentration (0.45 μg/g) in raw green peppers; however, the content of this carotenoid was 14.7 times higher in raw red pepper than in green peppers. In general, the concentrations of free *all-trans*-zeaxanthin found in this work for raw green and red peppers were similar to those reported in the literature for several pepper genotypes.[41] The free *all-trans*-zeaxanthin content was diminished by boiling (1.1−46.7%) and grilling (11.7−13.3%). In our work, *cis*-zeaxanthin was generated by heat treatments, especially by grilling. Milanowska and Gruszecky observed that heating (35−95 °C) favors the isomerization of *all-trans*-zeaxanthin into 13-*cis*-zeaxanthin.[42] In addition, low to moderate concentrations of zeaxanthin mono- and diesters (0.4−2.8 μg/g) were observed in raw red peppers. Heat treatments modified differentially the levels of these zeaxanthin esters; however, the effect of the esterification on zeaxanthin thermostability could not be observed clearly in this work. The concentration of esterified zeaxanthin in peppers has been scarcely reported.

Free *all-trans*-α- and β-cryptoxanthins were detected only in red peppers. Their concentrations in raw peppers were 0.4 and 2.6 μg/g, respectively. Heat treatments drastically reduced (27.0−59.5%) the levels of *all-trans*-α-cryptoxanthin. Boiling slightly decreased the levels of free *all-trans*-β-cryptoxanthin (4.3%); however, the concentration of this carotenoid was increased (12.9%) by grilling. This increase could be explained in terms of the dehydration of the fruit during heat treatment.[6] The levels of β-cryptoxanthin laurate were low in raw peppers (0.6 μg/g), and heat treatments reduced the levels of this

compound (1.7−6.8%). *all-trans-β*-Cryptoxanthin is a carotenoid synthesized de novo, and its absence in green peppers and subsequent appearance in red peppers have been reported previously.[8]

all-trans-β-Carotene and 9-*cis-β*-carotene were detected in fruits at both maturity stages (6.1 and 0.2 μg/g, respectively). The contents of *all-trans-β*-carotene and 9-*cis-β*-carotene increased by boiling (41.6 and 15.8%) and grilling (35.0 and 131.6%, respectively). Previous studies have demonstrated that the content of these carotenes in peppers is either unchanged or increased by heat treatments.[43]

Only esterified capsorubin was detected in red peppers. Capsorubin laurate myristate, capsorubin dimyristate, and capsorubin myristate palmitate were detected in small concentrations (0.2, 0.7, and 0.4 μg/g, respectively) in raw peppers. The concentration of capsorubin laurate myristate was increased (13.3%) by boiling and diminished (13.3%) by grilling, whereas capsorubin dimyristate increased with both treatments (36.1−44.4%) and capsorubin myristate palmitate was degraded completely. Again, these findings suggest an effect of the fatty acid moiety on the thermostability of carotenoid esters, probably as a consequence of the variations on the stability of the bond between carotenoids and fatty acids and differences in the thermostability of fatty acids.[39] However, the small variability of the fatty acid moiety in the structure of carotenoid esters made it difficult to determine the effect of fatty acids on the stability of carotenoids esters. Unidentified peaks (peaks 21, 22, 25, 26, 30, and 38) were quantified as *all-trans-β*-carotene and capsanthin (peaks 36 and 37) on the basis of their UV−vis data. Heat treatments altered differentially the levels of these compounds.

In general, the percentages of free, monoesterified, and diesterified carotenoids, with respect to the total carotenoid content, in raw red peppers were 52.8, 23.2, and 24.0%, respectively. These percentages are different from those reported (9.6% free carotenoids, 22.5% monoesters, and 67.9% diesters) for red peppers of unknown genotype.[37] However, the fatty acids that were esterified with carotenoids of jalapeño peppers were the same (lauric, myristic, and palmitic) that have been reported in the literature for carotenoid esters from other pepper genotypes.[32,36,37] The percentages of free, monoesterified, and diesterified carotenoids found in raw peppers were not changed by boiling (55.3, 22.0, and 22.7%) and grilling (54.3, 25.2, and 20.4%), indicating that the thermostability of carotenoids from jalapeño peppers is high. Some studies have demonstrated that capsaicinoids prevent the thermal degradation of carotenoids in peppers.[44] Cell disruption by heating has been demonstrated in fruits and vegetables, and therefore an interaction between carotenoids by capsaicinoids can be expected for heat-treated peppers in the present study.[10]

Effect of Heat Treatments on the Antioxidant Capacity of Pigments Extracts. The extracts of red peppers presented a higher antioxidant capacity than those of green peppers (Figure 2). The high antioxidant capacity of red peppers has been attributed to their high levels of capsanthin and carotenoid esters containing palmitic, myristic, and lauric acids, which also exert a high antioxidant capacity.[45−47] In our work, the antioxidant capacity values found for raw peppers by using the DPPH (32.3−40.9 μmol TE/g FW) and FRAP (358.2−703.7 μmol TE/g FW) assays were similar to those reported for jalapeño and other pepper genotypes using the same assays.[6,48] In general, boiling tended to decrease the antioxidant capacity of peppers, whereas grilling caused minor changes in the antioxidant capacity of peppers, except for red pepper using the FRAP assay. Chuah et al. also observed a diminishing of the antioxidant capacity of green peppers and green paprika after boiling,[49] presumably as a consequence of the suppression of antioxidant oxidation by the thermal inactivation of oxidative enzymes and the increase of the extraction efficiency of antioxidants mediated by heat treatments.[50] Minor effects of heat treatments on the antioxidant capacity of some peppers also have been reported.[49]

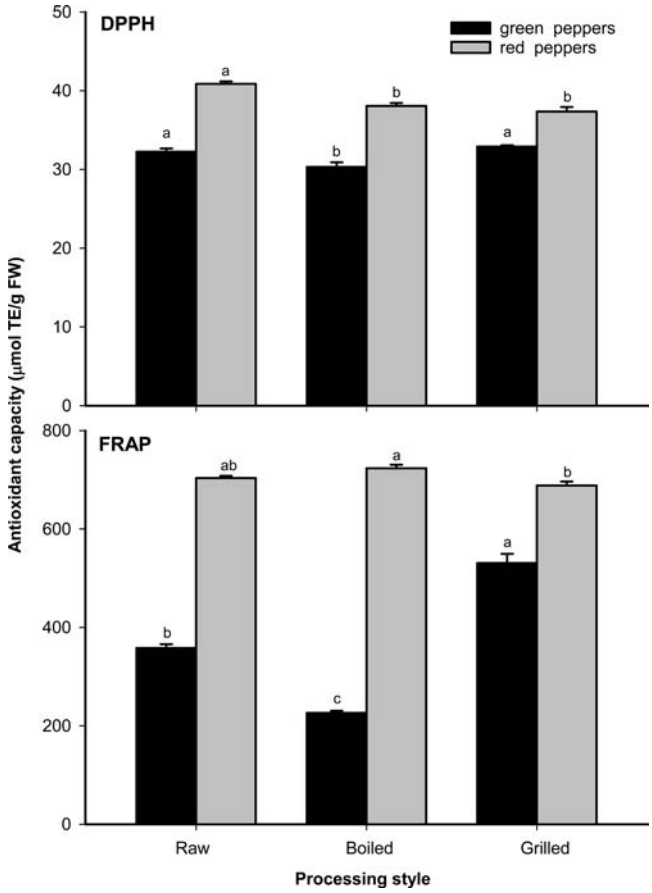

Figure 2. Antioxidant capacity of crude extracts from raw and heat-treated peppers determined by DPPH and FRAP assays. The data represent the mean of four individual measurements ± the standard error (slim bars).

In summary, the profiles of pigments and antioxidant capacity were completely different for green and red peppers. The effect of heat treatments depended on pigment speciation and food matrix. Our results suggest that heating is able to induce the esterification, de-esterification, degradation, isomerization, and transformation of pigments.

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Notes

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■ REFERENCES

(1) Howard, L. R.; Smith, R. T.; Wagner, A. B.; Villalon, B.; Burns, E. E. Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapeños. *J. Food Sci.* **1994**, *59*, 362−365.

(2) 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.

(3) Alvarez-Parrilla, E.; De la Rosa, L. A.; Amarowicz, R.; Shahidi, F. Protective effect of fresh and processed Jalapeño and Serrano peppers against food lipid and human LDL cholesterol oxidation. *Food Chem.* **2012**, *133*, 827−834.

(4) Levy, A.; Harel, S.; Palevitch, D.; Akiri, B.; Menagem, E.; Kanner, J. Carotenoid pigments and β-carotene in paprika fruits (*Capsicum* spp.) with different genotypes. *J. Agric. Food Chem.* **1995**, *43*, 362−366.

(5) O'Sullivan, L.; Jiwan, M. A.; Daly, T.; O'Brien, N. M.; Aherne, S. A. Bioaccessibility, uptake, and transport of carotenoids from peppers (*Capsicum* spp.) using the coupled in vitro digestion and human intestinal Caco-2 cell model. *J. Agric. Food Chem.* **2010**, *58*, 5374−5379.

(6) Ornelas-Paz, J. D. J.; Cira-Chávez, L. A.; Gardea-Béjar, A. A.; Guevara-Arauza, J. C.; Sepúlveda, D. R.; Reyes-Hernández, J.; Ruíz-Cruz, S. Effect of heat treatment on the content of some bioactive compounds and free radical-scavenging activity in pungent and non-pungent peppers. *Food Res. Int.* **2011**, DOI: 10.1016/j.foodres.2011.01.006.

(7) Guil-Guerrero, J. L.; Martínez-Guirado, C.; Rebolloso-Fuentes, M. d. M.; Carrique-Pérez, A. Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annuun*) varieties. *Eur. Food Res. Technol.* **2006**, *224*, 1−9.

(8) Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Formation and transformation of pigments during the fruit ripening of *Capsicum annuum* cv. *Bola* and *Agridulce*. *J. Agric. Food Chem.* **1994**, *42*, 30−44.

(9) Pérez-Gálvez, A.; Rios, J. J.; Mínguez-Mosquera, M. I. Thermal degradation products formed from carotenoids during a heat-induced degradation process of paprika oleoresins (*Capsicum annuum* L.). *J. Agric. Food Chem.* **2005**, *53*, 4820−4826.

(10) Rodríguez-Amaya, D. B.; Rodríguez, E. B.; Amaya-Farfan, J. Advances in food carotenoid research: chemical and technological aspects, implications in human health. *Malays. J. Nutr.* **2006**, *12*, 101−121.

(11) Zepka, L. Q.; Mercadante, A. Z. Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. *Food Chem.* **2009**, *117*, 28−34.

(12) Ferruzzi, M. G.; Blakeslee, J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutr. Res. (N.Y.)* **2007**, *27*, 1−12.

(13) Ornelas-Paz, J. D. J.; Yahia, E. M.; Gardea-Béjar, A. Identification and quantification of xanthophyll esters, carotenes, and tocopherols in the fruit of seven mexican mango cultivars by liquid chromatography−atmospheric pressure chemical ionization−time-of-flight mass spectrometry [LC-(APcI$^+$)-MS]. *J. Agric. Food Chem.* **2007**, *55*, 6628−6635.

(14) Corral-Aguayo, R. D.; Yahia, E. M.; Carrillo-López, A.; González-Aguilar, G. Correlation between some nutritional components and the total antioxidant capacity measured with six different assays in eight horticultural crops. *J. Agric. Food Chem.* **2008**, *56*, 10498−10504.

(15) Castro, S. M.; Saraiva, J. A.; Lopes-da-Silva, J. A.; Delgadillo, I.; Loey, A. V.; Smout, C.; Hendrickx, M. Effect of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits (*Capsicum annuum* L.). *Food Chem.* **2008**, *107*, 1436−1449.

(16) Gu, Y. S.; Howard, L. R.; Wagner, A. B. Physicochemical factors affecting firmness of pasteurized Jalapeno pepper rings. *J. Food Qual.* **1998**, *22*, 619−619.

(17) Guiné, R. P. F.; Barroca, M. J. Effect of drying treatments on texture and color of vegetables (pumpkin and green pepper). *Food Bioprod. Process.* **2011**, *90*, 58−63.

(18) Vega-Gálvez, A.; Di Scala, K.; Rodríguez, K.; Lemus-Mondaca, R.; Miranda, M.; López, J.; Pérez-Won, M. Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (*Capsicum annuum*, L. var. Hungarian). *Food Chem.* **2009**, *117*, 647−653.

(19) Castañer, M.; Gil, M. I.; Artes, F.; Tomas-Barberan, F. A. Inhibition of browning of harvested head lettuce. *J. Food Sci.* **1996**, *61*, 314−316.

(20) Molinari, A. F.; de Castro, L. R.; Antoniali, S.; Pornchaloempong, P.; Fox, A. J.; Sargent, S. The potential for bell pepper harvest prior to full color development. *Proc. Fla. State Hortic. Soc.* **1999**, *112*, 143−146.

(21) Mertz, C.; Brat, P.; Caris-Veyrat, C.; Gutana, Z. Characterization and thermal lability of carotenoids and vitamin C of tamarillo fruit (*Solanum betaceum* Cav.). *Food Chem.* **2010**, *119*, 653−659.

(22) de Rosso, V. V.; Mercadante, A. Z. HPLC−PDA−MS/MS of anthocyanins and carotenoids from dovyalis and tamarillo fruits. *J. Agric. Food Chem.* **2007**, *55*, 9135−9141.

(23) Rodríguez-Burruezo, A.; González-Mas, M. d. C.; Nuez, F. Carotenoid composition and vitamin A value in Ají (*Capsicum baccatum* L.) and Rocoto (*C. pubescens* R. & P.), 2 pepper species from the andean region. *J. Food Sci.* **2010**, *75*, 446−453.

(24) Lee, H. S.; Coates, G. A. Effect of thermal pasteurization on Valencia orange juice color and pigments. *LWT−Food Sci. Technol.* **2003**, *36*, 153−156.

(25) Meléndez-Martínez, A. J.; Britton, G.; Vicario, I. M.; Heredia, F. J. Identification of isolutein (lutein epoxide) as *cis*-antheraxanthin in orange juice. *J. Agric. Food Chem.* **2005**, *53*, 9369−9373.

(26) Kamffer, Z.; Bindon, K. A.; Oberholster, A. Optimization of a method for the extraction and quantification of carotenoids and chlorophylls during ripening in grape berries (*Vitis vinifera* cv. Merlot). *J. Agric. Food Chem.* **2010**, *58*, 6578−6586.

(27) Schweiggert, U.; Kammerer, D. R.; Carle, R.; Schieber, A. Characterization of carotenoids and carotenoid esters in red pepper pods (*Capsicum annuum* L.) by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2617−2628.

(28) Achir, N.; Randrianatoandro, V. A.; Bohuon, P.; Laffargue, A.; Avallone, S. Kinetic study of β-carotene and lutein degradation in oils during heat treatment. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 349−361.

(29) Tai, C. Y.; Chen, B. H. Analysis and stability of carotenoids in the flowers of daylily (*Hemerocallis disticha*) as affected by various treatments. *J. Agric. Food Chem.* **2000**, *48*, 5962−5968.

(30) Crupi, P.; Coletta, A.; Milella, R. A.; Palmisano, G.; Baiano, A.; La Note, E.; Antonacci, D. Carotenoid and chlorophyll-derived compounds in some wine grapes grown in Apulian region. *J. Food Sci.* **2010**, *75*, 191−198.

(31) Deli, J.; Matus, Z.; Tóth, G. Carotenoid composition in the fruits of *Capsicum annuum* cv. Szentesi Kosszarvú during ripening. *J. Agric. Food Chem.* **1996**, *44*, 711−716.

(32) Breithaupt, D. E.; Schwack, W. Determination of free and bound carotenoids in paprika (*Capsicum annuum* L.) by LC/MS. *Eur. Food Res. Technol.* **2000**, *211*, 52−55.

(33) Weller, P.; Breithaupt, D. E. Identification and quantification of zeaxanthin esters in plants using liquid chromatography−mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 7044−7049.

(34) Canjura, F. L.; Schwartz, S. Separation of chlorophyll compounds and their polar derivatives by high-performance liquid chromatography. *J. Agric. Food Chem.* **2001**, *39*, 1102−1105.

(35) Aparicio-Ruiz, R.; Mínguez-Mosquera, M. I.; Gandul-Rojas, B. Thermal degradation kinetics of lutein, β-carotene and β-cryptoxanthin in virgin olive oils. *J. Food Compos. Anal.* **2011**, *24*, 811−820.
(36) Hornero-Méndez, D.; Mínguez-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.
(37) Schweiggert, U.; Kurz, C.; Schieber, A.; Carle, R. Effects of processing and storage on the stability of free and esterified carotenoids of red peppers (*Capsicum annuum* L.) and hot chilli peppers (*Capsicum frutescens* L.). *Eur. Food Res. Technol.* **2007**, *225*, 261−270.
(38) Mínguez-Mosquera, M. I.; Hornero-Méndez, 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.
(39) Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Comparative study of the effect of paprika processing on the carotenoids in peppers (*Capsicum annuum*) of the Bola and Agridulce varieties. *J. Agric. Food Chem.* **1994**, *42*, 1555−1560.
(40) Mínguez-Mosquera, M. I.; Pérez-Gálvez, A. Study of lability and kinetics of the main carotenoid pigments of red pepper in the de-esterification reaction. *J. Agric. Food Chem.* **1998**, *46*, 566−569.
(41) Russo, V.; Howard, L. Carotenoids in pungent and non-pungent peppers at various developmental stages grown in the field and glasshouse. *J. Sci. Food Agric.* **2002**, *82*, 615−624.
(42) Milanowska, J.; Gruszecki, W. I. Heat-induced and light-induced isomerization of the xanthophyll pigment zeaxanthin. *J. Photochem. Photobiol. B* **2005**, *80*, 178−186.
(43) 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.
(44) Daood, H. G.; Kapitány, J.; Biacs, P.; Albrecht, K. Drying temperature, endogenous antioxidants and capsaicinoids affect carotenoid stability in paprika (red pepper spice). *J. Sci. Food Agric.* **2006**, *86*, 2450−2457.
(45) Kim, J. S.; Ahh, J.; Lee, S. J.; Moon, B.; Ha, T. H.; Kim, S. Phytochemicals and antioxidant activity of fruits and leaves of paprika (*Capsicum annuum* L., var. *Special*) cultivated in Korea. *J. Food Sci.* **2011**, *76*, 193−198.
(46) Henry, G. E.; Momin, R. A.; Nair, M. G.; Dewitt, D. L. Antioxidant and cyclooxygenase activities of fatty acids found in food. *J. Agric. Food Chem.* **2002**, *50*, 2231−2234.
(47) Hervert-Hernández, D.; Sáyago-Ayerdi, S. G.; Goñi, I. Bioactive compounds of four hot pepper varieties (*Capsicum annuum* L.), antioxidant capacity, and intestinal bioaccessibility. *J. Agric. Food Chem.* **2010**, *58*, 3399−3406.
(48) Deepa, N.; Kaur, C.; Sing, B.; Kapoor, H. C. Antioxidant activity in some red sweet pepper cultivars. *J. Food Compos. Anal.* **2006**, *19*, 572−578.
(49) 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.
(50) Yamaguchi, T.; Mizobuchi, T.; Kajikawa, R.; Kawashima, H.; Miyabe, F.; Terao, J. Radical-scavening activity of vegetables and the effect of cooking on their activity. *Food Sci. Technol. Res.* **2001**, *7*, 250−257.