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Effect of the Interaction of Heat-Processing Style and Fat Type on the Micellarization of Lipid-Soluble Pigments from Green and Red Pungent Peppers (*Capsicum annuum*)

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ABSTRACT: The high diversity of carotenoids and chlorophylls in foods contrasts with the reduced number of pigments that typically are investigated in micellarization studies. In this study, pepper samples (raw and heat-treated) contained 68 individual pigments, but only 38 of them were micellarized after *in vitro* digestion. The micellarization of pigments was majorly determined by the interaction effect of processing style (food matrix effect) and fat type (saturated and unsaturated). The highest micellarization was observed with raw peppers. Unsaturated fat increased the micellarization of carotenoid esters, while the impact of fat on the micellarization of free carotenoids seemed to be dependent on pigment structure. The micellarization efficiency was diminished as the esterification level of carotenoids increased. The type of fatty acid moiety and the polarity of the carotenoids modulated their micellarization. Chlorophylls were transformed into pheophytins by heat-processing and digestion, with the pheophytins being stable under gastrointestinal conditions. Micellarization of pheophytins was improved by fat.

KEYWORDS: *bioactive pigments, healthy vegetables, heat-processing, hydrophobicity, micellarization, saturated and unsaturated fat*

INTRODUCTION

The chlorophylls and carotenoids are two abundant classes of bioactive compounds in peppers, and their qualitative and quantitative profiles are well known.[1,2] However, the bioaccessibility (micellarization) and bioavailability of these pigments in peppers have only been partially characterized and limited to some carotenoids. The micellarization efficiency of a few free carotenoids has been determined in raw fruits of some pepper genotypes, comparing in some cases their bioaccessibility with that of carotenoids from other foods.[3−5] The effect of heat-processing styles on the bioaccessibility of this limited number of carotenoids has also been determined in sweet peppers.[6] The fate and micellarization efficiency of a small number of carotenoid esters of peppers have been determined only in sweet peppers.[7−9] Interestingly, the micellarization/bioavailability of capsanthin and capsorubin, two carotenoids that are exclusive of red peppers, has been scarcely studied, and the existing data are contradictory.[7,10,11] The limited number of carotenoids included in these studies contrasts with the high number of carotenoid species, chlorophylls, and chlorophyll derivatives that have been identified in peppers, which is of up to 60, 6, and 5, respectively.[2,12] Carotenoids in peppers are mainly esterified with fatty acids.[2] To date, the study of bioaccessibility of carotenoid esters in food matrixes has been limited to general groups (mono- and diesters) and therefore provided little insight into the possible impact of specific fatty acids on the micellarization of these pigments.[3,8,9] The number and type of fatty acids modulate the polarity and stability of carotenoids,[2] two properties involved in their micellarization.[13,14] The micellarization/bioavailability of chlorophyll pigments in complex food matrixes has been studied only in spinach and peas.[15,16]

Food matrix and dietary fat are known to influence the bioaccessibility and bioavailability of carotenoids.[6,17] Heat-processing is one of the most common ways of altering the food matrix. Heating favors the release of carotenoids and may thereby enhance their micellarization.[18] Heat-processing also modifies the structure of fibers,[19] which has the potential to affect the micellarization of carotenoids by altering the capacity of fibers to bind bile acids, reduce the activity of pancreatic lipase (lipolysis), modify the viscosity of the gastrointestinal contents, and decrease emulsification of fat.[20,21] Heat-processing affects the levels of carotenoids in foods,[2] which might alter their micellarization since with some foods the micellarization of carotenoids is directly proportional to their concentration in the food.[22] Heat-processing can also induce structural changes in carotenoids, mainly isomerization, altering their solubility and consequently their micellarization.[2,14] The extent of micellarization of carotenoids is generally increased as the intensity of heat-processing of the food increases.[18] This positive impact of heating is potentiated by the amount and type of dietary fat.[23,24] However, the effect of fat is selective for carotenoid type and seems to be dependent on the food matrix. Schweiggert et al. demonstrated that dietary fat increased the micellarization of lycopene from tomatoes but not from papaya, although lycopene from papaya is considered to be more bioavailable.[25] Micellarization of β-carotene was differentially increased by fat in mangoes of the

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Table 1. Content (μg/g FW) of Carotenoids, Chlorophylls, and Chlorophyll Derivatives in Raw and Heat-Processed Jalapeño Peppers (Green and Red)[a]

		green peppers			red peppers		
compound	abbreviation	raw	boiled	grilled	raw	boiled	grilled
all-*trans*-neoxanthin		1.5 ± 0.1 a	N.D.[b]	0.2 ± 0.0 b	N.D.	0.2 ± 0.0 b	0.3 ± 0.0 a
cis-neochrome		N.D.	0.3 ± 0.0 a	0.2 ± 0.0 b	N.D.	N.D.	N.D.
all-*trans*-neochrome		N.D.	0.4 ± 0.0 a	0.2 ± 0.0 b	N.D.	N.D.	N.D.
cis-violaxanthin		0.8 ± 0.1	N.D.	N.D.	1.3 ± 0.0 c	2.4 ± 0.0 b	2.5 ± 0.0 a
all-*trans*-violaxanthin		5.1 ± 0.4	N.D.	N.D.	N.D.	N.D.	N.D.
all-*trans*-luteoxanthin		0.5 ± 0.0 a	0.1 ± 0.0 b	N.D.	0.1 ± 0.0 c	0.2 ± 0.0 b	0.2 ± 0.0 a
capsanthin 5,6-epoxide		N.D.	N.D.	N.D.	0.8 ± 0.0 c	1.7 ± 0.0 a	1.5 ± 0.0 b
cis-capsanthin		N.D.	N.D.	N.D.	1.5 ± 0.2 b	4.8 ± 0.2 a	4.8 ± 0.1 a
all-*trans*-capsanthin		N.D.	N.D.	N.D.	42.2 ± 0.9 b	46.0 ± 0.7 a	43.1 ± 0.9 a,b
all-*trans*-antheraxanthin		1.0 ± 0.2 a	0.3 ± 0.0 b	0.2 ± 0.0 b	4.6 ± 0.6 a	4.9 ± 0.1 a	4.8 ± 0.1 a
all-*trans*-lutein		3.2 ± 0.0 a	7.8 ± 0.4 b	8.1 ± 0.2 b	N.D.	N.D.	N.D.
all-*trans*-mutatoxanthin		N.D.	N.D.	N.D.	3.5 ± 0.2 b	4.2 ± 0.1 a	3.9 ± 0.1 a,b
all-*trans*-zeaxanthin		0.8 ± 0.0 a	0.4 ± 0.0 b	0.4 ± 0.0 b	7.2 ± 0.6 a	7.7 ± 0.0 a	7.5 ± 0.1 a
all-*trans*-β-cryptoxanthin		N.D.	N.D.	N.D.	2.8 ± 0.1 a	2.3 ± 0.0 a	3.0 ± 0.0 a
all-*trans*-α-carotene		0.2 ± 0.0 c	0.3 ± 0.0 b	0.4 ± 0.0 a	N.D.	N.D.	N.D.
all-*trans*-β-carotene		3.1 ± 0.4 b	3.5 ± 0.1 b	5.1 ± 0.0 a	21.1 ± 0.5 b	21.3 ± 0.3 b	25.4 ± 0.3 a
9-*cis*-β-carotene		0.3 ± 0.0 c	0.4 ± 0.0 b	0.5 ± 0.0 a	N.D.	N.D.	N.D.
chlorophyll *b*		23.3 ± 1.5 a	1.2 ± 0.1 b	4.7 ± 0.1 b	N.D.	N.D.	N.D.
chlorophyll *b*′		0.8 ± 0.2 b	2.6 ± 0.1 a	2.2 ± 0.1 a	N.D.	N.D.	N.D.
chlorophyll *a*		46.7 ± 2.2 a	1.4 ± 0.1 b	1.8 ± 0.0 b	2.0 ± 0.2	N.D.	N.D.
pheophytin *b*′		0.3 ± 0.0 b	2.4 ± 0.2 a	2.7 ± 0.0 a	N.D.	N.D.	N.D.
pheophytin *b*-*a*′		8.4 ± 1.0 b	13.1 ± 0.1 a	13.9 ± 0.1 a	N.D.	N.D.	N.D.
pheophythin *a*		N.D.	N.D.	6.5 ± 0.3	N.D.	N.D.	N.D.
antheraxanthin-laurate	AL	N.D.	N.D.	N.D.	5.3 ± 0.3 a,b	4.6 ± 0.1 b	5.8 ±0.0 a
antheraxanthin-myristate	AM	N.D.	N.D.	N.D.	8.9 ± 0.0 b	7.3 ± 0.1 c	10.1 ± 0.0 a
capsanthin-laurate	CL	N.D.	N.D.	N.D.	4.4 ± 0.1 b	4.2 ± 0.1 b	4.9 ± 0.2 a
capsanthin-myristate	CM	N.D.	N.D.	N.D.	20.6 ± 0.6 b	20.1 ± 0.1 b	23.4 ± 0.4 a
capsanthin-palmitate	CP	N.D.	N.D.	N.D.	5.2 ± 0.2 b	5.8 ± 0.1 b	7.7 ± 0.4 a
zeaxanthin-myristate	ZM	N.D.	N.D.	N.D.	3.6 ± 0.1 b	2.8 ± 0.1 c	4.1 ± 0.0 a
β-cryptoxanthin-laurate	βCL	N.D.	N.D.	N.D.	3.4 ± 0.1 a	2.6 ± 0.1 b	3.5 ± 0.1 a
capsanthin-dilaurate	CDL	N.D.	N.D.	N.D.	5.1 ± 0.2 c	6.7 ± 0.1 b	8.0 ± 0.1 a
capsanthin-laurate-myristate	CLM	N.D.	N.D.	N.D.	20.7 ± 0.6 b	19.8 ± 0.4 b	24.8 ± 0.3 a
capsanthin-dimyristate	CDM	N.D.	N.D.	N.D.	15.5 ± 0.6 b	13.6 ± 0.2 c	17.4 ± 0.2 a
capsanthin-palmitate-laurate	CPL	N.D.	N.D.	N.D.	3.3 ± 0.1 c	4.8 ± 0.1 b	5.4 ± 0.0 a
capsanthin-palmitate-myristate	CPM	N.D.	N.D.	N.D.	5.6 ± 0.1 a	4.5 ± 0.1 b	5.6 ± 0.1 a
capsanthin-myristate-palmitate	CMP	N.D.	N.D.	N.D.	4.1 ± 0.2 a	3.5 ± 0.1 b	3.8 ± 0.2 a,b
mutatoxanthin-palmitate-laurate	MPL	N.D.	N.D.	N.D.	1.2 ± 0.0 b	1.4 ± 0.0 a	1.4 ± 0.0 a
zeaxanthin-dilaurate	ZDL	N.D.	N.D.	N.D.	1.3 ± 0.0 a	1.0 ± 0.0 b	1.3 ± 0.0 a
zeaxanthin-laurate-myristate	ZLM	N.D.	N.D.	N.D.	1.5 ± 0.0 a	1.0 ± 0.0 b	1.5 ± 0.0 a

[a]Values represent the mean of three independent measurements ± the standard error. Values in the same row, for each stage of ripening, with different letters are significantly different ($p < 0.05$). [b]N.D., not detected.

same genotype but varying in ripening stage.[17] Saturated and unsaturated fats differentially affected the micellarization of the same carotenoids in different food matrixes.[24,26] Collectively, these studies suggest the existence of an interaction of food matrix and dietary fat on the micellarization of carotenoids and perhaps chlorophylls. The objective of this study was to determine the digestive stability (DS) and micellarization efficiency of individual carotenoids (free and esterified), chlorophylls, and chlorophyll derivatives of green and red Jalapeño peppers as a function of heat-processing style and fat type.

MATERIALS AND METHODS

Chemicals and Solvents. All reagents and solvents of analytical or HPLC grade were purchased from J. T. Baker (Baker-Mallinckrodt Inc., Mexico) or Sigma-Aldrich (St. Louis, MO, USA). High-purity carotenoid and chlorophyll standards (all-*trans*-α-carotene, all-*trans*-β-carotene, all-*trans*-lutein, all-*trans*-β-cryptoxanthin, all-*trans*-zeaxanthin, all-*trans*-capsanthin, all-*trans*-violaxanthin, and chlorophylls *a* and *b*) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Southcot Inc. (Chapel Hill, NC, USA), or Carotenature GmbH (Lupsingen, Switzerland).

Plant Material and Fat Types. Green and red Jalapeño peppers (cv. Marajá) were collected from a commercial orchard in Chihuahua, Mexico. The fruits of each ripening stage were divided into three samples containing 40 fruits each. One sample was boiled (94 °C/12.5 min) and another was grilled (210 °C/13.2 min) according to Cervantes-Paz et al.[2] The fruit to water ratio for boiling of peppers was 1:4 (v/v). The third sample was used in raw form. The moisture content of green and red peppers (raw and heat-processed) ranged from 89.6% to 90.8% and from 86.1% to 87.9%, respectively. Subsamples of 10 fruits from each treatment were individually homogenized to a puree and immediately subjected to *in vitro* digestion.

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Table 2. Micellarization (%) and Digestive Stability (%, in Parentheses) of Carotenoids, Chlorophylls, and Chlorophyll Derivatives from Raw and Heat-Processed Green Jalapeño Peppers after *in Vitro* Digestions without Fat (WF), with Soybean Oil (SO), and with Beef Tallow (BT)[a]

	raw			boiled			grilled		
compound	WF	SO	BT	WF	SO	BT	WF	SO	BT
all-*trans*-neoxanthin	7.6 ± 1.1 a (9.5 ± 1.4) a	5.3 ± 0.4 a (5.1 ± 0.6) a	5.0 ± 0.7 a (9.2 ± 1.6) a	N.D.[b] (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
cis-neochrome	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	79.3 ± 4.6 a (90.6 ± 4.1) a	71.7 ± 1.1 a (89.9 ± 2.6) a	78.8 ± 3.7 a (97.8 ± 4.2) a	123.6 ± 3.7 a (146.1 ± 6.8) a	96.7 ± 3.1 b (117.0 ± 3.6) b	120.2 ± 3.1 a (115.8 ± 6.4) b
all-*trans*-neochrome	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	22.8 ± 1.4 a (35.9 ± 1.7) a	16.5 ± 1.9 b (21.8 ± 0.1) a	20.1 ± 1.5 a,b (24.0 ± 0.6) b	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
cis-violaxanthin	23.2 ± 1.6 a (29.1 ± 2.9) a	25.1 ± 0.4 a (28.9 ± 3.2) a	24.3 ± 2.0 a (24.2 ± 2.8) a	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
all-*trans*-violaxanthin	3.8 ± 0.4 a (4.0 ± 0.7) a	3.7 ± 0.2 a (3.9 ± 0.2) a	3.0 ± 0.6 a (4.6 ± 0.2) a	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
all-*trans*-antheraxanthin	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	15.5 ± 2.5 a (17.5 ± 0.5) a	11.9 ± 1.0 a (16.0 ± 0.4) a	15.9 ± 0.9 a (19.3 ± 1.4) a	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
all-*trans*-lutein	62.6 ± 2.3 a (66.5 ± 3.3) a	71.3 ± 2.7 a (69.8 ± 1.3) a	45.2 ± 1.2 b (72.7 ± 0.8) a	66.9 ± 2.7 a (64.7 ± 2.8) a	49.1 ± 0.1 c (57.5 ± 1.4) a	56.8 ± 0.5 b (61.1 ± 0.8) a	56.7 ± 0.5 a (62.7 ± 1.1) a	48.5 ± 1.1 b (61.2 ± 0.8) a	56.8 ± 1.0 a (64.8 ± 0.8) a
all-*trans*-zeaxanthin	75.6 ± 5.1 a (82.9 ± 3.9) a	70.1 ± 6.6 a (69.7 ± 1.1) a	44.3 ± 2.7 b (69.3 ± 5.8) a	63.9 ± 4.2 b (72.8 ± 5.3) a	83.6 ± 3.0 a (70.3 ± 2.9) a	64.0 ± 5.8 b (79.4 ± 0.2) a	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
all-*trans*-α-carotene	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (93.2 ± 1.9) a	33.7 ± 2.4 a (52.6 ± 1.8) b	23.7 ± 1.5 b (58.5 ± 4.7) b	13.1 ± 1.0 a (43.9 ± 2.7) a	19.0 ± 3.1 a (45.7 ± 4.1) a	16.6 ± 3.0 a (46.2 ± 8.5) a
all-*trans*-β-carotene	28.5 ± 3.9 a (114.5 ± 1.3) a	36.7 ± 7.5 a (114.9 ± 11.4) a	22.7 ± 1.7 a (77.7 ± 4.0) b	21.2 ± 0.3 a (74.4 ± 6.2) a	26.0 ± 4.6 a (68.5 ± 3.3) a	27.3 ± 1.5 a (71.8 ± 5.7) a	14.0 ± 0.7 b (62.4 ± 3.4) a	26.1 ± 1.4 a (56.5 ± 2.0) a	24.8 ± 1.1 a (55.1 ± 3.2) a
9-*cis*-β-carotene	39.4 ± 1.4 b (107.5 ± 11.6) a	79.7 ± 11.9 a (134.6 ± 26.1) a	33.7 ± 4.1 b (114.9 ± 9.7) a	35.8 ± 2.4 a (80.5 ± 2.9) b	32.4 ± 0.5 a (93.3 ± 4.4) a,b	39.6 ± 1.9 a (109.0 ± 5.3) a	N.D. (81.8 ± 1.2) a	76.4 ± 1.9 a (83.6 ± 6.3) a	55.8 ± 1.1 b (109.8 ± 11.8) a
chlorophyll *b*′	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	12.8 ± 1.4 b (18.4 ± 0.2) a	11.4 ± 1.3 b (15.3 ± 0.1) b	17.1 ± 0.4 a (13.2 ± 1.2) b	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)
pheophytin *b*′	239.6 ± 20.9 c (1498.6 ± 31.1) a	667.8 ± 15.1 a (1426.2 ± 20.2) a	379.0 ± 20.7 b (1480.5 ± 33.3) a	35.2 ± 3.0 b (118.6 ± 3.5) a	55.9 ± 2.8 a (111.2 ± 4.2) a,b	49.1 ± 0.4 a (102.4 ± 0.4) b	29.0 ± 1.5 b (118.6 ± 6.1) a	28.3 ± 2.1 b (111.6 ± 1.8) a	39.2 ± 5.2 a (121.7 ± 7.2) a
pheophytin *b*-*a*′	59.7 ± 4.1 b (212.8 ± 4.7) a	98.1 ± 9.4 a (199.4 ± 6.3) a	59.8 ± 4.6 b (205.0 ± 4.9) a	32.3 ± 0.7 b (102.4 ± 3.8) a	43.5 ± 1.3 a (104.8 ± 0.9) a	38.2 ± 1.8 a (99.2 ± 0.1) a	30.5 ± 0.7 c (107.1 ± 3.5) a	39.5 ± 1.3 a (106.3 ± 2.8) a	34.7 ± 0.5 b (101.3 ± 0.7) a
pheophytin *a*	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	N.D. (N.D.)	24.0 ± 2.9 a (92.8 ± 2.4) a	22.7 ± 1.7 a (89.7 ± 0.5) a	27.3 ± 2.6 a (86.8 ± 1.9) a

[a]Values represent the mean of three individual measurements ± the standard error. Values in the same row, for each heat-processing style, with different lowercase letters are significantly different ($p < 0.05$). [b]N.D., not detected.

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Table 3. Micellarization (%) and Digestive Stability (%, in Parentheses) of Free Carotenoids from Raw and Heat-Processed Red Jalapeño Peppers after *in Vitro* Digestions without Fat (WF), with Soybean Oil (SO), and with Beef Tallow (BT)[a]

	raw			boiled			grilled		
compound	WF	SO	BT	WF	SO	BT	WF	SO	BT
cis-violaxanthin	39.2 ± 4.0 b (79.4 ± 1.4) a	42.2 ± 0.9 a,b (58.2 ± 2.3) b	53.7 ± 2.9 a (65.3 ± 2.5) b	27.2 ± 1.2 a (34.8 ± 1.2) a	21.9 ± 1.2 b (30.2 ± 2.2) a	21.9 ± 0.6 b (29.7 ± 1.6) a	16.6 ± 0.7 a (49.2 ± 2.9) a	14.5 ± 0.7 a (29.6 ± 2.5) b	17.0 ± 1.1 a (32.0 ± 3.4) b
all-*trans*-luteoxanthin	307.0 ± 5.6 a (361.2 ± 2.3) b	180.1 ± 8.0 b (272.3 ± 10.5) c	308.1 ± 8.2 a (441.2 ± 8.0) a	92.7 ± 3.0 a (198.5 ± 14.9) a	77.3 ± 2.9 b (232.9 ± 4.0) a	88.2 ± 3.6 a,b (124.3 ± 8.6) b	246.3 ± 7.5 a (294.7 ± 9.6) a	162.8 ± 6.0 b (275.6 ± 9.6) a	260.7 ± 7.1 a (254.5 ± 13.9) a
capsanthin 5,6-epoxide	144.7 ± 7.0 a (174.7 ± 5.0) a,b	82.7 ± 1.4 c (184.7 ± 2.8) a	122.6 ± 3.0 b (165.8 ± 4.9) b	57.2 ± 1.7 a (91.6 ± 5.1) b	42.3 ± 1.8 b (114.7 ± 0.8) a,b	43.5 ± 2.7 b (122.8 ± 12.7) a	77.6 ± 3.6 a,b (108.8 ± 6.6) a	68.6 ± 4.3 b (110.6 ± 3.9) a	86.8 ± 2.3 a (112.8 ± 5.0) a
cis-capsanthin	96.0 ± 6.1 b (85.3 ± 4.3) a	69.7 ± 1.1 c (72.9 ± 2.9) a	91.3 ± 0.6 a (86.4 ± 4.2) a	71.6 ± 1.6 a (82.1 ± 1.1) a	52.0 ± 0.9 c (78.7 ± 2.2) a	65.3 ± 1.0 b (78.9 ± 2.2) a	77.4 ± 0.9 a (89.7 ± 2.8) a	57.1 ± 2.4 b (78.8 ± 2.3) a	77.2 ± 0.8 a (88.4 ± 4.1) a
all-*trans*-capsanthin	81.9 ± 1.9 b (102.9 ± 0.7) a	90.4 ± 0.4 a (99.7 ± 0.9) a	87.0 ± 1.0 a,b (96.2 ± 2.1) a	75.3 ± 2.9 a (95.1 ± 0.7) a,b	65.0 ± 1.1 b (100.1 ± 0.9) a	71.8 ± 0.6 a,b (90.7 ± 3.3) b	86.7 ± 1.2 a (101.0 ± 1.0) a	70.8 ± 1.4 b (100.1 ± 3.0) a	84.2 ± 1.4 a (99.5 ± 3.4) a
all-*trans*-antheraxanthin	68.5 ± 2.3 a (89.0 ± 0.7) a	60.1 ± 1.5 b (64.1 ± 3.0) c	75.2 ± 1.5 a (77.6 ± 1.5) b	58.3 ± 1.5 a (99.8 ± 0.1) a	50.0 ± 1.7 b (88.0 ± 1.4) a,b	56.5 ± 0.9 a (79.7 ± 3.3) b	71.1 ± 1.8 b (100.3 ± 1.9) a	56.2 ± 2.4 c (87.0 ± 2.8) b	81.9 ± 0.9 a (95.6 ± 3.0) a,b
all-*trans*-mutatoxanthin	82.5 ± 3.1 a (93.6 ± 1.6) a	77.8 ± 1.3 a (86.5 ± 2.1) a	85.4 ± 1.0 a (93.4 ± 2.6) a	67.6 ± 2.6 a (88.6 ± 3.4) a	52.0 ± 0.8 b (80.9 ± 1.8) a	60.9 ± 0.5 a (84.3 ± 3.8) a	80.7 ± 2.0 a (100.4 ± 1.5) a	61.5 ± 2.0 b (85.5 ± 1.2) b	80.5 ± 0.7 a (95.3 ± 3.4) a,b
all-*trans*-zeaxanthin	72.9 ± 2.8 b (106.5 ± 2.7) a	95.6 ± 1.6 a (104.2 ± 0.4) a	87.9 ± 0.7 a (101.5 ± 2.6) a	56.1 ± 1.5 a (81.0 ± 1.6)	55.3 ± 2.0 a (84.8 ± 3.6) a	58.3 ± 1.1 a (75.9 ± 2.6) a	76.7 ± 0.7 a (91.2 ± 1.2) a	64.2 ± 2.0 b (82.9 ± 3.0) a	74.3 ± 0.5 a (86.8 ± 1.5) a
all-*trans*-β-cryptoxanthin	44.5 ± 1.3 c (85.2 ± 4.1) a	57.2 ± 1.5 b (85.7 ± 1.7) a	63.0 ± 0.6 a (77.3 ± 3.7) a	30.4 ± 2.7 c (73.1 ± 1.1) a	48.0 ± 2.1 b (77.9 ± 2.1) a	59.3 ± 0.6 a (70.4 ± 3.5) a	51.3 ± 2.1 b (70.6 ± 2.5) b	46.7 ± 1.2 b (80.8 ± 2.4) a	59.4 ± 2.0 a (77.9 ± 0.6) a,b
all-*trans*-β-carotene	7.2 ± 0.3 c (75.6 ± 0.7) b	34.8 ± 1.4 a (84.0 ± 0.7) a	28.3 ± 2.1 b (72.2 ± 1.6) b	8.7 ± 0.7 b (60.2 ± 1.1) b	25.6 ± 1.2 a (71.2 ± 1.8) a	25.7 ± 2.2 a (69.5 ± 1.7) a	8.1 ± 0.6 c (67.9 ± 2.3) a	18.1 ± 0.1 b (73.8 ± 3.4) a	30.2 ± 1.4 a (69.4 ± 4.1) a

[a]Values represent the mean of three individual measurements ± the standard error. Values in the same row, for each heat-processing style, with different lowercase letters are significantly different ($p < 0.05$).

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Soybean oil (SO) and beef tallow (BT) were used as sources of fat. The SO and BT differ highly in the level of saturation of their fatty acids. SO was obtained from a local grocery, while BT was extracted from beef fat by slight heating. Both fat types were cooked (114.3 ± 3.5 °C/7.5 ± 0.2 min) following traditional procedures. The fats were cooled at room temperature, blanketed with nitrogen gas, and stored at −70 °C until utilization.

Simulated *in Vitro* Digestion. The *in vitro* digestions were performed according to Garrett et al.[27] Reactions contained 2 g of pepper puree and 120 μL of SO or BT. This fat amount was similar to that typically used in other *in vitro* studies regarding carotenoid micellarization.[26,27] Control digestions did not contain exogenous fat. The gastric phase of the digestion process was performed at pH 2 in anaerobiosis. The reaction contained porcine pepsin at a final concentration of 0.4 mg/mL and was kept at 37 °C during 1 h under reciprocal shaking (95 rpm). The pH of the reaction was increased to 6 with sodium bicarbonate before addition of porcine pancreatin, porcine pancreatic lipase, and bile extract to final concentrations of 0.4, 0.4, and 2.4 mg/mL, respectively, to initiate the small intestinal phase of digestion. The porcine pancreatic lipase is able to efficiently cleave carotenoid esters from peppers.[7] The pH was increased to 7, and samples were incubated for 2 h at the same temperature and agitation as indicated above. After completion of the digestion process, aliquots of chyme were collected and stored at −70 °C. Other aliquots of chyme (10 mL) were centrifuged (15000*g*/20 min/4 °C) (centrifuge Allegra 64R, Beckman Coulter Inc., IN, USA) to separate the micellar fraction from undigested materials. Pilot studies were conducted to determine appropriate conditions for centrifugation to facilitate recovery of carotenoid-rich mixed micelles.[9] The micellar fraction was recovered, filtered (0.22 μm pore size; Millipore Corp., MA, USA) to remove suspended particles, and stored under nitrogen gas at −70 °C until analysis. Aliquots of puree, chyme, and micellar fraction were analyzed for pigment composition to determinate the DS and bioaccessibility of pigments. DS represents the percentage of individual pigments in the test food recovered in the digestate, whereas bioaccessibility reflects the percentage of individual pigments in the micellar fraction, as defined previously.[4,9,18]

Pigment Analysis by HPLC-DAD-MS. Pigments from puree (4 g) were sequentially extracted by methanol, acetone, and hexane, according to our previously reported methodology.[2] Pigments in chyme and the micellar fraction (3 mL) were extracted into a mixture of petroleum ether and acetone (4 mL, 2:1 v/v).[17] Solvents were evaporated with a stream of gas nitrogen, and the residue was reconstituted in acetone (2 mL), filtered (0.45 μm pore size; Millipore Corp., Bedford, MA, USA), and injected (20−100 μL) into an Agilent 1200 Series HPLC system (Agilent, Palo Alto, CA, USA), equipped with a diode array detector. The separation of pigments was carried out on a C_{30} reversed-phase column (4.6 × 150 mm, 3 μm) (YMC Inc., Milford, MA, USA) at 15 °C. The mobile phase was composed of water, methanol, and methyl *tert*-butyl ether according to Cervantes-Paz et al.[2] Each pigment extract was also subjected to MS analysis using a 6210 time-of-flight (TOF) mass spectrometer (Agilent Inc., Palo Alto, CA, USA) equipped with an atmospheric pressure chemical ionization source (APCI+). The operating conditions for the MS system have been described previously.[2] The identification of pigments was carried out by cochromatography with pure standards and analysis of UV−vis (λ_{max}, %III/II) and MS (m/z = 100−1200) spectra. Quantitative analysis was performed by calibration curves constructed with pure compounds. *Cis* isomers of carotenoids and chlorophyll derivatives were quantified as all-*trans* carotenoids or their precursors, respectively. Quantification of pheophytins and chlorophylls *a* and *a*′ was performed at λ = 430 nm. Chlorophylls *b* and *b*′ as well as the different forms of capsanthin were quantified at λ = 470 nm. Violaxanthin forms were monitored at λ = 440. The rest of the carotenoids were monitored at λ = 452 nm.

Data Analysis. All experiments and measurements were made in triplicate. Data are presented as the mean ± the standard error. Data from green and red peppers were separately analyzed. The DS and micellarization values for each compound were analyzed using a completely randomized design with a factorial arrangement, considering the style of heat-processing of peppers and fat type as the factors. The Tukey-Kramer honestly significant difference test was also applied (α = 0.05). The JMP (SAS Institute, Inc., Cary, NC, USA) statistical software was used.

RESULTS AND DISCUSSION

Bioaccessible Pigments. The profile of pigments of tested peppers was composed of 68 compounds, 67 of which we recently characterized in the same food matrix.[2] The additional compound was identified as zeaxanthin-dipalmitate on the basis of its UV−vis (λ_{max} = 429, 453, and 478 nm) and MS (m/z at 1045, 789, and 533) characteristics. In general, the effect of heat-processing on the concentration of individual pigments was independent of moisture content of peppers and similar to that recently reported in our previous study of raw and heat-processed peppers from the same genotype.[2] However, the concentration of some pigments in the peppers that were used in this study was slightly higher than that found previously, likely due to crop variability. Only 38 of the detected pigments were incorporated into the mixed micelles, and therefore only these compounds were considered in this study. The concentration of these compounds in the pepper purees is listed in Table 1. The concentration of chlorophylls *a* and *b* in peppers, which were not micellarized, are also given in Table 1 since they were used to calculate values of DS and micellarization for total chlorophylls (sum of precursor and derivatives). Six *cis* isomers of xanthophylls, chlorophyll *a*, chlorophyll *b*, α-cryptoxanthin, auroxanthin, nine carotenoid diesters, and 11 unidentified pigments were not micellarized. These carotenoids were detected at very low concentrations, while chlorophylls *a* and *b* were completely degraded to pheophytins during digestion, explaining their absence in the micelles.[15,22]

Effect of Heat-Processing Style and Fat Type on Digestive Stability of Free Pigments. The DS of individual free pigments was highly variable among them (Tables 2 and 3), finding that besides the effect of interaction between fat type and processing style of peppers, the pigment structure also played an important role in their DS. In general, the DS of the majority of free carotenoids was greater with raw than with processed peppers, except for all-*trans*-α-carotene and all-*trans*-antheraxanthin. Similarly, the DS of carotenoids from carrots was greater with raw than with boiled or steamed carrots.[23,28] It has been suggested that cells from some raw vegetable tissues are more susceptible to breaking during digestion than cooked cells, increasing the release of carotenoids.[28] On the other hand, dietary fat either reduced or did not alter the DS of the majority of free xanthophylls, while DS of β-carotene isomers was increased by the addition of fat. These results demonstrate the influence of the polarity of carotenoids and dietary fat on DS of these pigments since the DS of carotenes (nonpolar) and xanthophylls (polar) was increased and reduced by fat, respectively. This effect could be mediated by the localization of carotenoids into the lipid droplets according to their polarity. The xanthophylls are located on the surface, while the carotenes are located in the core of lipid droplets,[13] with xanthophylls being more exposed to the degradative conditions of the digestive medium than carotenes.

BT increased the DS of capsanthin-5,6-epoxide with heat-treated peppers but caused the opposite with raw peppers. BT also increased the DS of 9-*cis*-β-carotene. SO tended to increase the DS of all-*trans*-β-carotene in digestions with red peppers, although in some cases statistical differences were not found.

We did not observe a specific tendency for DS of this carotene with green peppers as a function of fat type. These results demonstrate the effect of the interaction of heat-processing of peppers (food matrix effect) and the fat type on DS of free carotenoids.

The *cis* isomers of violaxanthin, neochrome, and *β*-carotene showed higher DS than their all-*trans* forms in any treatment, which could be a consequence of the formation of these compounds by *cis* isomerization of carotenoids in the acid conditions of the chyme.[14] These findings contrast with those reported for *cis* and all-*trans* *β*-carotene from beadlets and cassava, where both isomers showed similar DS.[22,29] In our study, the DS of some carotenoids (*cis*-neochrome, capsanthin-5,6-epoxide, luteoxanthin, 9-*cis*-*β*-carotene, and all-*trans*-*β*-carotene) exceeded 100%, indicating that the formation/release of these compounds occurred during the digestion process. Capsanthin-5,6-epoxide might be formed by the oxidation of capsanthin, as suggested previously.[30] Luteoxanthin could be formed from violaxanthin by the isomerization of the 5,6-epoxide groups into 5,8-furanoid groups in the acidic medium of the chyme.[31] Neochrome can be formed from neoxanthin.[31,32] The DS for some of these compounds has been previously reported as the sum of precursors and derivatives, avoiding DS values higher than 100% but underestimating information for individual compounds. Thus under this consideration, the DS of violaxanthin (violaxanthin + luteoxanthin) ranged from 6.8% to 7.7% with green peppers and from 38.0% to 96.5% with red fruits, respectively. Similarly, the DS of neoxanthin (neoxanthin + *cis*-neochrome + all-*trans*-neochrome) ranged from 5.2% to 58.0% in digestions with green peppers. We were unable to calculate DS values for capsanthin considering precursors and derivatives due to the diversity of capsanthin forms that were detected. The 9-*cis*-*β*-carotene could be formed from all-*trans*-*β*-carotene under the acidic conditions of the chyme.[14] The high DS of *β*-carotene with raw green peppers could be caused by an increased extractability of this carotene, driven by the hydrolysis of *β*-carotene−protein complexes under the acid conditions of the gastric medium, as demonstrated previously for green tissues.[33]

Chlorophylls and their derivatives were observed only in green peppers and their digestates (Tables 1 and 2). Chlorophylls *a* and *b* were completely degraded during the *in vitro* digestions, leading to high concentrations of pheophytins *a* and *b*, which showed high DS values. The instability of chlorophylls in the gastric chyme has been demonstrated previously.[15] Interestingly, chlorophyll *b*′ was not completely degraded by heating and showed some resistance to digestive conditions (13.2−18.4%) with boiled green peppers. Some studies have demonstrated that chlorophyll *b* and their derivatives are more stable than the *a* series.[16,34] The DS of all pheophytins was typically above 100%, except for pheophytin *a*, which presented DS values from 86.8% to 92.8%. The DS of chlorophylls and their derivatives was diminished by heat-processing and was altered only by fat with boiled peppers. The DS of chlorophylls can also be expressed as the sum of precursors and derivatives. Under this mode of data presentation, the DS of total chlorophylls (chlorophylls + pheophytins) varied between 26.8%, in digestions with raw peppers, and 74.0−88.4% with heat-processed fruits. The DS of chlorophylls and their derivatives had not been previously determined in peppers.

Effect of Heat-Processing Style and Fat Type on Digestive Stability of Carotenoid Monoesters. Seven carotenoid monoesters were observed in red peppers and their digestates. The abbreviated name and DS for these compounds are shown in Tables 1 and 4. The DS of monoesters followed a more homogeneous behavior as a function of heat-processing and fat type than that of free pigments. This suggests that the strong effect of the chemical structure of free carotenoids on their DS is diminished by the fatty acid moiety in the carotenoid monoesters. The DS of CM, CP, and *β*CL was significantly influenced by the interaction of heat-processing and fat type, while the DS for the other monoesters was not determined by this interaction. Interestingly, the DS of myristates of capsanthin and antheraxanthin always tended to be greater than those of laurates and palmitates. The DS of CL also was always greater than that of CP. Similarly, *β*-cryptoxanthin-myristate from citrus juices had greater DS values than *β*CL.[35] To date, only the DS of myristates and laurates of *β*-cryptoxanthin have been compared.[35] Considering the elution time of carotenoid myristates and laurates, which is based on their polarity, we infer that myristates were less polar, being placed in the core of lipid droplets, and therefore they were more protected by fat from the degradative gastrointestinal medium and from hydrolytic enzymes than laurates, as reported for free carotenoids varying in polarity.[13] However, under this consideration a higher DS of carotenoid palmitates was expected, which was not observed. This suggests that the relationship between DS and polarity of carotenoids may exist only in a range of polarity values or that other factors are involved in this phenomenon. The DS of monoesters was greater with raw than with cooked peppers, as described for free carotenoids. The effect of heat-processing on the DS of carotenoids seems to be food matrix dependent.[6,23] The SO and BT promoted the DS of carotenoid monoesters by 6−37%, probably by a protective effect of fat.[13] This fat-dependent increase was greater in digestions with raw and boiled than with grilled peppers, indicating the effect of interaction between heating and fat type. The DS for all monoesters was greater with SO than with BT, although in some cases statistical differences were not observed. This indicates that the protective effect of fat was modulated by the solubility of carotenoids in these fat types, as demonstrated previously.[13] We infer that carotenoid monoesters were more soluble in SO than in BT, conferring SO a higher protection of these compounds.

Effect of Heat-Processing Style and Fat Type on Digestive Stability of Carotenoid Diesters. Nine carotenoid diesters were observed in red peppers and their digestates. The abbreviated name and DS for these compounds are shown in Tables 1 and 4. The DS of carotenoid diesters also followed a homogeneous behavior as a function of heating of peppers and fat type, again demonstrating that the variability of DS for free carotenoids is diminished by the fatty acid moieties. The DS of the majority of diesters tended to be greater in digestions with raw than with heat-treated peppers, as described for free and monoesterified carotenoids, except for CPM and CMP, where the opposite was observed. Fat only altered slightly the DS of CDM, CPM, and ZLM.

In general, the DS of free all-*trans*-capsanthin, all-*trans*-antheraxanthin, all-*trans*-zeaxanthin, all-*trans*-mutatoxanthin, and all-*trans*-*β*-cryptoxanthin was statistically greater than their respective mono- and diesterified forms in all treatments. Interestingly, the proportion of free all-*trans*-capsanthin, all-*trans*-antheraxanthin, all-*trans*-zeaxanthin, all-*trans*-mutatoxanthin, and all-*trans*-*β*-cryptoxanthin, relative to their esterified forms, was 7.3−10.3%, 3.5−18.4%, 10.0−15.0%, 4.5−12.8%, and 9.1−16.8%, respectively, higher in digestates than in pepper samples, indicating that the increased DS of the free forms was favored by the hydrolysis of carotenoid esters.

Table 4. Micellarization (%) and Digestive Stability (%, in Parentheses) of Mono- and Diesterified Xanthophylls from Raw and Heat-Processed Red Jalapeño Peppers after *in Vitro* Digestions without Fat (WF), with Soybean Oil (SO), and with Beef Tallow (BT)[a]

	raw			boiled			grilled		
compound	WF	SO	BT	WF	SO	BT	WF	SO	BT
				Monoesterified Xanthophylls					
antheraxanthin-laurate	4.6 ± 0.4 c (52.6 ± 2.1) b	23.9 ± 0.5 a (69.4 ± 1.4) a	17.4 ± 0.1 b (59.4 ± 1.3) b	N.D.[b] (39.0 ± 0.8) b	20.5 ± 1.6 a (58.3 ± 2.5) a	20.8 ± 0.5 a (53.5 ± 3.4) a,b	10.4 ± 0.3 b (49.7 ± 1.2) b	12.8 ± 1.0 b (61.5 ± 2.5) a	18.6 ± 1.9 a (54.1 ± 1.0) a,b
antheraxanthin-myristate	3.2 ± 0.1 b (61.7 ± 4.4) a	25.6 ± 2.2 a (73.5 ± 0.6) a	N.D. (63.2 ± 1.7) a	5.2 ± 0.5 c (49.3 ± 2.6) b	23.9 ± 0.9 a (75.7 ± 6.4) a	18.9 ± 1.4 b (61.0 ± 2.0) a,b	7.1 ± 0.7 b (47.6 ± 1.2) c	15.3 ± 0.5 a (68.5 ± 0.8) a	17.6 ± 1.0 a (57.8 ± 2.2) b
capsanthin-laurate	6.2 ± 0.3 c (57.9 ± 0.7) b	45.2 ± 1.7 a (90.3 ± 1.3) a	24.6 ± 1.1 b (78.6 ± 5.1) a	5.3 ± 0.4 c (61.7 ± 3.0) a	33.0 ± 0.4 a (77.8 ± 4.0) a	31.1 ± 0.1 b (65.3 ± 0.6) a	7.8 ± 0.1 b (56.8 ± 0.6) c	23.9 ± 0.8 a (78.9 ± 1.9) a	26.2 ± 1.3 a (67.5 ± 1.2) b
capsanthin-myristate	N.D. (77.6 ± 2.7) c	52.1 ± 3.4 a (100.0 ± 1.3) a	35.4 ± 1.1 b (89.4 ± 1.2) b	6.2 ± 0.5 b (66.8 ± 1.9) b	40.7 ± 0.9 a (86.8 ± 2.2) a	35.9 ± 2.6 a (80.0 ± 1.2) a	15.6 ± 1.0 c (69.0 ± 1.1) b	27.7 ± 1.1 b (81.2 ± 1.8) a	35.5 ± 2.1 a (78.5 ± 1.1) a
capsanthin-palmitate	N.D. (48.0 ± 2.1) b	33.5 ± 0.5 a (72.4 ± 5.1) a	20.8 ± 0.1 b (55.4 ± 1.9) b	N.D. (51.7 ± 2.5) a,b	22.9 ± 0.1 a (60.3 ± 0.0) a	18.1 ± 0.8 b (44.7 ± 3.2) b	4.2 ± 0.5 b (37.1 ± 1.9) a	14.9 ± 0.2 a (42.4 ± 1.7) a	17.9 ± 2.1 a (42.9 ± 0.8) a
zeaxanthin-myristate	N.D. (59.7 ± 3.1) b	41.2 ± 1.9 a (85.1 ± 0.3) a	28.4 ± 1.5 b (75.7 ± 3.9) a	N.D. (48.4 ± 1.6) b	33.5 ± 0.2 a (74.8 ± 3.1) a	27.6 ± 1.4 b (61.2 ± 3.9) b	5.6 ± 2.8 c (48.6 ± 1.4) c	20.0 ± 0.5 b (68.2 ± 0.5) a	26.0 ± 0.9 a (56.9 ± 1.2) b
β-cryptoxanthin-laurate	N.D. (44.9 ± 1.2) b	22.2 ± 2.1 a (74.8 ± 4.7) a	15.3 ± 1.1 b (52.8 ± 1.6) b	N.D. (36.9 ± 3.1) b	17.5 ± 1.0 a (57.5 ± 4.9) a	21.4 ± 3.2 a (56.7 ± 3.1) a	5.2 ± 0.9 c (42.7 ± 1.9) c	8.9 ± 0.7 b (58.8 ± 1.1) a	15.5 ± 0.2 a (50.5 ± 0.3) b
				Diesterified Xanthophylls					
capsanthin-dilaurate	N.D. (72.3 ± 1.1) a	12.6 ± 0.5 a (71.3 ± 3.0) a	6.8 ± 0.2 b (69.0 ± 0.3) a	N.D. (64.2 ± 3.1) a	9.1 ± 0.6 a (67.6 ± 2.4) a	8.0 ± 0.6 a (63.7 ± 1.9) a	3.9 ± 0.6 b (67.9 ± 0.6) a	5.2 ± 0.4 b (67.3 ± 2.4) a	7.8 ± 0.7 a (63.5 ± 0.3) a
capsanthin-laurate-myristate	N.D. (82.3 ± 1.5) a	16.2 ± 0.5 a (75.4 ± 2.4) a	7.6 ± 0.1 b (75.4 ± 0.9) a	N.D. (70.9 ± 2.2) a	11.5 ± 0.9 a (68.9 ± 0.8) a	11.6 ± 0.8 a (66.4 ± 1.4) a	3.7 ± 0.2 c (72.8 ± 0.5) a	7.8 ± 0.3 b (66.2 ± 2.4) a	11.1 ± 0.5 a (67.5 ± 0.9) a
capsanthin-dimyristate	N.D. (66.0 ± 0.7) a	10.5 ± 0.2 a (62.2 ± 2.7) a,b	7.1 ± 0.3 b (59.4 ± 1.1) b	N.D. (52.7 ± 2.5) a	9.6 ± 0.8 a (54.4 ± 0.9) a	7.0 ± 0.8 a (52.9 ± 1.2) a	3.3 ± 0.8 b (57.0 ± 1.0) a	4.6 ± 0.1 a,b (55.4 ± 1.0) a	8.8 ± 1.8 a (51.0 ± 0.9) b
capsanthin-palmitate-laurate	N.D. (72.6 ± 1.9) a	11.9 ± 0.3 a (69.1 ± 3.1) a	5.4 ± 1.1 b (67.1 ± 0.5) a	N.D. (34.8 ± 0.6) a	N.D. (37.5 ± 1.2) a	N.D. (37.2 ± 1.1) a	N.D. (35.9 ± 0.6) a	N.D. (37.34 ± 0.7) a	N.D. (35.1 ± 1.5) a
capsanthin-palmitate-myristate	N.D. (61.3 ± 1.1) a	14.4 ± 0.7 a (58.3 ± 0.7) a	9.1 ± 0.5 b (57.5 ± 2.4) a	N.D. (56.2 ± 4.7) b	11.9 ± 0.5 b (68.7 ± 2.1) a	13.1 ± 0.0 a (68.1 ± 2.6) a	8.8 ± 0.7 a (70.2 ± 2.9) a	N.D. (63.4 ± 1.5) a	11.0 ± 0.9 a (61.6 ± 1.6) a
capsanthin-myristate-palmitate	N.D. (58.2 ± 3.3) a	18.6 ± 0.8 a (58.5 ± 2.2) a	14.0 ± 0.7 b (59.8 ± 2.6) a	N.D. (91.2 ± 1.3) a	23.2 ± 1.7 a (82.9 ± 3.7) a	13.0 ± 0.9 b (82.5 ± 3.4) a	19.1 ± 1.9 a (75.5 ± 4.1) a	N.D. (59.9 ± 3.7) a	21.9 ± 1.6 a (64.0 ± 3.9) a
mutatoxanthin-palmitate-laurate	N.D. (73.0 ± 1.1) a	17.8 ± 2.2 a (75.1 ± 2.7) a	N.D. (69.0 ± 0.5) a	N.D. (50.1 ± 3.2) a	N.D. (49.8 ± 0.2) a	N.D. (52.9 ± 3.6) a	13.9 ± 0.3 a (45.6 ± 2.5) a	N.D. (55.1 ± 2.5) a	10.2 ± 2.6 a (48.2 ± 1.6) a
zeaxanthin-dilaurate	N.D. (63.7 ± 2.5) a	N.D. (64.9 ± 1.4) a	N.D. (63.6 ± 0.9) a	N.D. (65.0 ± 3.2) a	13.2 ± 0.1 (62.4 ± 2.6) a	N.D. (64.3 ± 2.0) a	13.1 ± 1.5 (57.2 ± 3.3) a	7.8 ± 0.2 (65.3 ± 2.0) a	10.5 ± 3.3 (60.8 ± 1.2) a
zeaxanthin-laurate-myristate	N.D. (88.6 ± 3.0) a	30.6 ± 1.9 a (72.3 ± 2.2) b	N.D. (77.7 ± 3.4) a,b	N.D. (55.0 ± 5.6) a	N.D. (51.1 ± 2.3) a	N.D. (50.2 ± 4.1) a	N.D. (48.2 ± 4.7) a	N.D. (55.2 ± 0.6) a	26.3 ± 3.3 (51.0 ± 3.3) a

[a]Values represent the mean of three individual measurements ± the standard error. Values in the same row, for each heat-processing style, with different lowercase letters are significantly different ($p < 0.05$). [b]N.D., not detected.

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Effect of Heat-Processing Style and Fat Type on Micellarization of Free Pigments. The micellarization efficiency for free pigments with green and red peppers is shown in Tables 2 and 3. Our values of micellarization for free all-*trans*-lutein (45.2−71.3%), all-*trans*-zeaxanthin (44.3−95.6%), all-*trans*-β-cryptoxanthin (30.4−63.0%), and all-*trans*-β-carotene (8.1−36.7%) were in the typical ranges reported (36.3−106.2%, 47−106.9%, 20−112.8%, and 6.2−76.7%, respectively) for these carotenoids from raw and processed peppers (sweet and pungent).[3−6,9] The α-cryptoxanthin was not micellarized, contrasting with the results of Granado-Lorencio et al., who reported bioaccessibility values of up to 100% for this xanthophyll in red peppers.[9] The study of the bioaccessibility of pepper carotenoids has been generally limited to these free carotenoids. The bioaccessibility of 9-*cis*-β-carotene, neoxanthin, antheraxanthin, and violaxanthin (*cis* and all-*trans*) has been determined in some food matrixes, except in peppers. In our study, the micellarization of 9-*cis*-β-carotene (32.4−79.7%) was higher than that previously reported in the literature (14.4−48.3%) for this carotene from *Dunaliella salina*, broccoli, kale, spinach, and savoy cabbage.[29,36] The bioaccessibility of all-*trans*-α-carotene (13.1−33.7%) was lower than that of its β counterpart, contrasting with the results of Hornero-Méndez and Mínguez-Mosquera.[23] According to our results, the bioaccessibility of neoxanthin from Jalapeño peppers (5.0−7.6%) is considerably lower than that reported for this compound (24−30.1%) from spinach.[31,32] The micellarization values for antheraxanthin were high (11.9−81.9%), contrasting with the results of Cha et al., who found that the antheraxanthin from *C. ellipsoidea* is not micellarized because it is totally transformed into its 5,8-furanoid derivative during the digestion.[37] Our bioaccessibility values for *cis*- (14.5−53.7%) and all-*trans*-violaxanthin (3.0−3.8%) were similar to those reported for these xanthophylls (4.3−48.5%) from butternut squash, grapefruit, mangoes, spinach, and papaya.[32,38] The instability of violaxanthin under gastrointestinal conditions has been demonstrated, its bioaccessibility being determined in some cases as the sum of it and its 5,8-furanoid derivatives (auroxanthin and luteoxanthin).[32] Finally, the micellarization of some free carotenoids was determined in this study for the first time, including that of all-*trans*-mutatoxanthin (52.0−85.4%), all-*trans*-luteoxanthin (77.3−308.1%), all-*trans*-neochrome (16.5−22.8%), *cis*-neochrome (71.7−123.6%), all-*trans*-capsanthin (65.0−90.4%), *cis*-capsanthin (52.0−96.0%), and capsanthin 5,6-epoxide (42.3−144.7%). The considerably high micellarization of luteoxanthin and neochrome could be a consequence of their formation during the digestion by the transformation of violaxanthin and neoxanthin in these compounds.[31,32] The bioaccessibility of violaxanthin and neoxanthin has been previously determined as the sum of micellarized precursors and their derivatives. Under this consideration, the micellarization values for violaxanthin (violaxanthin + luteoxanthin) were 6.2−72.5% with raw peppers and 26.3−38.4% with heat-processed peppers, while the bioaccessibility of neoxanthin (neoxanthin + *cis*-neochrome + all-*trans*-neochrome) from raw and processed peppers was 5.1−7.6% and 37.7−48.6%, respectively. This study also demonstrated that free capsanthin is micellarized, although the opposite has been hypothesized.[11] Capsanthin has been detected in human plasma.[10]

The effect of pepper processing on micellarization of free pigments was not clearly observed in digestions with green peppers, probably because only a few compounds from raw and heat-treated peppers were micellarized, limiting the number of possible comparisons. This could also be caused by differences in the food matrix of green and red peppers. Castro et al. demonstrated that cells of green and red peppers show different susceptibility to disruption by heating and high-pressure, probably by differences in solubility and resistance to heating of their pectins.[20] Pectins from the same fruit but at different ripening stages alter differentially the micellarization of carotenoids.[17] The micellarization of the majority of free pigments from red peppers as a function of heat-processing was clearly in the order raw > grilled > boiled. These findings contrast with those of many other studies, where the bioavailability/bioaccessibility of carotenoid pigments is increased by cooking.[6,18,23,36] However, there are reports where the opposite has been observed. Ryan et al. demonstrated that heat-processing did not increase the micellarization of lycopene, lutein, and β-cryptoxanthin of peppers, hypothesizing that cooking does not always destroy the physical barriers that limit the release of carotenoids from vegetable cells.[6] Tydeman et al. suggested that the breaking of the organelles' membranes during digestion leads to the formation of intracellular droplets with materials from disrupted membranes that are able to capture carotenoids during digestion, impeding their absorption.[28] In another study, Tydeman et al. indicated that cooked cells also tend to separate intact, leaving their content encapsulated.[39] On the other hand, the majority of free xanthophylls of green and red peppers had their highest bioaccessibility in digestions without fat (WF) or with BT. Borel et al. demonstrated that xanthophylls are mostly incorporated into the emulsion when a stabilizer is present.[13] It has been demonstrated that unsaturated fatty acids reduce the stability of emulsions of fat in water.[40] The less polar carotenoids such as all-*trans*-β-cryptoxanthin and β-carotene isomers showed a greater partition within micelles by the addition of any fat type, but mainly with SO. Similarly, the addition of avocado oil to a salad increased significantly the postprandial levels of β-carotene but not those of lutein.[41] Goltz et al. demonstrated that the absorption of the low-polar lycopene was higher when it was coconsumed with two vegetable oils (rich in mono- and polyunsaturated fatty acids) instead of butter (rich in saturated fatty acids).[26] The bioaccessibility of xanthophylls was ∼8 times greater than that of β-carotene in digestions with red peppers (raw or cooked) WF, while in digestions with fat (SO or BT) the bioaccessibility of xanthophylls was only ∼2.5 times greater than that of β-carotene. Similar findings have been reported for other food matrixes.[5,6,42] This phenomenon is mediated by the differential localization of carotenoids according to their polarity into the emulsified lipid droplets.[13]

Fat increased the bioaccessibility of chlorophylls and their derivatives; however this variable was modulated by the interaction of the two factors. To date the effect of fat on chlorophyll micellarization has not been studied. Chlorophyll *b*′ was the unique micellarized chlorophyll (11.4−17.1%). Chlorophyll derivatives were highly micellarized. Pheophytins *b*-*a*′ and *b*′ showed their highest bioaccessibility (98.1% and 667.8%, respectively) in digestions with raw peppers and SO. The bioaccessibility of chlorophyll *b* derivatives is also higher with fresh spinach than with heat- and acid-treated spinach.[15] However, the opposite has been reported for chlorophyll derivatives from peas.[16] The high bioaccessibility of pheophytin *b*′ was probably derived from its formation by the degradation of chlorophylls *b* and *b*′ and from its high polarity. This is the first time that micellarization values are reported for chlorophyll

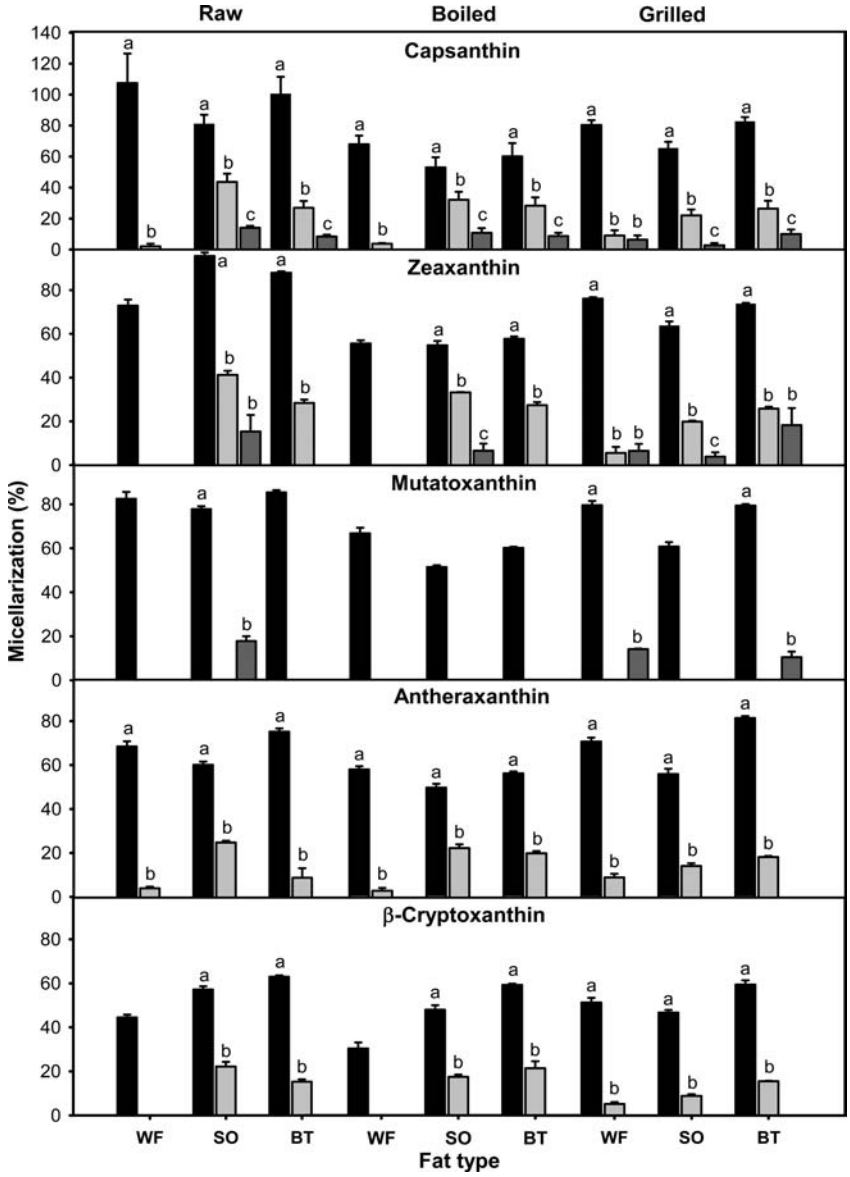

Figure 1. Micellarization of free (black bars), monoesterified (light gray bars), and diesterified (dark gray bars) xanthophylls from red peppers in digestions without fat (WF), with soybean oil (SO), and with beef tallow (BT). Data represent the mean of three replicates ± the standard error of the mean (narrow bars). Significant differences between the values of free and mono- and diesterified carotenoids in each treatment (processing style and fat type) are indicated with different letters ($p < 0.05$).

b′ and pheophytin *b*′. The individual bioaccessibility of pheophytins *b* and *a*′ could not be determined because they coeluted. The bioaccessibility of chlorophyll *a* derivatives from spinach have been estimated to be 10.0−40.0%.[15] Pheophytin *a* was observed only in micelles from digestions with grilled peppers, and its bioaccessibility was unaffected by the presence of either SO or BT. The bioaccessibility of chlorophylls and their derivatives from other food matrixes has been previously expressed as the sum of these compounds. Under this consideration, the micellarization of total chlorophyll pigments (chlorophylls + pheophytins) ranged from 7.1% to 8.5% with raw peppers and from 20.9% to 38.4.9% with processed fruits.

Effect of Heat-Processing Style and Fat Type on Micellarization of Carotenoid Monoesters. The micellarization efficiency for carotenoid monoesters is listed in Table 4, ranging from 0 to 52.1%. These values are similar to those reported (13.7−44.0%) for a limited number of carotenoid monoesters (zeaxanthin and *β*-cryptoxanthin) from peppers, butternut squash, wolfberries, and citrus juices.[8,35] In this study, the individual bioaccessibility of many carotenoid monoesters from peppers is given. The factorial analysis showed that the monoesters' bioaccessibility was determined by the interaction of heat-processing of peppers and fat type; however, the micellarization of these compounds was clearly favored by the addition of any fat type. The micellarization efficiency for monoesters from raw and boiled peppers followed the descending order SO > BT > WF. The micellarization of monoesters with these heat treatments was 10.3−14.5 times greater in the presence of fat than WF. Similarly, the levels of circulating lutein were increased by the consumption of a high-fat (unsaturated) diet rich in esterified lutein.[43] Heat-processing modulated the micellarization of monoesters in digestions with SO (raw > boiled > grilled). All carotenoid monoesters showed the highest micellarization values in digestions of raw peppers

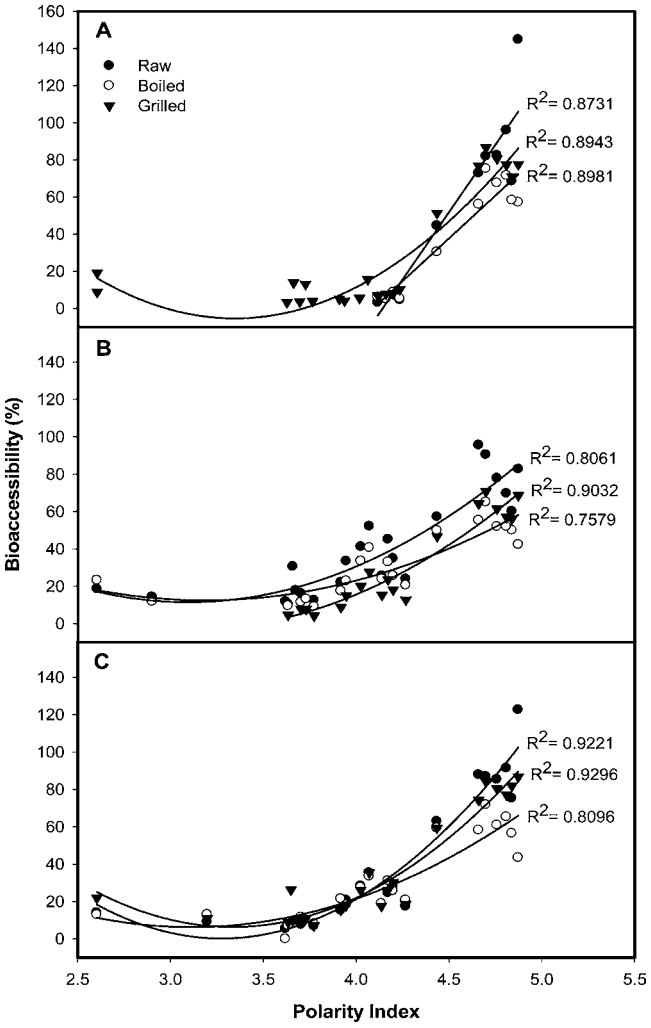

Figure 2. Relationship between the micellarization efficiency (individual values) of free and esterified carotenoids from red Jalapeño peppers and the polarity index of the mobile phase at their elution time. Graphics represent results of digestions reactions without fat (A), with soybean oil (B), and with beef tallow (C). Correlation coefficients (R^2) are given for each treatment.

with SO. The effect of fat type on monoester micellarization with grilled peppers was different from that described above for raw and boiled peppers since BT caused a greater micellarization than SO, demonstrating the interaction effect of the two factors. In these reactions (grilled peppers), the micellarization of monoesters was increased only 2.2−2.8 times by fat. Additionally, grilling favored the micellarization of all monoesters in the absence of fat, while in digestions of raw or boiled peppers WF the number of micellarized monoesters was low, indicating the effect of heat-processing on the change of the food matrix effect. Similarly, Cilla et al. demonstrated that the micellarization of carotenoids from heat- and high-pressure-treated juices was different between animal (milk fat) and vegetal fats (soybean).[44] Heat-processing did not alter the micellarization of monoesters in digestions with BT. βCL, CP, and ZM were not micellarized with raw and boiled red peppers WF. Interestingly, these compounds showed the highest retention times in the group of monoesters in the HPLC-MS analysis, an indication of their low polarity, and their micellarization values were very low (0−5.6%) in the treatments WF, suggesting that fat has a greater role in the micellarization of the least polar carotenoid monoesters. Borel et al. also demonstrated that the fat hydrolysis is more required for the micellarization of nonpolar than for polar carotenoids.[13] On the other hand, our data suggest an effect of the fatty acid moiety on the micellarization efficiency of carotenoid monoesters since the micellarization of capsanthin monoesters followed a clear trend in all treatments (myristate > laurate > palmitate). Dhuique-Mayer et al. also demonstrated that the fatty acid moiety modulates the micellarization of β-cryptoxanthin monoesters.[35] In general, the effect of the fatty acid moiety on micellarization of carotenoid monoesters has been scarcely studied.

Effect of Heat-Processing Style and Fat Type on Micellarization of Carotenoid Diesters. The micellarization of carotenoid diesters in this study ranged from 0 to 30.6%. To date, only micellarization values (11%) for zeaxanthin diesters, as a group, from peppers, butternut squash, and wolfberries, have been reported.[8] The bioaccessibility of carotenoid diesters was also influenced by the interaction of heat-processing and fat type. The absence of fat consistently inhibited the micellarization of diesters in digestions of raw and boiled peppers, while grilling tended to reduce the negative effect of the absence of fat on diester micellarization. Fat favored the micellarization of carotenoid diesters from raw and boiled peppers in the order SO > BT, as described for carotenoid monoesters. The micellarization efficiency was greater with BT than with SO in digestions with grilled peppers, as described for carotenoid monoesters. We infer that boiling and grilling modified differentially the fibers of peppers, as reported previously for preheated (50 °C/0−60 min) rings of Jalapeño peppers,[19] affecting also differentially the emulsion and lipolysis of fat during digestion, two indispensable steps for carotenoid micellarization.[13,17] Pasquier et al. demonstrated that emulsification and lipolysis of fat depend on the chemical structure of fibers.[21] The number of micellarized carotenoid diesters ranged from 8, in digestion with raw and grilled red peppers with SO and BT, respectively, to 0, with raw and boiled red peppers WF. This suggests that fat is more important for the micellarization of diesters than monoesters of carotenoids since even WF a small number of carotenoid monoesters were micellarized (Table 4). The highest micellarization rate of carotenoid diesters was observed with raw red peppers with SO (10.5−30.6%), as occurred for carotenoid monoesters, demonstrating again the positive effect of SO on micellarization of carotenoid esters. The number of micellarized carotenoid diesters and their micellarization efficiency in digestions with raw and boiled peppers decreased in the order SO > BT > WF (Table 4), as occurred for carotenoid monoesters (Table 4). In contrast, this order was different for digestions with grilled peppers (BT > WF > SO), demonstrating that the effect of fat type is food matrix dependent. This could explain the conflicting results of the literature regarding the effect of fat type on carotenoids micellarization.[24,26] The increase of micellarization of mono- and diesters of carotenoids in digestions with grilled peppers was greater with BT than with SO. On the other hand, the capsanthin diesters (CDM, CDL, CLM, CPM, and CMP) were micellarized in the majority of the treatments, in contrast to other xanthophyll diesters. ZLM showed the highest micellarization efficiencies (26.3−30.6%), although this diester was micellarized in only two treatments.

Other Considerations. Free and esterified carotenoids coexisted only in red peppers and their digestates and micellar fractions. The bioaccessibility of carotenoids from red peppers

that were found in free and in at least one esterified form is shown in Figure 1, grouping free and mono- and diesterified forms. The bioaccessibility of these carotenoids was greater in the free form than in the mono- and diesterified forms in all treatments, and in most cases, the micellarization of monoesters was greater than that of diesters. The trends shown in Figure 1 are similar to those typically reported for free and mono- and diesterified forms of β-cryptoxanthin, and zeaxanthin from some foods,[8,35] hypothesizing that carotenoid esters are situated in the core of emulsified lipid droplets, making their incorporation into the mixed micelles difficult.[45] This type of data analysis allows the comparison of the bioaccessibility of free and esterified carotenoids, which possesses high importance from a nutritional point of view, but underestimates the effect of the chemistry of the esterification on the micellarization of individual carotenoids.

Some studies have demonstrated for a limited number of carotenoids that the micellarization of these compounds depends on their polarity.[8,13,42] Since in our study the chromatographic separation of pigments differed according to their polarity, the micellarization efficiency of all carotenoids was related to the polarity of the mobile phase at their elution time, which is very close to the polarity of the compounds.[46] This correlation was not clearly observed for pigments from green peppers (data not shown), probably due to the small number of pigments that were incorporated into the mixed micelles. In contrast, a high correlation (R^2 = 0.76−0.93) between micellarization efficiency of pigments and the polarity index of the mobile phase at the time of elution was observed in digestions with red peppers, mainly from a polarity index of 3.6 onward (Figure 2). At polarity index values below 3.6 the micellarization efficiency seemed to be independent of polarity index and the micellarization did not exceed 30%. The ranges of polarity index of the mobile phase at the elution time for free and mono- and diesterified carotenoids were 4.2−4.9, 3.9−4.3, and 2.6−3.8, respectively. This relationship of micellarized carotenoids from digestions WF and with raw and boiled peppers was linear (Figure 2A) because only free carotenoids were micellarized in these treatments. This linear behavior turned to an exponential type by grilling and fat, which favored the micellarization of mono- and diesters of carotenoids (Figure 2A−C). Others studies have also demonstrated that the polarity of free carotenoids is positively correlated with their bioaccessibility.[27,42] Herein, we demonstrate that this is true for free and monoesterified carotenoids but not for carotenoid diesters, which are considerably nonpolar.

In conclusion, the trends of DS and bioaccessibility of free pigments as a function of processing style and fat type were more irregular than those for esterified forms, suggesting a strong role of the fatty acid moiety on DS and bioaccessibility of pigments. The free xanthophylls were more bioaccessible than their respective mono- and diesterified forms. There was a strong relationship between the polarity of pigments and their micellarization efficiency. Boiling and grilling decreased the DS and bioaccessibility of most free and esterified pigments of Jalapeño peppers. Fat favored the DS and bioaccessibility of the less polar pigments (carotenes, β-cryptoxanthin, and esterified xanthophylls), while the highest values of these variables for free xanthophylls were observed WF and with BT. Our results have to be validated by *in vivo* studies, and the limitations of the *in vitro* model, which have been previously described,[47] must be considered.

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Notes

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