JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effect of Ripening, Heat Processing, and Fat Type on the Micellarization of Pigments from Jalapeño Peppers

Claudia I. Victoria-Campos,[†] José de Jesús Ornelas-Paz,*[,†] Elhadi M. Yahia,[‡] Jorge A. Jiménez-Castro,[§] Braulio Cervantes-Paz,[†] Vrani Ibarra-Junquera,[#] Jaime David Pérez-Martínez,[⊥] Paul B. Zamudio-Flores,[†] and Pilar Escalante-Minakata[#]

[†]Centro de Investigación en Alimentación y Desarrollo A. C. (CIAD), Unidad Cuauhtémoc, Avenida Río Conchos S/N, Parque Industrial, C.P. 31570 Cd. Cuauhtémoc, Chihuahua, Mexico

[‡]Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Avenida de las Ciencias S/N, C.P. 76230 Juriquilla, Querétaro, Mexico

[§]Universidad Autónoma de Chihuahua, Avenida Escorza No. 900, Zona Centro, C.P. 31000 Chihuahua, Chihuahua, Mexico

[#]Bioengineering Laboratory, Universidad de Colima, Km. 9 carretera Coquimatlán-Colima, C.P. 28400 Coquimatlán, Colima, Mexico

[⊥]Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, Manuel Nava No. 6, Zona Universitaria, C.P. 78210 San Luis Potosí, Mexico

Supporting Information

ABSTRACT: Raw and heat-processed (boiled and grilled) jalapeño peppers at three intermediate ripening stages (brown, 50% red, and 75% red) were digested in vitro without fat and in the presence of soybean oil (SO) or beef tallow (BT), and the micellarization of their lipid soluble pigments (LSP) was measured. The micelles from digestions with brown, 50% red, and 75% red peppers contained up to 27, 35, and 29 different LSP, respectively. Boiling and grilling decreased the micellarization of LSP from brown peppers, whereas the opposite was observed with 75% red peppers. Heat processing did not clearly affect the micellarization of LSP from 50% red fruits. The impact of fat on LSP micellarization was ripening-dependent, but the micellarization of the less polar carotenoids was always increased by SO or BT. This positive effect of fat was higher with SO than with BT.

KEYWORDS: *healthy vegetables, Capsicum annuum, food matrix effect, bioactivity, bioavailability*

INTRODUCTION

Ripening and heat processing of peppers extensively alter some of the SLAMENGHI factors that are related with the food matrix and modulate the bioaccessibility of carotenoids and other lipid soluble pigments (LSP) such as chlorophylls.[1] Ripening modifies the pigment species (S factor) in peppers. Some important LSP from green peppers (chlorophylls, neoxanthin, and lutein) disappear, or their concentration is considerably diminished during ripening, other LSP (capsanthin, β-carotene, zeaxanthin, capsorubin, β-cryptoxanthin, and several xanthophyll esters) being the most abundant in red peppers.[2] Heat processing also modifies the LSP species in peppers by the isomerization, epoxidation, oxidation, and/or degradation of pigments.[3,4] Several studies have demonstrated that the micellarization of LSP is species dependent.[5,6] Ripening and heat processing also alter the molecular linkage (L factor) of LSP. The ripening-related transformation of chloroplast into chromoplasts in peppers leads to the breaking of linkages between LSP and proteins or lipids.[7] The esterification of carotenoids with fatty acids increases during pepper ripening,[3] reducing their polarity and micellarization potential.[2,6] Heat processing breaks protein−carotenoid complexes, increasing the extractability and bioaccessibility of carotenoids.[8] Ripening and heat processing modify the amount (A factor) of LSP in peppers and, therefore, the amount of consumed LSP. The initial content of LSP in peppers increases up to 20−32 times during ripening.[9,10] In contrast, the total carotenoid content in peppers is reduced up to 53% by boiling, grilling, or microwave-heating.[11,12] The bioaccessibility of LSP depends, in some cases, on their concentration in the food.[13] Ripening and heat processing modify the matrix in which the LSP are incorporated (M factor), especially the pectin, which is one of the most abundant components of peppers (8.1−9.2%, on a dry weight basis).[14] Changes in the quantity, polymerization level, solubility, sugar composition, and esterification of pectin occur during pepper ripening.[15,16] Ripening-related changes of pectins alter the in vitro micellarization of LSP.[17] Heat processing causes the softening of peppers, making matrix disruption easier during digestion and increasing the liberation and bioaccessibility of LSP. This heating-induced softening has been attributed to the solubilization, depolymerization, and demethylation of pectins.[18,19] On the other hand, dietary fat is an effector (E factor) of LSP bioaccessibility,[1] typically increasing their bioaccessibility; however, this effect depends on the fat type and food matrix.[20−22] A strong effect of the

Received: July 22, 2013
Revised: September 18, 2013
Accepted: September 19, 2013
Published: September 19, 2013

Table 1. Micellarization Values (Percent) of Lipid-Soluble Pigments from Raw and Heat-Processed Brown Peppers after in Vitro Digestion without Exogenous Fat (WF) or with Soybean Oil (SO) or Beef Tallow (BT) as Bioaccessibility Effector[a]

			raw			boiled			grilled		
no.	compound	abbrev	WF	SO	BT	WF	SO	BT	WF	SO	BT
1	*cis*-violaxanthin	*cis*-Viol	29.0 ± 0.4 a	22.9 ± 1.3 b	24.4 ± 0.8 b	29.7 ± 0.6 b	17.3 ± 1.3 c	35.3 ± 0.5 a	32.9 ± 1.9 a	27.5 ± 1.4 a	26.1 ± 1.9 a
2	*all-trans*-violaxanthin	Viol	8.8 ± 0.2 a	6.7 ± 0.4 b	6.4 ± 0.3 b	ND[b]	ND	ND	ND	ND	ND
3	*all-trans*-luteoxanthin	luteox	ND	64.8 ± 3.6 b	96.9 ± 4.1 a	71.9 ± 4.0 a	35.8 ± 0.4 c	51.5 ± 4.2 b	42.6 ± 1.2 a	40.5 ± 1.4 a	35.9 ± 3.3 a
4	capsanthin-5,6-epoxide	cap5,6ep	20.5 ± 1.8 b	53.1 ± 5.0 a	50.2 ± 1.2 a	ND	ND	ND	ND	ND	ND
5	*all-trans*-antheraxanthin	ant	32.4 ± 0.9 b	42.1 ± 3.8 a	43.7 ± 2.4 a	64.7 ± 3.3 a	37.2 ± 4.7 b	71.7 ± 5.4 a	23.9 ± 1.1 a	18.5 ± 0.9 b	26.3 ± 0.5 a
6	*cis*-antheraxanthin	*cis*-ant	16.5 ± 0.7 b	49.9 ± 3.1 a	53.6 ± 1.5 a	17.5 ± 0.0 a	12.6 ± 0.9 b	12.2 ± 0.8 b	12.2 ± 0.7 a	11.1 ± 0.6 a	13.9 ± 0.8 a
7	chlorophyll *b*	chl *b*	13.7 ± 0.2 a	11.9 ± 0.4 b	13.2 ± 0.4 ab	12.2 ± 0.2 a	10.9 ± 0.6 ab	9.1 ± 0.3 b	28.3 ± 0.2 a	26.2 ± 0.9 a	28.2 ± 0.7 a
8	*all-trans*-mutatoxanthin	mut	ND	ND	ND	ND	ND	ND	31.1 ± 3.3 a	18.0 ± 1.4 a	31.1 ± 1.2 a
9	*all-trans*-lutein	lut	75.0 ± 1.1 a	75.0 ± 2.1 a	76.7 ± 1.3 a	109.3 ± 4.0 a	68.9 ± 3.6 b	103.6 ± 2.3 a	ND	ND	ND
10	*cis*-mutatoxanthin	*cis*-mut	ND	ND	ND	164.8 ± 0.9 a	118.9 ± 1.7 c	127.0 ± 1.8 b	ND	ND	ND
11	*all-trans*-capsanthin	cap	81.2 ± 0.3 a	77.7 ± 1.2 b	81.1 ± 0.4 a	82.1 ± 4.6 a	70.7 ± 3.4 a	78.1 ± 1.2 a	80.8 ± 3.4 a	69.2 ± 2.9 a	75.7 ± 1.7 a
12	*all-trans*-zeaxanthin	zea	87.1 ± 1.9 ab	85.6 ± 1.1 b	91.2 ± 0.1 a	77.8 ± 2.2 a	66.8 ± 1.3 b	72.5 ± 1.8 ab	81.5 ± 1.2 a	71.0 ± 3.2 b	72.6 ± 1.6 ab
13	*all-trans*-β-cryptoxanthin	β-cx	44.6 ± 4.0 b	86.7 ± 3.4 a	93.9 ± 4.2 a	47.9 ± 3.4 b	49.7 ± 1.0 b	73.1 ± 6.1 a	60.1 ± 2.7 a	42.3 ± 2.5 b	56.7 ± 6.0 ab
14	pheophytin *b*′	phe *b*′	ND	ND	ND	29.5 ± 1.8 a	28.1 ± 0.5 a	23.6 ± 5.0 a	27.4 ± 1.4 a	34.8 ± 2.4 a	27.9 ± 3.9 a
15	*all-trans*-α-carotene	α-car	ND	ND	ND	ND	ND	ND	4.1 ± 0.3 b	11.5 ± 1.5 a	ND
16	pheophytin *b*	phe *b*	194.4 ± 14.0 b	620.6 ± 20.9 a	593.7 ± 39.3 a	28.0 ± 2.0 a	35.5 ± 0.9 a	34.3 ± 3.5 a	24.3 ± 0.6 b	39.0 ± 1.4 a	34.0 ± 2.6 a
17	*all-trans*-β-carotene	β-car	26.2 ± 0.2 b	48.4 ± 1.2 a	46.8 ± 2.2 a	26.8 ± 2.4 a	30.0 ± 5.5 a	33.0 ± 2.2 a	17.4 ± 1.3 b	32.5 ± 0.6 a	29.0 ± 3.3 a
18	pheophytin *a*	phe *a*	ND	96.4 ± 11.1 a	73.7 ± 5.5 b	29.9 ± 1.2 c	67.0 ± 3.6 a	44.4 ± 4.6 b	30.6 ± 2.4 b	64.6 ± 0.6 a	57.0 ± 3.8 a
19	antheraxanthin-laurate	al	ND	18.3 ± 1.2 a	19.1 ± 3.6 a	ND	ND	ND	ND	ND	ND
20	antheraxanthin-myristate	am	15.4 ± 0.5 b	35.0 ± 2.7 a	29.2 ± 4.3 a	17.9 ± 1.8 a	23.6 ± 3.4 a	20.5 ± 2.9 a	N.D.	28.0 ± 3.0 a	21.2 ± 2.9 a
21	capsanthin-myristate	cm	15.1 ± 1.6 c	76.1 ± 3.3 a	60.9 ± 4.4 b	28.3 ± 2.4 b	62.1 ± 1.2 a	36.4 ± 4.6 b	20.7 ± 1.7 c	49.4 ± 1.2 a	32.6 ± 1.8 b
22	zeaxanthin-myristate	zm	ND	20.5 ± 0.9	ND	ND	ND	ND	ND	ND	ND
23	capsanthin-palmitate	cp	ND	41.6 ± 1.7 a	33.8 ± 1.7 b	ND	ND	ND	ND	ND	ND
24	capsanthin-laurate-myristate	clm	ND	17.1 ± 0.8 b	33.3 ± 4.9 a	10.7 ± 1.8 ab	7.4 ± 0.7 b	13.2 ± 0.9 a	ND	9.9 ± 0.8 a	7.0 ± 0.5 b
25	capsanthin-dimyristate	cdm	ND	19.1 ± 0.4 a	20.8 ± 0.9 a	8.9 ± 1.2 b	9.7 ± 2.8 ab	16.7 ± 3.1 a	ND	16.5 ± 1.2 a	13.2 ± 1.4 a
26	capsanthin-palmitate-myristate	cpm	ND	ND	38.0 ± 1.8	ND	ND	ND	ND	ND	ND
27	capsanthin-myristate-palmitate	cmp	ND	ND	63.9 ± 7.8	ND	ND	ND	ND	ND	ND

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

dx.doi.org/10.1021/jf4032124 | J. Agric. Food Chem. 2013, 61, 9938−9949

Table 2. Micellarization Values (Percent) of Lipid-Soluble Pigments from Raw and Heat-Processed 50% Red Peppers after in Vitro Digestion without Exogenous Fat (WF) or with Soybean oil (SO) or Beef Tallow (BT) as Bioaccessibility Effector[a]

			raw			boiled			grilled		
no.	compound	abbrev	WF	SO	BT	WF	SO	BT	WF	SO	BT
1	*cis*-violaxanthin	*cis*-Viol	24.5 ± 3.3 a	28.1 ± 1.3 a	31.9 ± 2.1 a	33.0 ± 0.3 a	34.8 ± 1.5 a	30.8 ± 3.2 a	28.4 ± 1.3 a	29.7 ± 1.3 a	29.7 ± 1.8 a
2	*all-trans*-luteoxanthin	luteox	130.1 ± 1.3 a	143.4 ± 10.0 a	135.9 ± 19.7 a	116.7 ± 1.6 a	127.1 ± 5.7 a	75.1 ± 4.4 b	97.0 ± 4.5 a	73.7 ± 3.3 b	50.4 ± 5.4 c
3	capsanthin-5,6-epoxide	cap5,6ep	46.7 ± 5.1 a	41.5 ± 0.5 a	52.9 ± 2.2 a	64.6 ± 3.3 a	56.8 ± 0.7 a	57.7 ± 0.6 a	49.8 ± 1.64 a	39.2 ± 0.5 a	42.7 ± 5.0 a
4	*all-trans*-antheraxanthin	ant	36.2 ± 1.2 a	35.2 ± 0.1 a	25.5 ± 1.5 b	75.6 ± 1.3 a	61.6 ± 2.7 b	65.1 ± 2.0 b	73.9 ± 2.3 a	64.5 ± 0.7 b	71.0 ± 2.5 ab
5	*cis*-antheraxanthin	*cis*-ant	65.3 ± 1.4 b	63.0 ± 2.0 b	89.2 ± 2.0 a	242.9 ± 4.1 a	249.5 ± 11.4 a	213.5 ± 13.3 a	ND[b]	ND	ND
6	*cis*-capsanthin	*cis*-cap	43.9 ± 1.7 a	43.4 ± 1.5 a	43.9 ± 2.2 a	32.8 ± 1.4 a	32.6 ± 2.5 a	29.8 ± 0.6 a	85.7 ± 3.3 a	74.7 ± 1.1 a	92.6 ± 7.0 a
7	*all-trans*-mutatoxanthin	mut	34.7 ± 5.0 b	31.2 ± 2.3 b	52.4 ± 2.1 a	89.1 ± 2.5 a	79.3 ± 3.2 a	83.6 ± 0.7 a	37.8 ± 3.5 a	28.0 ± 0.6 b	35.1 ± 1.1 ab
8	chlorophyll *b*	chl *b*	24.0 ± 0.1 a	25.1 ± 1.0 a	20.3 ± 0.3 b	ND	ND	ND	49.5 ± 0.4 a	46.2 ± 0.8 a	47.3 ± 1.2 a
9	*all-trans*-lutein	lut	ND	ND	ND	80.0 ± 2.9 a	76.6 ± 2.7 a	77.0 ± 1.5 a	ND	ND	ND
10	*cis*-mutatoxanthin	*cis*-mut	175.4 ± 7.1 a	195.8 ± 8.3 a	179.9 ± 2.5 a	ND	ND	ND	92.4 ± 6.2 a	85.7 ± 1.3 a	92.0 ± 2.0 a
11	*all-trans*-capsanthin	cap	65.6 ± 2.8 b	74.3 ± 1.6 a	76.4 ± 0.3 a	83.4 ± 2.0 a	78.7 ± 4.1 a	74.4 ± 1.2 a	74.4 ± 1.7 a	72.7 ± 0.4 a	76.0 ± 2.8 a
12	*all-trans*-zeaxanthin	zea	59.3 ± 4.1 b	81.1 ± 1.4 a	76.8 ± 1.3 a	73.7 ± 1.2 a	65.9 ± 0.5 b	67.1 ± 0.5 b	74.6 ± 0.4 a	69.7 ± 1.8 a	73.0 ± 3.5 a
13	*all-trans*-β-cryptoxanthin	β-cx	49.5 ± 1.5 b	77.8 ± 2.7 a	77.1 ± 1.4 a	48.4 ± 3.1 a	61.5 ± 3.0 a	52.4 ± 3.5 a	55.8 ± 1.9 b	66.0 ± 1.2 a	66.3 ± 3.4 a
14	pheophytin *b*′	phe *b*′	ND	ND	ND	ND	ND	ND	ND	34.1 ± 0.3 a	30.5 ± 3.3 a
15	pheophytin *b*	phe *b*	ND	ND	ND	19.2 ± 1.6 c	42.4 ± 1.8 a	31.9 ± 0.5 b	22.6 ± 3.1 c	45.5 ± 0.8 a	33.3 ± 0.3 b
16	*all-trans*-β-carotene	β-car	10.6 ± 1.5 b	30.6 ± 3.2 a	34.5 ± 2.1 a	11.0 ± 1.6 c	38.2 ± 2.0 a	28.7 ± 0.6 b	16.7 ± 1.0 c	38.7 ± 1.2 a	29.1 ± 0.6 b
17	antheraxanthin-laurate	al	ND	22.1 ± 2.4 a	20.3 ± 0.8 a	ND	24.1 ± 2.5 a	13.5 ± 0.4 b	ND	23.4 ± 1.1 a	15.3 ± 2.1 b
18	capsanthin-laurate	cl	13.2 ± 2.4 b	46.2 ± 3.1 a	40.1 ± 0.3 a	13.1 ± 1.9 c	43.5 ± 2.6 a	26.3 ± 0.8 b	14.0 ± 0.8 c	44.4 ± 2.3 a	31.7 ± 0.6 b
19	antheraxanthin-myristate	am	3.6 ± 0.3 b	26.9 ± 2.6 a	26.0 ± 1.6 a	ND	24.3 ± 1.8 a	15.8 ± 0.4 b	2.8 ± 0.1 c	38.6 ± 1.6 a	22.0 ± 1.5 b
20	mutatoxanthin-myristate	mm	ND	33.6 ± 2.5 b	53.4 ± 1.0 a	ND	38.0 ± 1.1 b	60.2 ± 1.3 a	ND	62.3 ± 5.7 a	48.6 ± 3.8 a
21	capsanthin-myristate	cm	10.4 ± 2.1 b	49.1 ± 3.1 a	44.2 ± 1.0 a	10.6 ± 1.8 c	51.6 ± 3.5 a	35.8 ± 0.9 b	18.7 ± 1.5 c	57.4 ± 2.1 a	42.8 ± 0.7 b
22	zeaxanthin-myristate	zm	ND	45.1 ± 4.3 a	31.2 ± 1.7 b	ND	37.8 ± 3.1 a	30.3 ± 1.0 a	10.6 ± 1.5 c	56.3 ± 1.5 a	36.4 ± 2.3 b
23	capsanthin-palmitate	cp	ND	43.8 ± 3.1 a	30.0 ± 2.7 b	ND	31.2 ± 1.5 a	20.4 ± 0.7 b	ND	43.5 ± 2.5 a	33.5 ± 1.6 b
24	β-cryptoxathin-laurate	β-cxl	ND	5.3 ± 0.5 b	23.9 ± 2.1 a	ND	ND	ND	ND	23.9 ± 1.9 a	14.8 ± 2.8 b
25	capsanthin-dilaurate	cdl	ND	9.1 ± 2.0 a	13.5 ± 0.8 a	ND	ND	ND	ND	16.8 ± 0.3 a	9.4 ± 2.5 b
26	capsanthin-laurate-myristate	clm	2.1 ± 0.1 b	10.8 ± 1.2 a	12.5 ± 2.3 a	5.2 ± 0.7 b	14.6 ± 2.0 a	11.0 ± 0.1 a	ND	ND	ND
27	capsorubin-dimyristate	crdm	ND	14.1 ± 3.3 a	14.7 ± 0.4 a	ND	ND	ND	ND	16.1 ± 1.2 a	10.9 ± 1.3 b
28	capsanthin-dimyristate	cdm	2.7 ± 0.3 b	8.8 ± 1.6 a	9.8 ± 1.3 a	ND	8.7 ± 1.2 a	8.2 ± 0.4 a	5.9 ± 0.4 c	14.4 ± 0.3 a	8.7 ± 0.5 b
29	capsanthin-palmitate-laurate	cpl	ND	ND	ND	ND	ND	ND	ND	11.1 ± 2.2	ND
30	capsorubin-myristate-palmitate	crmp	ND	ND	ND	ND	ND	ND	ND	25.9 ± 2.5	ND
31	capsanthin-palmitate-myristate	cpm	ND	ND	15.7 ± 1.3	ND	ND	ND	ND	28.8 ± 1.8	ND
32	capsanthin-myristate-palmitate	cmp	ND	ND	25.9 ± 0.5	ND	ND	ND	ND	44.8 ± 1.9	ND
33	zeaxanthin-myristate-palmitate	zmp	ND	ND	12.9 ± 1.2	ND	17.5 ± 3.2 a	13.1 ± 0.3 a	ND	19.2 ± 0.8 b	25.6 ± 1.7 a
34	capsanthin-dipalmitate	cdp	ND	ND	15.0 ± 1.4	ND	18.3 ± 1.9 a	18.6 ± 0.4 a	ND	30.9 ± 0.5 a	20.7 ± 1.8 b
35	zeaxanthin-dipalmitate	zdp	ND	ND	9.4 ± 1.3	ND	27.7 ± 4.5 a	16.1 ± 2.8 a	ND	20.5 ± 1.3 a	26.8 ± 2.3 a

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

dx.doi.org/10.1021/jf4032124 | J. Agric. Food Chem. 2013, 61, 9938−9949

Table 3. Micellarization Values (Percent) of Lipid-Soluble Pigments from Raw and Heat-Processed 75% Red Peppers after in Vitro Digestion without Exogenous Fat (WF) or with Soybean Oil (SO) or Beef Tallow (BT) as Bioaccessibility Effector[a]

			raw			boiled			grilled		
no.	compound	abbrev	WF	SO	BT	WF	SO	BT	WF	SO	BT
1	*cis*-violaxanthin	*cis*-Viol	23.4 ± 1.0 b	24.6 ± 1.6 b	32.4 ± 1.2 a	33.4 ± 1.5 a	30.5 ± 1.1 a	31.0 ± 2.7 a	23.0 ± 1.3 b	20.8 ± 2.8 b	35.7 ± 3.0 a
2	*all-trans*-luteoxanthin	luteox	348.9 ± 9.9 a	319.5 ± 48.4 a	344.3 ± 13.8 a	172.4 ± 7.6 a	132.2 ± 2.6 b	157.9 ± 10.8 ab	130.4 ± 10.4 a	71.59 ± 3.9 b	83.06 ± 1.6 b
3	capsanthin-5,6-epoxide	cap5,6ep	68.8 ± 5.9 a	57.5 ± 4.4 a	64.3 ± 5.6 a	78.9 ± 3.4 a	63.8 ± 1.3 b	77.3 ± 1.7 a	76.4 ± 0.9 a	69.5 ± 3.0 a	74.7 ± 4.8 a
4	*all-trans*-antheraxanthin	ant	56.6 ± 0.9 a	56.2 ± 1.5 a	58.5 ± 0.1 a	80.4 ± 2.6 a	62.8 ± 1.4 b	75.2 ± 3.6 a	57.8 ± 1.3 ab	44.8 ± 6.4 b	67.0 ± 2.5 a
5	*cis*-capsanthin	*cis*-cap	37.0 ± 1.3 a	21.4 ± 1.0 b	36.6 ± 1.8 a	75.9 ± 2.9 a	60.7 ± 0.3 b	78.7 ± 3.0 a	62.9 ± 3.4 a	53.7 ± 4.5 a	64.2 ± 2.4 a
6	*all-trans*-mutatoxanthin	mut	74.2 ± 0.1 a	73.4 ± 1.7 a	75.4 ± 0.4 a	80.9 ± 1.4 a	63.2 ± 0.4 b	78.9 ± 2.2 a	88.4 ± 4.4 a	75.6 ± 1.3 b	72.1 ± 1.4 b
7	*all-trans*-capsanthin	cap	69.2 ± 1.4 b	76.8 ± 1.2 a	71.4 ± 0.5 b	79.7 ± 0.6 a	72.3 ± 0.9 b	76.0 ± 2.7 ab	75.0 ± 4.2 a	65.5 ± 2.6 a	67.5 ± 3.5 a
8	*all-trans*-zeaxanthin	zea	47.4 ± 1.1 c	68.4 ± 0.4 a	55.8 ± 1.5 b	69.5 ± 1.9 a	68.5 ± 0.6 a	70.9 ± 0.8 a	72.7 ± 2.2 a	63.9 ± 2.6 a	68.3 ± 1.2 a
9	*all-trans*-β-cryptoxanthin	β-cx	40.0 ± 1.1 c	59.7 ± 0.8 a	55.7 ± 0.5 b	42.7 ± 3.3 b	66.5 ± 3.4 a	56.2 ± 2.8 ab	41.4 ± 0.5 b	42.9 ± 2.0 b	51.7 ± 1.6 a
10	*all-trans*-β-carotene	β-car	12.1 ± 1.2 c	34.2 ± 0.9 a	22.5 ± 0.5 b	11.3 ± 1.5 c	31.6 ± 1.2 a	21.6 ± 1.5 b	8.5 ± 1.0 b	23.4 ± 1.0 a	19.6 ± 1.2 a
11	antheraxanthin-laurate	al	ND[b]	21.7 ± 0.2 a	13.4 ± 2.1 b	ND	19.4 ± 0.4 a	11.1 ± 1.3 b	ND	16.5 ± 1.8 a	13.4 ± 2.2 b
12	capsanthin-laurate	cl	13.9 ± 2.6 c	43.8 ± 2.3 a	28.5 ± 1.1 b	11.5 ± 0.9 c	45.5 ± 2.7 a	23.9 ± 1.3 b	6.9 ± 0.2 c	28.4 ± 1.4 a	21.4 ± 0.8 b
13	antheraxanthin-myristate	am	5.4 ± 0.6 c	28.2 ± 0.5 a	10.5 ± 0.7 b	7.5 ± 0.5 c	29.5 ± 1.6 a	16.4 ± 1.4 b	ND	22.3 ± 1.4 a	17.1 ± 0.7 b
14	mutatoxanthin-myristate	mm	ND	54.3 ± 2.7 a	39.4 ± 2.5 b	ND	76.4 ± 2.1 a	40.2 ± 6.0 b	ND	28.8 ± 0.9 a	29.0 ± 1.8 a
15	capsanthin-myristate	cm	12.6 ± 1.5 c	49.3 ± 0.8 a	30.5 ± 1.2 b	14.5 ± 0.4 c	48.4 ± 2.7 a	31.7 ± 2.7 b	7.0 ± 1.1 b	32.3 ± 2.2 a	29.8 ± 2.1 a
16	zeaxanthin-myristate	zm	ND	36.4 ± 0.8 a	21.6 ± 0.8 b	ND	35.5 ± 1.4 a	22.7 ± 2.4 b	ND	27.5 ± 2.5 a	27.2 ± 2.3 a
17	capsanthin-palmitate	cp	ND	43.6 ± 1.5 a	22.4 ± 1.3 b	ND	25.4 ± 2.3 a	21.1 ± 0.2 a	ND	18.0 ± 1.0 a	23.1 ± 4.8 a
18	β-cryptoxathin-laurate	β-cxl	ND	19.1 ± 2.3 a	11.3 ± 1.8 b	ND	23.0 ± 3.2 a	11.2 ± 0.4 b	ND	14.5 ± 1.9 a	12.1 ± 0.3 a
19	capsanthin-dilaurate	cdl	ND	ND	ND	ND	13.4 ± 0.5 a	9.3 ± 0.4 b	ND	10.2 ± 0.9 a	9.0 ± 0.9 a
20	capsanthin-laurate-myristate	clm	3.6 ± 0.6 b	11.6 ± 1.5 a	6.0 ± 0.6 b	6.2 ± 0.7 c	15.3 ± 0.6 a	10.3 ± 0.2 b	ND	12.8 ± 1.1 a	11.1 ± 1.1 a
21	capsorubin-dimyristate	crdm	ND	14.6 ± 1.8	ND	ND	ND	ND	ND	19.1 ± 2.4 a	12.3 ± 0.9 b
22	zeaxanthin-laurate-myristate	zlm	ND	ND	ND	ND	ND	ND	ND	17.4 ± 1.7 a	12.4 ± 0.9 b
23	capsanthin-dimyristate	cdm	2.5 ± 0.3 b	7.6 ± 1.2 a	4.1 ± 0.6 b	4.5 ± 0.1 c	11.1 ± 0.3 a	7.2 ± 0.2 b	ND	10.9 ± 1.4 a	7.6 ± 0.5 a
24	capsanthin-palmitate-laurate	cpl	ND	10.4 ± 2.3	ND	ND	11.4 ± 1.3	N.D.	ND	12.3 ± 0.4	ND
25	capsanthin-palmitate-myristate	cpm	ND	ND	ND	ND	22.1 ± 0.5 a	15.2 ± 4.3 a	ND	15.8 ± 0.9 a	10.2 ± 2.2 b
26	capsanthin-myristate-palmitate	cmp	ND	ND	ND	ND	25.4 ± 1.1 a	16.5 ± 0.2 b	ND	28.3 ± 1.0 a	28.5 ± 2.4 a
27	zeaxanthin-myristate-palmitate	zmp	ND	16.6 ± 0.1 a	8.3 ± 0.5 b	ND	13.2 ± 1.4 a	7.8 ± 0.2 b	ND	10.0 ± 0.5 a	7.6 ± 1.23 a
28	capsanthin-dipalmitate	cdp	ND	16.2 ± 1.1 a	11.1 ± 1.1 b	ND	22.7 ± 1.0 a	16.3 ± 0.7 b	ND	16.9 ± 1.7 a	14.8 ± 1.2 a
29	zeaxanthin-dipalmitate	zdp	ND	20.7 ± 5.4 a	8.4 ± 1.3 ab	ND	23.0 ± 4.1 a	24.5 ± 2.3 a	ND	17.3 ± 1.2 a	16.2 ± 1.0 a

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

dx.doi.org/10.1021/jf4032124 | J. Agric. Food Chem. 2013, 61, 9938−9949

interaction between dietary fat and food matrix on the micellarization of individual LSP has been proposed, but it has been scarcely studied.[6]

The study of the impact of the changes of each SLAMENGHI factor as a function of ripening and heat processing on the micellarization of LSP is virtually impossible for individual LSP from food matrices containing a high number of pigments. Peppers contain up to 67 different LSP.[3] The effect of the mathematical interactions (I factor) between pigments and between the SLAMENGHI factors adds complexity to the study of micellarization of individual LSP.[1,5] Thus, the bioaccessibility of LSP must be determined from foods at the different edible stages of ripening, considering the processing styles commonly used. To date, the impact of heat processing on the micellarization of LSP has been well investigated;[23−26] however, the impact of ripening on the micellarization of LSP has received little attention and has been determined with limitations only in mangoes and peppers.[17,27] O'Sullivan et al. determined the micellarization of four carotenoids in green and red peppers belonging to different genotypes, making it difficult to make comparisons between ripening stages.[27] Victoria-Campos et al. found wide micellarization differences for many LSP from green and red peppers;[6] however, these differences were expected because green and red pepper matrices vary greatly, the effect of ripening on LSP micellarization remaining unclear. Experiments with peppers at intermediate ripening stages might provide new insights about the impact of ripening on LSP micellarization because the differences between these matrices are smaller than those between green and red peppers. The objective of this study was to evaluate the effect of ripening, heat processing, and dietary fat type on the micellarization of LSP from jalapeño peppers.

MATERIALS AND METHODS

Chemicals and Standards. Solvents, reagents, digestive enzymes, and porcine bile were purchased from J. T. Baker (Baker-Mallinckrodt Inc., Mexico) or Sigma-Aldrich (St. Louis, MO, USA). High-purity standards of *all-trans*-β-cryptoxanthin, *all-trans*-β-carotene, *all-trans*-lutein, *all-trans*-violaxanthin, *all-trans*-zeaxanthin, chlorophylls (*a* and *b*), and *all-trans*-capsanthin were obtained from Sigma-Aldrich, CaroteNature GmbH (Lupsingen, Switzerland) and Southcot Inc. (Chapel Hill, NC, USA).

Collection and Preparation of Samples. Jalapeño peppers (cv. Marajá) were harvested from a local orchard in Chihuahua, Mexico, at three intermediate ripening stages (brown, 50% red, and 75% red). Three samples of peppers at each ripening stage were boiled (94 °C/12.5 min), three samples were grilled (210 °C/13.2 min), and three samples were used in raw form. Each sample was composed of 10 peppers. Boiling and grilling represent two typical processing styles for peppers in Mexico.

Simulated in Vitro Digestions. Pepper samples (10 fruits) were homogenized to puree using a kitchen blender. Two grams of pepper puree was mixed with 120 μL of soybean oil (SO) or beef tallow (BT) and subjected to in vitro digestion. Digestions without fat were used as control reactions. The SO was bought in a local grocery and cooked (114.3 °C/7.5 min). BT was obtained by heating beef fat (208.7 ± 7.0 °C/37.5 min). The gastric and duodenal phases of digestions were simulated according to the method of Garrett et al.[28] Porcine pancreatic lipase (100−400 units/mg protein) was added during the intestinal phase at a final concentration of 0.4 mg/mL to stimulate the hydrolysis of carotenoid esters. The micellar phase was recovered after centrifugation of digestate (15000*g*/20 min/4 °C) (Allegra 64R, Beckman Coulter Inc., Indianapolis, IN, USA) and was then filtered through a membrane of 0.22 μm of pore size (Millipore Corp., Bedford, MA, USA). Aliquots of pepper puree, digestate, and micellar fraction were stored at −70 °C until LSP analysis, which occurred in the following five days. The digestive stability was calculated as the percentage of LSP of peppers present in digesta, whereas the micellarization efficiency represented the percentage of LSP of peppers present in the micellar fraction.

Pigment Extraction and Analysis. The LSP were extracted from pepper puree (4 g) by sequential washes with methanol (120 mL) and a mixture of hexane/acetone (75 mL; 1:1, v/v). The total extraction time was 1.25 h. The carotenoid-rich hexane layer was recovered, evaporated at 40 °C under reduced pressure conditions, and dissolved in acetone (4 mL). Pigments from digestates and micellar fractions were extracted by a liquid−liquid extraction using a mixture of petroleum ether/acetone (4 mL; 2:1 v/v).[17] The petroleum ether extracts were dried under a slight nitrogen flow, dissolved in acetone (2 mL), and filtered through a membrane of 0.45 μm pore size. Aliquots of pigment extracts from peppers (20 μL), digestates (100 μL), and micellar fractions (100 μL) were manually injected into an Agilent 1200 series HPLC system (Agilent, Palo Alto, CA, USA). The HPLC system was equipped with a C_{30} reversed-phase column (4.6 × 150 mm, 3 μm) and a diode array detector (YMC Inc., Milford, MA, USA). The UV−vis spectra were recorded for each LSP. The mobile phase consisted of water as eluent A, methanol as eluent B, and methyl *tert*-butyl ether as eluent C 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 flow rate of the mobile phase was 0.75 mL/min. Capsanthin forms and chlorophylls *b* and *b*′ were monitored at λ = 470 nm. Pheophytins and chlorophylls *a* and *a*′ were detected at λ = 430 nm. Violaxanthin forms were observed at λ = 440 nm. The other carotenoids were monitored at λ = 452 nm. The mass spectrum of each LSP was obtained using a TOF-MS, which was equipped with an APCI+ source. The MS operating conditions have been previously described.[3] Quantification of LSP was performed using an external standard method. *cis* Isomers and esterified forms of carotenoids were quantified as their correspondent free *all-trans* forms. When reference compounds were not available, carotenoids were quantified as *all-trans*-β-carotene. The concentration of chlorophyll derivatives was determined using the calibration curves of their respective precursors, chlorophyll *a* or *b*.

Data Analysis. All measurements were made in triplicate. The micellarization values were analyzed by an ANOVA and Tukey−Kramer tests. The relationship between the polarity or content of pigments and their micellarization efficiency was assessed by regression analysis. The micellarization values for LSP from peppers at each ripening stage were subjected to a principal component analysis (PCA). These analyses were performed using the JMP (SAS Institute, Inc., Cary, NC, USA) and XLSTAT (Addinsoft, France) statistical software.

RESULTS AND DISCUSSION

Pigment Composition of Peppers and Bioaccessible Pigments. Tested peppers contained similar numbers of LSP (71−77 different LSP); however, only 39 of them were micellarized after in vitro digestion. The concentration of these LSP in peppers is shown in the Supporting Information (Supplementary Table 1). All of the bioaccessible LSP were identified according to their chromatographic behavior, UV−vis characteristics, and MS spectra. Micelles from digestions of brown, 50% red, and 75% red peppers contained up to 27, 35, and 29 different LSP, respectively, although these numbers depended on dietary fat and heat-processing style of peppers (Tables 1, 2, and 3). Tested peppers contained a higher number of bioaccessible LSP than green (15) and red (26) peppers of the same genotype.[6] This could be due to the coexistence of chlorophylls and esterified carotenoids in tested peppers. The micelles from digestions with tested peppers contained some LSP (*cis*-antheraxanthin, *cis*-mutatoxanthin, mutatoxanthin-myristate, capsanthin-dipalmitate, capsorubin-dimyristate, capsorubin-myristate-palmitate, zeaxanthin-myris-

tate-palmitate, and zeaxanthin-dipalmitate) that were not bioaccessible from green or red jalapeño peppers, whereas other pigments (*all-trans*-neoxanthin, *all-trans*-α-carotene, mutatoxanthin-palmitate-laurate, and zeaxanthin-dilaurate) that had been micellarized with green or red peppers were not bioaccessible from tested peppers.[6]

Micellarization of Individual LSP from Peppers at Intermediate Ripening Stages. Some studies have demonstrated that the content of certain pigments is higher in peppers at intermediate ripening stages than in green or red peppers.[9,10] Peppers at intermediate ripening stages also exert higher antioxidant activity than fruits at other ripening stages,[29] probably due to their content of both chlorophylls and esterified carotenoids.[3] However, the bioaccessibility of these pigments had not been determined in peppers at intermediate stages of ripening until now, despite their edibility. The micellarization values for individual LSP are shown in Tables 1, 2, and 3. In general, the micellarization efficiency for the characteristic free carotenoids from peppers *all-trans*-capsanthin (65.5−83.4%), *all-trans*-antheraxanthin (18.5−80.4%), *all-trans*-lutein (0−109.3%), *all-trans*-zeaxanthin (47.4−91.2%), *all-trans*-β-cryptoxanthin (40.0−93.9%), and *all-trans*-β-carotene (8.5−48.4%) showed variations as a function of treatments, as seen for carotenoids from other pepper genotypes.[26,27] This variability has been attributed to the differential hydrolysis of xanthophyll esters during digestion, variation in the gastrointestinal stability of pigments, ripening or cooking mediated changes of the food matrix, and interaction between fat type and food matrix.[6,17] In our study, the micellarization of free xanthophylls was higher than that of carotenes, as reported in peppers and other fruits.[5,27,28] This phenomenon was probably governed by the polarity of the LSP, with the less lipophilic compounds being more easily transferred from the emulsified oil droplets to the mixed micelles.[28] Our results demonstrated that peppers at intermediate ripening stages do not present a big advantage over the green and red peppers in regard to the micellarization efficiency of free carotenoids. Only *all-trans*-lutein and *all-trans*-β-cryptoxanthin from tested peppers showed higher micellarization values (0−109.3 and 40.0−77.8%, respectively) than those reported with green (45.2−71.3%) and red (30.4−63.0%) peppers.[6] The micellarization values for the majority of the free xanthophylls from tested peppers were similar to those previously reported for this and other pepper genotypes at the green and red stages of ripening.[6,27]

Only chlorophyll *b* from brown and 50% red peppers was incorporated into the micelles (Tables 1 and 2). Some studies have demonstrated that chlorophylls *a* and *b* from spinach, pea, and green pepper are not bioaccessible;[4,6,24] however, in our study chlorophyll *b* from tested peppers was highly micellarized (9.1−49.5%) in some treatments. Pheophytins *b* and *b*′ were found in micelles from digestive processes with brown and 50% red peppers, whereas pheophytin *a* was observed only with brown peppers. The micellarization of pheophytin *b* exceeded 100% in some cases, whereas the micellarization values for pheophytins *b*′ and *a* ranged between 0 and 96.4% (Tables 1 and 2). Tested peppers show advantages over green peppers with regard to the bioaccessibility of pheophytin *b*′ but disadvantages in terms of micellarization of pheophytin *a*, according to a previous study.[6] The micellarization efficiency of pheophytins from peas (15−100%) was similar to that of tested peppers.[4] Considering total chlorophylls (chlorophylls + pheophytins) of the *a* and *b* series, we observed that the micellarization of the total chlorophylls of the *b* series was similar with brown (18.9−42.0%) and 50% red (13.8−45.5%) peppers. The micellarization of total chlorophylls of the *a* series was more variable, being minimal (0−4.9%) with raw peppers and high (11.0−54.3%) with processed peppers. In general, total chlorophylls of the *a* and *b* series showed similar bioaccessibilities. Conversely, total chlorophylls from series *a* are more bioaccessible than those of the *b* series in spinach and pea.[4,24] Recently, the micellarization differences between total chlorophylls of the *a* and *b* series could not be established in green peppers.[6]

The number of micellarized carotenoid esters varied between treatments, ranging from 4 to 9, with brown peppers, and up to 19, with 50% red and 75% red peppers (Tables 1, 2, and 3). On the basis of these data and considering the number of bioaccessible carotenoid esters reported for red peppers (16), we conclude that the number of bioaccessible carotenoid esters peaks in 50% red and 75% red peppers.[6] The micellarization efficiency of carotenoid monoesters and diesters ranged from 0 to 76.4% and from 0 to 44.8%, respectively. These values were highly altered by fat and were in many cases higher than those reported previously for carotenoid esters from citrus juices, wolfberry, orange pepper, and red pepper (11−44%).[30,31] Some carotenoid esters (antheraxanthin-myristate, capsanthin-myristate, capsanthin-palmitate, capsanthin-laurate-myristate, capsanthin-dimyristate, capsanthin-palmitate-myristate, capsanthin-myristate-palmitate) from tested peppers showed higher micellarization values than those reported for the same pigments from red peppers.[6] Thus, tested peppers (intermediate stages of ripening) have an advantage in this regard compared with fully ripened peppers.

The myristates of capsanthin and antheraxanthin had higher micellarization values than their respective laurates with 50% red and 75% red peppers (Tables 2 and 3). On the other hand, capsanthin-laurate had higher micellarization values than capsanthin-palmitate. Interestingly, the concentrations of myristates of capsanthin and antheraxanthin were higher than those of laurates and palmitates, which could increase their micellarization (Supplementary Table 1).[13] However, the difference between the micellarization of capsanthin-laurate and capsanthin-palmitate might be explained by the effect of the fatty acid in the solubilization of monoesters into the micelles. Similar results were obtained with red peppers.[6] Previously, Dhuique-Mayer et al. reported higher micellarization values for β-cryptoxanthin-laurate than for β-cryptoxanthin-myristate from citrus juices, in which the concentrations of both monoesters were similar.[31] Thus, the micellarization efficiency for the same carotenoid might depend on the polarity provided by its fatty acid moiety (laurate > myristate > palmitate). Despite the fact that some studies suggest that foods rich in esterified carotenoids (β-cryptoxanthin, lutein, and zeaxanthin) cause greater carotenoid absorption, the effect of the fatty acid moiety on the micellarization/absorption of esterified carotenoids remains unclear.[32−34] Victoria-Campos et al. demonstrated that the polarity of some groups of LSP (carotenoid monoesters, carotenes, and free xanthophylls) from red peppers was positively correlated (lineal order) with their micellarization values, that the micellarization values were not related to their digestive stability, and that the most polar free xanthophylls showed the highest micellarization values.[6] In tested peppers (intermediate stages of ripening), the polarity of all of the LSP groups was also correlated positively with their micellarization values (R^2 = 0.55−0.95) but following a

polynomial order in most of the treatments. The micellarization of the most polar pigments (many free xanthophylls, chlorophyll *b*, and pheophytin *b*) positively correlated (R^2 = 0.779−0.973) with their digestive stability (data not shown), and the most polar carotenoids (*cis*-violaxanthin, *all-trans*-luteoxanthin, capsanthin-5,6-epoxide, *all-trans*-antheraxanthin, *cis*-capsanthin, and *all-trans*-mutatoxanthin) did not show the highest micellarization values (Tables 1, 2, and 3). These findings demonstrate that the changes of the food matrix by ripening and heat processing may decrease the effect of the chemical characteristics of LSP that normally modulate their micellarization efficiency.[5,25]

Effect of the Interaction between Heat-Processing Style and Dietary Fat Type on the Micellarization of LSP. The micellarization of free and esterified LSP was significantly influenced by the interaction between dietary fat and heat processing, according to the ANOVA. PCA allowed visualizing the effect of heat-processing style and dietary fat type on the micellarization of all of the LSP from jalapeño peppers at each ripening stage. Figure 1 shows the score plots for the first two principal components (PCs) for peppers at each ripening stage. These PCs explained 61.7, 64.0, and 70.0% of the total variability of the micellarization values in digestions with brown, 50% red, and 75% red peppers, respectively. In the plots, the LSP were localized near the treatments that favored their micellarization. Interesting, only *cis*-violaxanthin, *all-trans*-antheraxanthin, and *all-trans*-lutein were consistently micellarized at high percentages in digestions with heat-processed peppers in a ripening-independent fashion; thus, in all PC plots they were located near treatments where heat processing was involved (Figure 1; Tables 1, 2 and 3). Contrarily, in other studies with green and red peppers the micellarization of lutein and *cis*-violaxanthin was decreased by different cooking methods (boiling, grilling, microwave heating, and steaming).[6,26] This difference might be attributed to variations in firmness and metabolism of cell wall polysaccharides between pepper genotypes, which might differentially modulate the impact of heat processing on tissue integrity and liberation and micellarization of LSP.[14,35] However, the effect of the heat processing on the micellarization of the rest of LSP depended on the ripening stage of peppers. The separation of treatments in PC1 axis indicates that the LSP micellarization was more influenced by heat processing (PC1 45.2%) than by fat type in digestions with brown peppers (Figure 1A). Contrarily, the variability of the micellarization efficiency was mainly explained by fat type in digestions with 50% red (PC1, 43.5%) and 75% red (PC1, 53.7%) peppers (Figure 1B,C). By comparison of the clusters of treatments and pigments according to heat processing of brown peppers, it can be noted that a great number of pigments showed their highest micellarization values with raw fruits, being located in the positive side of PC1 (Figure 1A; Table 1), whereas the most bioaccessible pigments from heat-treated peppers were located in the negative side of PC1. However, the separation of treatments and pigments in the PC2 axis showed that the micellarization of specific pigments was increased by boiling (*cis*-violaxanthin, *cis*-mutatoxanthin, *all-trans*-antheraxanthin, *all-trans*-capsanthin, and *all-trans*-lutein) and grilling (pheophytin *b*′, *all-trans*-mutatoxanthin, chlorophyll *b*, and *all-trans*-α-carotene) (Figure 1A; Table 1). In general, the bioaccessibility of LSP from brown peppers was higher with raw than with heat-treated peppers.

With 50% red peppers, a clear effect of heat processing on LSP micellarization was not observed, probably because the

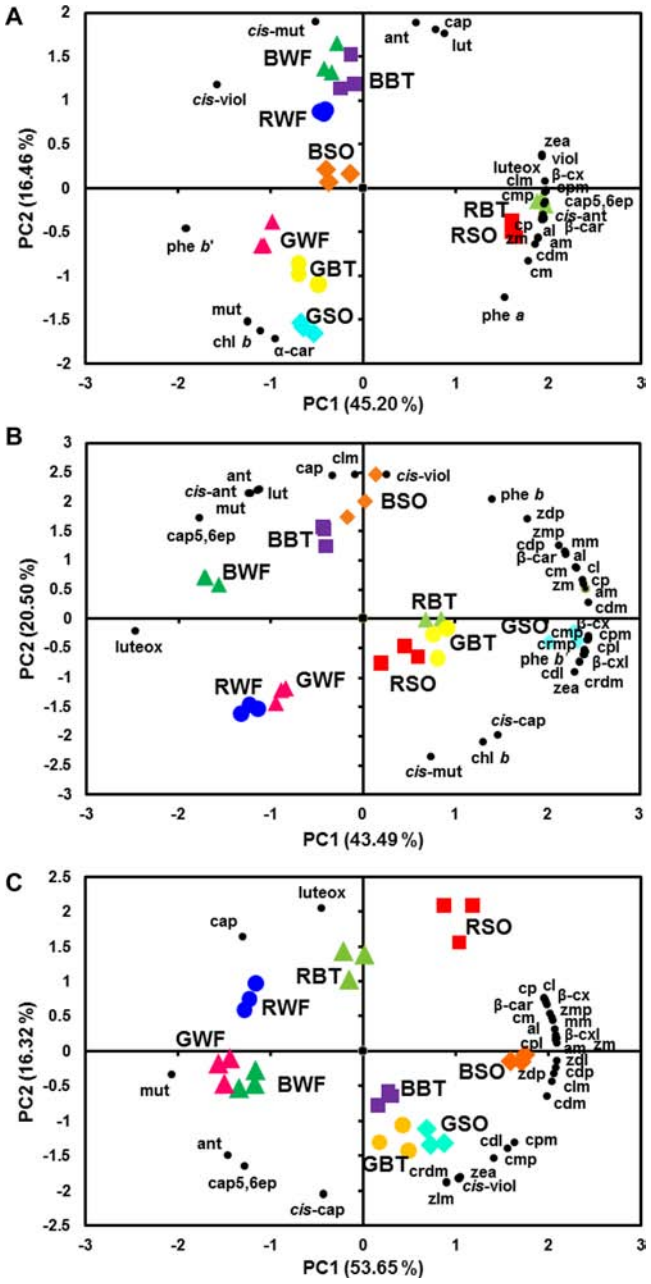

Figure 1. Biplots of the principal component analysis of the micellarization values for lipid-soluble pigments from peppers at three intermediate ripening stages (A, brown peppers; B, 50% red peppers; C, 75% red peppers). The name of treatments was assigned by the combination of the heat-processing style (R, raw; B, boiled; G, grilled) and the different dietary fat types (WF, without fat; SO, soybean oil; BT, beef tallow). The full and abbreviated names of the lipid-soluble pigments are shown in Tables 1, 2, and 3. Pheophytin *b* was removed from the analysis of brown peppers due to its extremely high micellarization values.

majority of LSP showed similar micellarization values in all of the treatments (Figure 1B; Table 2). However, the distribution of pigments and treatments in Figure 1B showed that pigments localized in the positive side of PC1 and negative side of PC2 had higher micellarization values with raw or grilled peppers than with boiled peppers, whereas the opposite was observed for the pigments localized in the negative side of PC1 and the positive side of PC2. Interestingly, grilling increased substan-

tially the micellarization of carotenoid diesters (Table 2). On the other hand, the micellarization of pigments localized in the positive sides of PC1 and PC2 was similarly increased by both heat-processing styles, compared with the micellarization of these pigments with raw peppers. The *all-trans*-luteoxanthin showed its highest micellarization values with raw peppers, but it was not included in any cluster (Figure 1B; Table 2).

Heat processing clearly increased the micellarization of a large number of pigments from 75% red peppers, compared with raw peppers (Figure 1C; Table 3). These pigments were localized in the negative side of PC2 of Figure 1C. The micellarization of carotenoid diesters depended on heat processing in the following order: boiled > grilled > raw. Only luteoxanthin showed its highest micellarization values in digestions with raw peppers, whereas the highest micellarization values for capsanthin were observed with raw and boiled peppers. Thus, these carotenoids were localized in the positive side of PC2.

In summary, the heat processing decreased the micellarization of LSP from brown peppers, whereas the opposite was observed for LSP from 75% red peppers. Heat processing did not systematically affect the micellarization of LSP from 50% red fruits. In a previous work, Victoria-Campos et al. demonstrated that heat processing decreased the micellarization of LSP from green and red peppers.[6] Ryan et al. also demonstrated that the micellarization of a great number of carotenoids was higher with raw than with cooked red peppers.[26] These findings collectively suggest that the effect of heat processing on LSP micellarization is ripening-dependent. Pectins undergo extensive chemical changes during pepper ripening, and they are one of the most abundant components of this food and therefore one of the most critical determinants of the food matrix effect on LSP micellarization.[17] Castro et al. reported higher firmness losses in red peppers than in green peppers after thermal and pressure treatments and were attributed to the ripening-induced differences in the esterification level of pectins.[35] Ornelas-Paz et al. showed strong evidence about the impact of ripening-induced changes of pectic materials on carotenoid micellarization.[17] The heat processing could also cause important chemical changes in the pectin of peppers, as has been reported for other foods.[19] Heat processing increases the amount of water-soluble pectin and decreases the content of chelator- and alkali-soluble pectins in carrots.[19,36] The heat-induced depolymerization of pectin depends on treatment intensity and their initial degree of methyl esterification, which is altered by ripening.[35,36] The heat processing also decreases differentially the degree of methyl esterification of different pectic fractions according to their solubility and heat treatment conditions (intensity, time, and pressure).[19,36] Sila et al. reported that heat treatment increases the concentration of neutral sugars in water-soluble pectin, whereas these sugars decrease in alkali-soluble pectins.[36] All of these ripening- or heat-induced chemical changes of pectins might modulate differentially the negative effect these polysaccharides on LSP micellarization. Dongowski demonstrated that the interaction of bile salts with pectins depends on their degree of methylation, acetylation, and amidation.[37] This interaction might alter the formation of micelles because the levels of bile salts are diminished, causing an increase of the droplet size of emulsified fat and therefore reducing their subsequent lypolysis, two indispensable steps for micelle formation.[38] Bile salts are also important components of mixed micelles.[17] The chemical characteristics of pectins also modify the viscosity of the duodenal medium, altering the emulsion of fat and micelle formation.[38] To date, the effects of chemical characteristics of pectins on binding of bile salts, fat emulsion, and lipolysis, and the subsequent LSP micellarization, remain untouched.

The effect of dietary fat on LSP micellarization depended on heat processing style in digestions with brown peppers. The digestions with raw brown peppers without fat were clearly separated from digestions with SO and BT, according to the distribution of treatments in the PC1 axis of Figure 1A. The SO and BT induced a higher micellarization of the less polar pigments such as β-cryptoxanthin, β-carotene, pheophytins, and esterified carotenoids, compared with digestions without fat. These differences were greater with raw than with processed peppers (Table 1); thus, the digestions with boiled and grilled peppers without fat and with both fat types had similar locations in Figure 1A.

Several clusters of pigments were clearly observed in the PC1 axis in plots of 50% red and 75% red peppers as a function of dietary fat (Figure 1B,C). In these plots, the treatments without fat were located in the most negative side of the PC1 axis. Similarly, the free xanthophylls capsanthin-5,6-epoxide, *all-trans*-capsanthin, *all-trans*-mutatoxanthin, *all-trans*-antheraxanthin, and *all-trans*-luteoxanthin were located in the negative side of PC1 of these plots (Figure 1B,C), showing their lowest micellarization values in digestions with 50% red and 75% red peppers with both fat types (Tables 2 and 3). Other xanthophylls (*cis*-violaxanthin, *cis*-mutatoxanthin, *all-trans*-zeaxanthin, *cis*-capsanthin, etc.) were near zero in the PC1 axis and showed similar micellarization values with and without fat (Figure 1B,C; Tables 2 and 3). The less polar pigments such as β-cryptoxanthin, β-carotene, pheophytins, and esterified carotenoids had higher micellarization values with SO and BT in digestions with raw and heat-processed peppers (Tables 2 and 3). Thus, these pigments and the treatments with fat were located in the positive region of the PC1 (Figure 1B,C). In digestions with 75% red peppers, the SO consistently caused a higher micellarization of the less polar pigments than BT in digestions with raw and heat-treated peppers. Thus, the distribution of treatments as a function of the fat was in the order SO > BT > WF from right to left of the PC1 axis (Figure 1C; Table 3). This order was similar for the digestions with boiled and grilled 50% red peppers, although this order was less clear with raw peppers (Figure 1B; Table 2).

In summary, the impact of dietary fat on LSP micellarization was ripening-dependent, but the micellarization of the less polar carotenoids was always increased by SO and BT. The positive effect of fat on LSP micellarization was more evident with raw than with processed brown peppers, whereas this positive effect was observed with raw and heat-treated peppers at the other ripening stages. The positive effect of fat on the micellarization of lipophilic pigments is well-known and has been observed in heat-processed carrots, orange-fleshed sweet potatoes, mangoes, and other foods.[17,23,39] However, this is one of the scarce studies about the interaction effect of fat type and heat-processing style in foods at edible intermediate ripening stages. The information regarding the effect of the different dietary fat sources or different fatty acids on LSP micellarization is controversial. Huo et al. reported that long-chain fatty acids promote the micellarization of α-carotene, β-carotene, and lycopene, whereas short-chain fatty acids favor the micellarization of lutein.[22] They reported that the degree of unsaturation did not affect the micellarization of carotenoids from a salad.

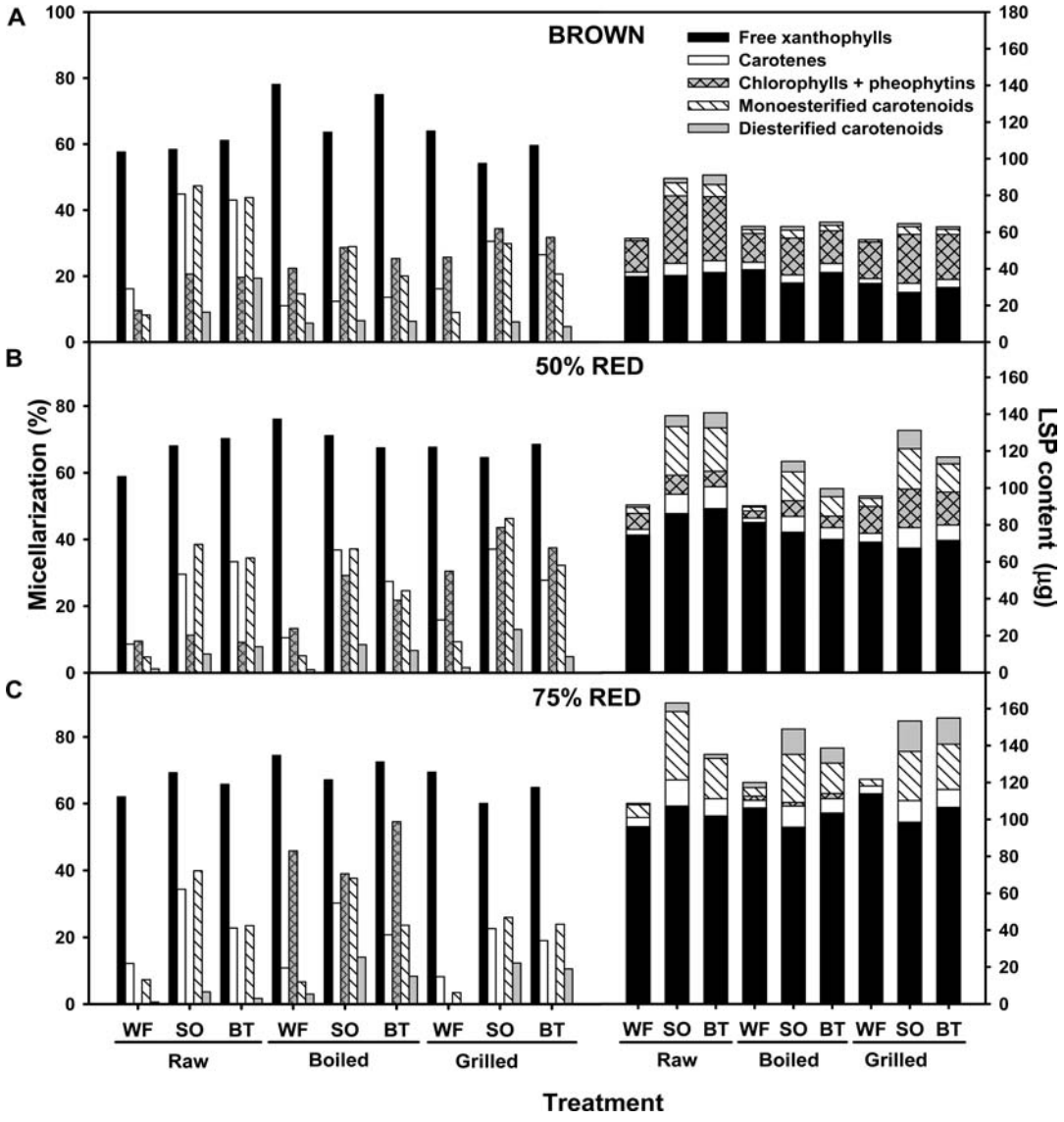

Figure 2. Micellarization values (%) and content of lipid-soluble pigments (LSP) groups in the micellar fraction (μg) of digestions with raw and heat-processed peppers at three intermediate ripening stages (A, brown peppers; B, 50% red peppers; C, 75% red peppers), without exogenous fat (WF), with soybean oil (SO), or with beef tallow (BT). Data represent the mean values obtained from data given in the Supporting Information (Supplementary Table 1) and Tables 1, 2, and 3.

Contrarily, Hu et al. found that the co-ingestion of a meal and saturated fat improved the β-carotene absorption, compared with the co-consumption of the meal with unsaturated fat.[21] Recently, Goltz et al. demonstrated that monounsaturated fatty acids cause a higher absorption of carotenoids from a vegetable salad than saturated fatty acids.[20] These differences found in the literature and those found in our work for the impact of fat type on LSP micellarization might be consequences of the differential interaction of each fat type with the food matrix, which can be extensively altered by ripening and heat processing.

Micellarization of LSP as Groups and Their Distribution in the Micelles. The micellarization of pigments is generally expressed as the proportion of each pigment in the food matrix that is transferred to the mixed micelles; however, micellarization efficiency trends and content of LSP in the micelles as a function of several factors are sometimes different.[27,31] The micellarization efficiency and content of LSP in the micelles, as groups, from digestions with tested peppers are given in Figure 2. Considering all of the treatments, the micellarization efficiency of free xanthophylls ranged similarly at each ripening stage, varying between 54.2 and 78.1%. The difference between the highest and lowest micellarization values for these ranges did not exceed 9.1% between ripening stages. Thus, ripening did not significantly affect the micellarization percentage of free xanthophylls as a group; however, the content of free xanthophylls in micelles dramatically increased during ripening. The content ranges were 27.1−39.61, 67.4−88.9, and 95.8−114.0 μg for all treatments with brown, 50% red, and 75% red peppers, respectively, demonstrating the strong effect of ripening on the transference of this pigment group from the peppers to the mixed micelles. The micellarization efficiency of free xanthophylls was significantly higher with boiled (8.6−22.4%) than with raw or grilled peppers, whereas the concentration of these pigments in micelles was diminished up to 19.3% by boiling and grilling in brown and 50% red peppers. Heat processing slightly increased (up to 4.5%) the concentration of

free xanthophylls in micelles of digestions with 75% red peppers. The fat either increased (3.8−17.4%) or did not alter the micellarization and content of free xanthophylls in the micelles with raw peppers, but the addition of fat to the digestions with heat-processed peppers decreased these values (1.6−11.2%).

The micellarization efficiency and content of carotenes in micelles were minimally altered by ripening because the levels and proportions of these pigments were similar in digestions of peppers at each tested ripening stage. Heat processing decreased the micellarization (35.6−70.3%) and content (14.8−22.6%) of carotenes in the micellar fractions in digestions with brown peppers with both fat types, but did not alter the micellarization and content of carotenes in the micellar fractions of digestions with peppers at the other ripening stages. Fat dramatically increased the micellarization efficiency of carotenes (up to 2.67 times), except in digestions with boiled brown peppers; however, these increases represented only 0.7−8.1 μg of more carotenes in the micelles.

The micellarization efficiency values for chlorophylls plus pheophytins showed similar variation between treatments (0−54.6%) for each ripening stage. However, the content of these compounds in the micelles decreased gradually through ripening, from 15.7−36.8 μg with brown peppers to 2.0−2.8 μg with 75% red peppers. Boiling and grilling increased (1.5−3.7 times) the micellarization of the chlorophylls plus pheophytins with peppers at the three ripening stages. However, heat processing decreased (19.8−39.5%) the amount of chlorophylls plus pheophytins in the micellar fraction of digestions with brown peppers and with boiled 50% red peppers. The addition of both fat types increased (2.1−109.8%) the micellarization and the amount of this LSP group in the micellar fraction of all treatments.

The variability of the micellarization efficiency for mono- (4.7−46.3%) and diesterified (0−14.1%) carotenoids was similar with 50% and 75% red peppers; however, the amount of monoesters in the micelles increased gradually as ripening advanced, varying from 1.2−7.1 μg, with brown peppers to 3.5−37.0 μg, with 75% red peppers, respectively. The content of diesters in the micelles was also increased by ripening, ranging from 0−5.2 to 0−16.6 μg with brown and 75% red peppers, respectively. The effect of heat processing on micellarization of carotenoid esters was inconsistent; however, the amount of monoesterified carotenoids in the micellar fractions was consistently decreased (16.6−46.7%) by heat processing. Heat processing also decreased (4.0−60.6%) the content of diesterified carotenoids in the micelles from digestions with brown and 50% red peppers. The micellarization and content of mono- and diesterified carotenoids in the micelles increased significantly (1.1−7.7 times) with the addition of SO or BT.

The in vitro bioaccessibility of LSP is typically expressed as micellarization percentages, underestimating the content of LSP in the micelles.[4−6,22,24,26,39] Our findings demonstrate that the LSP content in the micelles and micellarization percentages provide different points of view about the effect of ripening, heat processing, and fat type on the quantity of potentially absorbable LSP. Others have also obtained different conclusions from their works considering either micellarization efficiencies or pigment content in the micelles. Dhuique-Mayer et al. found that although the micellarization efficiency of β-cryptoxanthin from lemon juice (40%) was higher than that of other citrus juices (16−22%), the content of this carotenoid was ≈3 times higher in the micellar fraction from digestions with mandarins than with lemons.[31] Similarly, the micellar fractions from digestions with mandarin juices had the highest β-carotene content, but the micellarization values for this carotene were similar to those obtained with other citrus juices (26−33%).[31] The micellarization efficiency for 9-*cis*- and 13-*cis*-β-carotene from raw and cooked kale, spinach, and savoy cabbage was higher than that of their *all-trans* counterpart; however, the *all-trans*-β-carotene was the most abundant in the micellar fraction for all of the tested vegetables.[25] O'Sullivan et al. found that β-carotene and β-cryptoxanthin had their highest micellarization values in digestions with green and yellow bell peppers compared with red bell peppers, but these carotenoids were more abundant in the micelles from digestions with red bell peppers, whereas the opposite was observed for lutein.[27] In this study, the initial content of the different pigment groups did not correlate well with their micellarization values (R^2 = 0.000−0.460).

In conclusion, the number of micellarized LSP was higher with peppers at intermediate ripening stages than those reported for green and red fruits; however, their micellarization values seemed to be independent of the ripening stage for the majority of individual LSP. Our data demonstrate that peppers at intermediate ripening stages represent an advantage over the green and red peppers in regard to the bioaccessibility of only a limited number of LSP (pheophytin *b*′, *all-trans*-lutein, *all-trans*-β-cryptoxanthin, antheraxanthin-myristate, capsanthin-myristate, capsanthin-palmitate, capsanthin-laurate-myristate, capsanthin-dimyristate, capsanthin-palmitate-myristate, capsanthin-myristate-palmitate), according to the literature. However, the quantity of LSP in the micelles must be also considered. Ripening-induced changes in the food matrix influenced the effect of heat processing on LSP micellarization and interfered with the micellarization efficiency of each pigment according to their polarity. The dietary fat increased the micellarization of the less polar carotenoids, and this effect was independent of ripening stage and heat processing.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(J.J.O.-P.) Phone: +52-625-5812920, ext. 110. Fax: +52-625-5812920. E-mail: jornelas@ciad.mx.

Funding

This research was funded by the Fondo Sectorial de Investigación para la Educación (Investigación Básica SEP-CONACYT; Project Clave 103391). The authors are members of the research group on "Pigmentos Naturales (Enfasis en Carotenoides)" and "IBERCAROT" (CYTED).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Olivia Patricia Ramos Aguilar and Emilio Ochoa Reyes for their technical assistance.

REFERENCES

(1) West, C.; Castenmiller, J. Quantification of the "SLAMENGHI" factors for carotenoid bioavailability and bioconversion. *Int. J. Vitam. Nutr. Res.* **1998**, *68*, 371−377.

(2) Mínguez-Mosquera, M. I.; Hornero-Mendez, D. Changes in carotenoid esterification during the fruit ripening of *Capsicum annuum* cv. Bola. *J. Agric. Food Chem.* **1994**, *42*, 640−644.

(3) Cervantes-Paz, B.; Yahia, E. M.; Ornelas-Paz, J. de J.; Gardea-Béjar, A. A.; Ibarra-Junquera, V.; Pérez-Martínez, J. D. Effect of heat processing on the profile of pigments and antioxidant capacity of green and red jalapeño peppers. *J. Agric. Food Chem.* **2012**, *60*, 10822−10833.

(4) Gallardo-Guerrero, L.; Gandul-Rojas, B.; Mínguez-Mosquera, M. I. Digestive stability, micellarization, and uptake by Caco-2 human intestinal cell of chlorophyll derivatives from different preparations of pea (*Pisum sativum* L.). *J. Agric. Food Chem.* **2008**, *56*, 8379−8386.

(5) Tyssandier, V.; Lyan, B.; Borel, P. Main factors governing the transfer of carotenoids from emulsion lipid droplets to micelles. *Biochim. Biophys. Acta* **2001**, *1533*, 285−292.

(6) Victoria-Campos, C. I.; Ornelas-Paz, J. de J.; Yahia, E. M.; Failla, M. L. 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*). *J. Agric. Food Chem.* **2013**, *61*, 3642−3653.

(7) 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.

(8) Howard, L. A.; Wong, A. D.; Perry, A. K.; Klein, B. P. β-Carotene and ascorbic acid retention in fresh and processed vegetables. *J. Food Sci.* **1999**, *64*, 929−936.

(9) Deli, J.; Matus, Z.; Szabolcs, J. Carotenoid composition in the fruits of black paprika (*Capsicum annuum* variety *longum nigrum*) during ripening. *J. Agric. Food Chem.* **1992**, *40*, 2072−2076.

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

(11) 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.

(12) Ornelas-Paz, J. de J.; Cira-Chávez, L. A.; Gardea-Béjar, A. A.; Guevara-Arauza, J. C.; Sepúlveda, D. R.; Reyes-Hernández, J.; Ruiz-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.* **2013**, *50*, 519−525.

(13) Thakkar, S. K.; Maziya-Dixon, B.; Dixon, A. G. O.; Failla, M. L. β-Carotene micellarization during in vitro digestion and uptake by Caco-2 cells is directly proportional to β-carotene content in different genotypes of cassava. *J. Nutr.* **2007**, *137*, 2229−2233.

(14) Gu, Y. S.; Howard, L. R.; Wagner, A. B. Physicochemical factors affecting firmness of pasteurized jalapeño peppers rings. *J. Food Qual.* **1999**, *22*, 619−629.

(15) Conforti, F. D.; Zinck, J. B. Hydrocolloid-lipid coating affect on weight loss, pectin content, and textural quality of green bell peppers. *J. Food Sci.* **2002**, *67*, 1360−1363.

(16) Sethu, K. M. P.; Prabha, T. N.; Tharanathan, R. N. Post-harvest biochemical changes associated with the softening phenomenon in *Capsicum annuum* fruits. *Phytochemistry* **1996**, *42*, 961−966.

(17) Ornelas-Paz, J. de J.; Failla, M. L.; Yahia, E. M.; Gardea-Bejar, A. Impact of the stage of ripening and dietary fat on in vitro bioaccessibility of β-carotene in 'Ataulfo' mango. *J. Agric. Food Chem.* **2008**, *56*, 1511−1516.

(18) Howard, L. R.; Burma, P.; Wagner, A. B. Firmness and cell wall characteristics of pasteurized jalapeno pepper rings as affected by preheating and storage. *J. Food Sci.* **1997**, *62*, 89−92.

(19) De Roeck, A.; Sila, D. N.; Duvetter, T.; Van Loey, A.; Hendrickx, M. Effect of high pressure/high temperature processing on cell wall pectic substances in relation to firmess of carrot tissue. *Food Chem.* **2008**, *107*, 1225−1235.

(20) Goltz, S. R.; Campbell, W. W.; Chitchumroonchokchai, C.; Failla, M. L.; Ferruzzi, M. G. Meal triacylglycerol profile modulates postprandial absorption of carotenoids in humans. *Mol. Nutr. Food Res.* **2012**, *56*, 866−877.

(21) Hu, X.; Jandacek, R. J.; White, W. S. Intestinal absorption of β-carotene ingested with a meal rich in sunflower oil or beef tallow: postprandial appearance in triacylglycerol-rich lipoproteins in women. *Am. J. Clin. Nutr.* **2000**, *71*, 1170−1180.

(22) Huo, T.; Ferruzzi, M. G.; Schwartz, S. J.; Failla, M. L. Impact of fatty acyl composition and quantity of triglycerides on bioaccessibility of dietary carotenoids. *J. Agric. Food Chem.* **2007**, *55*, 8950−8957.

(23) Bengtsson, A.; Brackmann, C.; Enejder, A.; Alminger, M. L.; Svanberg, U. Effects of thermal processing on the in vitro bioaccessibility and microstructure of β-carotene in orange-fleshed sweet potato. *J. Agric. Food Chem.* **2010**, *58*, 11090−11096.

(24) Ferruzzi, M. G.; Failla, M. L.; Schwartz, S. J. Assessment of degradation and intestinal cell uptake of carotenoids and chlorophyll derivatives from spinach puree using an in vitro digestion and Caco-2 human cell model. *J. Agric. Food Chem.* **2001**, *49*, 2082−2089.

(25) O'Sullivan, L.; Galvin, K.; Aherne, S. A.; O'Brien, N. M. Effects of cooking on the profile and micellarization of 9-*cis*-, 13-*cis*- and all-*trans*-β-carotene in green vegetables. *Food Res. Int.* **2010**, *43*, 1130−1135.

(26) Ryan, L.; O'Connell, O.; O'Sullivan, L.; Aherne, S. A.; O'Brien, N. M. Micellarisation of carotenoids from raw and cooked vegetables. *Plant Foods Hum. Nutr.* **2008**, *63*, 127−133.

(27) 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.

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

(29) Fox, A. J.; Del Pozo-Insfran, D.; Lee, J. H.; Sargent, S. A.; Talcott, S. T. Ripening-induced chemical and antioxidant changes in bell peppers as affected by harvest maturity and postharvest ethylene exposure. *HortScience* **2005**, *40*, 732−736.

(30) Chitchumroonchokchai, C.; Failla, M. L. Hydrolysis of zeaxanthin esters by carboxyl ester lipase during digestion facilitates micellarization and uptake of the xanthophyll by Caco-2 human intestinal cells. *J. Nutr.* **2006**, *136*, 588−594.

(31) Dhuique-Mayer, C.; Borel, P.; Reboul, E.; Caporiccio, B.; Besancon, P.; Amiot, M. β-Cryptoxanthin from *Citrus* juices: assessment of bioaccessibility using an *in vitro* digestion/Caco-2 cell culture model. *Br. J. Nutr.* **2007**, *97*, 883−890.

(32) Bowen, P. E.; Herbst-Espinosa, S. M.; Hussain, E. A.; Stacewicz-Sapuntzakis, M. Esterification does not impair lutein bioavailability in humans. *J. Nutr.* **2002**, *132*, 3668−3673.

(33) Breithaupt, D. E.; Weller, P.; Wolters, M.; Hahn, A. Comparison of plasma responses in human subjects after the ingestion of 3*R*,3*R*′-zeaxanthin dipalmitate from wolfberry (*Lycium barbarum*) and non-esterified 3*R*,3*R*′-zeaxanthin using chiral high-performance liquid chromatography. *Br. J. Nutr.* **2004**, *91*, 707−713.

(34) Wingerath, T.; Stahl, W.; Sies, H. β-Cryptoxanthin selectively increases in human chylomicrons upon ingestion of tangerine concentrate rich in β-cryptoxanthin esters. *Arch. Biochem. Biophys.* **1995**, *324*, 385−390.

(35) Castro, S. M.; Saraiva, J. A.; Lopes-da-Silva, J. A.; Delgadillo, I.; Van Loey, A.; 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.

(36) Sila, D. N.; Doungla, E.; Smout, C.; Van Loey, A.; Hendrickx, M. Pectin fraction interconversions: insight into understanding texture evolution of thermally processed carrots. *J. Agric. Food Chem.* **2006**, *54*, 8471−8479.

(37) Dongowski, G. Influence of pectin structure on the interaction with bile acids under in vitro conditions. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 390−398.

(38) Pasquier, B.; Armand, M.; Castelain, C.; Guillon, F.; Borel, P.; Lafont, H.; Lairon, D. Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fibres in acidic gastric medium *in vitro*. *Biochem. J.* **1996**, *314*, 269−275.
(39) Hornero-Méndez, D.; Mínguez Mosquera, M. I. Bioaccessibility of carotenes from carrots: effect of cooking and addition of oil. *Innovative Food Sci. Emerging Technol.* **2007**, *8*, 407−412.