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Impact of *in vitro* digestion phases on the stability and bioaccessibility of carotenoids and their esters in mandarin pulps

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Electronic Supplementary Material

Table S1. Carotenoid contents in pulps of mandarin cvs. 'Ponkan', 'Rio' and 'Murcott', and in the aqueous phase containing the bioaccessible carotenoids after *in vitro* digestion

Carotenoid	Fresh fruit (μg/100g fresh weight)			Supernatant digesta (μg/100g fresh weight)		
	Ponkan	Rio	Murcott	Ponkan	Rio	Murcott
(13*Z*)- or (15*Z*)-β-cryptoxanthin	2.3 ± 0.4	3.3 ± 0.7	nd	2.8 ± 0.7	0.7 ± 0.08	nd
(all-*E*)-β-cryptoxanthin	101.1 ± 3.2	84.8 ± 2.2	83.5 ± 2.9	41.9 ± 1.8	27.7 ± 0.2	32.9 ± 2.1
phytoene	35.7 ± 2.1	24.5 ± 1.1	46.5 ± 1.7	11.1 ± 0.4	5.3 ± 0 .4	14.8 ± 1.2
phytofluene isomer 1	38.8 ± 0.6	23.7 ± 0.6	60.5 ± 2	7.6 ± 1.6	3.2 ± 0.5	17.2 ± 2.2
phytofluene isomer 2	2.5 ± 0.4	0.6 ± 0.2	nd	1.2 ± 0 .2	0.5 ± 0.1	nd
ζ-carotene isomer 1	124.0 ± 9.3	30.0 ± 1.1	206.5 ± 6 .6	20.1 ± 4.4	4.8 ± 0.5	52.2 ± 7.7
ζ-carotene isomer 2	10.2 ± 0.5	nd	17.3 ± 1.3	2.8 ± 0 .2	nd	4.9 ± 0.6
ζ-carotene isomer 3	23.6 ± 6.2	6.9 ± 0.7	28.9 ± 1.4	11.1 ± 1.9	1.9 ± 0.2	13.9 ± 0.5
(all-*E*)-β-carotene	75.6 ± 1.6	59.6 ± 0.4	67.7 ± 2.3	11.8 ± 1.7	16.4 ± 2.3	24.6 ± 4.6
ζ-carotene isomer 4	9.2 ± 2.3	0.9 ± 0.4	6.3 ± 1.5	3.1 ± 0.8	0.3 ± 0.09	3.9 ± 0.3
(9*Z*)-violaxanthin dilaurate	27.0 ± 0.1	5.5 ± 1.1	nd	nd	nd	nd
(13*Z*)- or (15*Z*)-β-cryptoxanthin laurate	73.9 ± 4.0	20.9 ± 2.0	77.4 ± 13.9	13.5 ± 0.4	5.0 ± 0.9	23.7 ± 3.3
(9*Z*)-violaxanthin laurate-myristate	48.7 ± 1.4	17.8 ± 4.0	nd	nd	nd	nd
ni. (all-*E*)-β-cryptoxanthin ester	45.5 ± 2.2	nd	27.4 ± 5.6	6.9 ± 1.3	nd	9.7 ± 2.6
(13*Z*)- or (15*Z*)-β-cryptoxanthin	48.9 ± 1.1	14.2 ± 0.5	48.9 ± 2.1	6.7 ± 0.6	2.5 ± 0. 4	15.5 ± 3.0

myristoleate						
(all-*E*)-β-cryptoxanthin laurate	339.7 ± 3.4	150.4 ± 4.7	340.0 ± 7.2	56.1 ± 3.8	33.7 ± 5.4	109.8 ± 13.9
(9*Z*)-β-cryptoxanthin laurate	29.9 ± 1.0	12.7 ± 2.5	46.9 ± 4.8	5.8 ± 0.3	3.9 ± 1. 2	15.9 ± 3.2
(13*Z*)- or (15*Z*)-β-cryptoxanthin palmitoleate	58.2 ± 2.7	36.7 ± 1.3	57.5 ± 4.6	9.6 ± 0.7	9.7 ± 2. 1	18.2 ± 2.7
(all-*E*)-violaxanthin myristate oleate + (9Z)- or (9'Z)-antheraxanthin dilaurate + (13*Z*)/(15*Z*)-β-cryptoxanthin oleate	23.8 ± 1.2	12.2 ± 2.5	39.9 ± 6.1	4.6 ± 0.5	2.2 ± 0. 6	10.4 ± 1.7
(all-*E*)-antheraxanthin laurate-myristate + (9*Z*)- or (9'*Z*)-luteoxanthin laurate-palmitate	22.1 ± 0.9	7.6 ± 0.5	18.3 ± 1.7	2.9 ± 0.2	1.8 ± 0.4	5.6 ± 1.2
(all-*E*)-β-cryptoxanthin myristate	280.5 ± 6.6	213.8 ± 6.2	267.8 ± 6.3	49.5 ± 6.3	52.3 ± 9.7	85.7 ± 12.5
(9*Z*)-β-cryptoxanthin myristate	nd	nd	12.9 ± 0.4	nd	nd	4.8 ± 1.0
(all-*E*)-β-cryptoxanthin oleate	31.1 ± 1.7	47.8 ± 1.1	46.5 ± 1.1	6.8 ± 0.7	12.2 ± 2 .4	17.9 ± 2.7
(9*Z*)-β-cryptoxanthin oleate + (9*Z*)-antheraxanthin laurate-myristate + (9*Z*)-zeinoxanthin myristate	38.7 ± 3.9	19.8 ± 2.2	46.5 ± 7.6	8.0 ± 1.3	4.2 ± 0. 9	14.4 ± 2.1
(13*Z*)- or (15*Z*)-β-cryptoxanthin palmitate + (all-*E*)-antheraxanthin dimyristate	24.8 ± 1.1	24.9 ± 1.1	24.9 ± 2.3	2.8 ± 0.4	4.4 ± 1. 0	7.8 ± 1.6
lutein 3-O-myristate-3'-O-laurate + lutein 3-O-laurate-3-O'-myristate	19.8 ± 1.5	17.1 ± 1.2	41.6 ± 1.2	2.7 ± 0.2	4.3 ± 1. 3	12.4 ± 2.7
(all-*E*)-β-cryptoxanthin palmitate	73.1 ± 2.8	103.3 ± 6.0	129.8 ± 4.6	15.1 ± 1.9	29.1 ± 6.3	43.5 ± 7.8
lutein 3-O-palmitate-3'-O-laurate	3.6 ± 0.7	6.9 ± 0.4	11.3 ± 1.6	0.2 ± 0.1	1.2 ± 0.5	3.5 ± 1.7
(9*Z*)-β-cryptoxanthin palmitate	24.5 ± 0.6	19.8 ± 0.7	25.5 ± 2.9	4.7 ± 0.2	4.9 ± 0. 9	7.7 ± 1.9
Total carotenoids	1647 ± 53	965 ± 22	1772 ± 57	309 ± 23	235 ± 37	593 ± 88

Results are expressed as mean ± standard deviation of triplicate analysis (n = 3).

Table S2. Sum of the contents of *Z*- and all-*E*- isomers of β-cryptoxanthin (free and esterified with lauric, myristic and palmitic acids), and respective percentages, in frozen pulp and in the different phases of *in vitro* digestion

Carotenoid	Carotenoid contents (μg/100g)					
	Frozen pulp	Oral phase	Oral + Gastric phases		Oral + Gastric + Duodenal phases	
			Digesta	Sup + Ppt	Digesta	Sup + Ppt
(13*Z*)- or (15*Z*)-β-cryptoxanthin	9.4 ± 1.7 (9.1%)	9.2 ± 2.3 (8.9%)	10.2 ± 0.1 (11.6%)	7.4 ± 1.1 (9.2%)	10.6 ± 1.1 (9.9%)	8.9 ± 1.1 (8.9%)
(all-*E*)-β-cryptoxanthin	93.5 ± 1.5 (90.9%)	93.8 ± 7.7 (91.1%)	78.6 ± 7.6 (88.4%)	73.3 ± 6.0 (90.8%)	96.5 ± 3.9 (90.1%)	91.1 ± 8.3 (91.1%)
Σ β-cryptoxanthin	**102.9 ± 3.2**	**103.0 ± 9.2**	**88.9 ± 7.4**	**80.7 ± 6.2**	**107.1 ± 2.9**	**100.0 ± 8.3**
(13Z)- or (15Z)-β-cryptoxanthin laurate	99.0 ± 8.3 (18.3%)	94.9 ± 6.5 (18.2%)	75.6 ± 11.8 (17.4%)	77.4 ± 4.5 (17.6%)	73.4 ± 6.6 (17.8%)	70.7 ± 0.3 (17.5%)
(all-*E*)-β-cryptoxanthin laurate	382.3 ± 6.4 (70.6%)	357.9 ± 24.5 (68.5%)	306.6 ± 51.3 (70.7%)	308.4 ± 19.1 (70.1%)	287.7 ± 24.3 (69.9%)	282.4 ± 1.5 (69.9%)
(9*Z*)-β-cryptoxanthin laurate	60.2 ± 4.7 (11.1%)	69.4 ± 8.0 (13.3%)	51.2 ± 13.9 (11.8%)	54.3 ± 4.3 (12.3%)	50.6 ± 3.9 (12.3%)	51.0 ± 0.1 (12.6%)
Σ β-cryptoxanthin laurate	**541.5 ± 8.9**	**522.2 ± 37.4**	**433.4 ± 47.0**	**440.2 ± 27.9**	**411.7 ± 33.8**	**404.2 ± 1.4**
(all-*E*)-β-cryptoxanthin myristate	281.4 ± 5.1 (94.4%)	265.6 ± 19.8 (94.6%)	223.5 ± 45.9 (94.8%)	226.5 ± 13.5 (94.8%)	205.4 ± 16.3 (94.9%)	205.9 ± 2.3 (95.0%)
(9*Z*)-β-cryptoxanthin myristate	16.8 ± 2.1 (5.6%)	15.3 ± 1.8 (5.4%)	12.1 ± 3.7 (5.2%)	12.4 ± 1.2 (5.2%)	10.9 ± 0.6 (5.1%)	10.8 ± 0.7 (5.0%)
Σ β-cryptoxanthin myristate	**298.3 ± 4.1**	**280.9 ± 21.5**	**235.7 ± 49.6**	**238.9 ± 14.2**	**216.3 ± 16.5**	**216.7 ± 1.9**
(all-*E*)-β-cryptoxanthin palmitate	127.8 ± 4.8 (82.7%)	120.3 ± 11.2 (82.6%)	99.8 ± 28.5 (83.1%)	103.9 ± 6.1 (83.2%)	90.5 ± 6.1 (83.1%)	95.1 ± 2.5 (81.1%)
(9*Z*)-β-cryptoxanthin palmitate	26.7 ± 5.2 (17.3%)	25.4 ± 4.3 (17.4%)	20.4 ± 5.1 (16.9%)	20.9 ± 3.0 (16.8%)	18.4 ± 1.4 (16.9%)	22.1 ± 3.9 (18.9%)
Σ β-cryptoxanthin palmitate	**154.6 ± 9.9**	**145.7 ± 15.1**	**120.1 ± 33.5**	**124.9 ± 9.1**	**108.9 ± 6.4**	**117.3 ± 6.4**
Σ (all-*E*)-β-cryptoxanthin esters	872.0 ± 6.2 (73.4%)	818.6 ± 58.1 (72.0%)	689.1 ± 139.0 (73.9%)	697.5 ± 41.6 (73.1%)	641.3 ± 48.7 (72.6%)	642.0 ± 2.1 (72.2%)

Σ (Z)-β-cryptoxanthin esters	316.4 ± 21.8 (26.6%)	317.8 ± 26.4 (28.0%)	242.6 ± 40.2 (26.1%)	257.2 ± 18.5 (26.9%)	241.6 ± 16.1 (27.4%)	246.8 ± 2.3 (27.3)
Σ β-cryptoxanthin esters	1188.4 ± 16.4	1136.4 ± 84.4	931.7 ± 179.9	954.6 ± 60.1	882.9 ± 63.4	888.9 ± 2.5

Digesta refers to the whole material resultant from each digestion phase, containing the compounds liberated from the matrix and the undigested material. When digesta was submitted to a centrifugation process, a supernatant fraction (Sup) containing the carotenoids liberated from the matrix and a precipitated fraction (Ppt) corresponding to the undigested material was obtained. Percent value of each carotenoid was calculated in relation to the sum of all isomers of this carotenoid.

Table S3. Sum of the contents of all isomer forms of phytofluene and ζ-carotene and respective percentages, in frozen pulp and in the different phases of *in vitro* digestion

Carotenoid	Carotenoid contents (µg/100g)					
	Frozen pulp	Oral phase	Oral + Gastric phases		Oral + Gastric + Duodenal phases	
			Digesta	Sup + Ppt	Digesta	Sup + Ppt
phytofluene isomer 1	63.6 ± 2.4 (100%)	56.3 ± 3.8 (100%)	48.5 ± 5.9 (87.7%)	54.3 ± 3.8 (91.3%)	40.9 ± 0.7 (91.1%)	40.7 ± 2.1 (88.9%)
phytofluene isomer 2	<LOQ	<LOQ	6.8 ± 1.8 (12.3%)	5.2 ± 0.3 (8.7%)	4.0 ± 0.5 (8.9%)	5.1 ± 0.6 (11.1%)
Σ phytofluene	63.6 ± 2.4	56.3 ± 3.8	**55.3 ± 7.7**	**59.5 ± 3.9**	**44.9 ± 0.2**	**45.8 ± 2.5**
ζ-carotene isomer 1	203.4 ± 8.3 (82.2%)	187.1 ± 11.9 (81.8%)	147.2 ± 19.3 (69.6%)	140.8 ± 14.6 (64.6%)	115.4 ± 8.4 (66.3%)	112.3 ± 4.4 (66.9%)
ζ-carotene isomer 2	9.3 ± 0.3 (3.8%)	8.2 ± 1.7 (3.6%)	11.4 ± 0.3 (5.4%)	12.3 ± 0.5 (5.6%)	9.9 ± 1.8 (5.7%)	9.2 ± 0.9 (5.5%)
ζ-carotene isomer 3	28.7 ± 0.3 (11.6%)	25.9 ± 2.7 (11.3%)	43.0 ± 7.7 (20.3%)	49.3 ± 1.8 (22.6%)	40.3 ± 6.9 (23.1%)	37.9 ± 3.1 (22.6%)
ζ-carotene isomer 4	5.8 ± 2.4 (2.3%)	7.9 ± 2.5 (3.4%)	9.8 ± 0.4 (4.6%)	15.4 ± 2.6 (7.1%)	8.5 ± 0.4 (4.9%)	8.6 ± 1.3 (5.1%)
Σ ζ-carotene	**247.5 ± 5.8**	**229.1 ± 15.9**	**211.4 ± 27.1**	**217.8 ± 10.8**	**174.1 ± 7.9**	**167.9 ± 5.2**

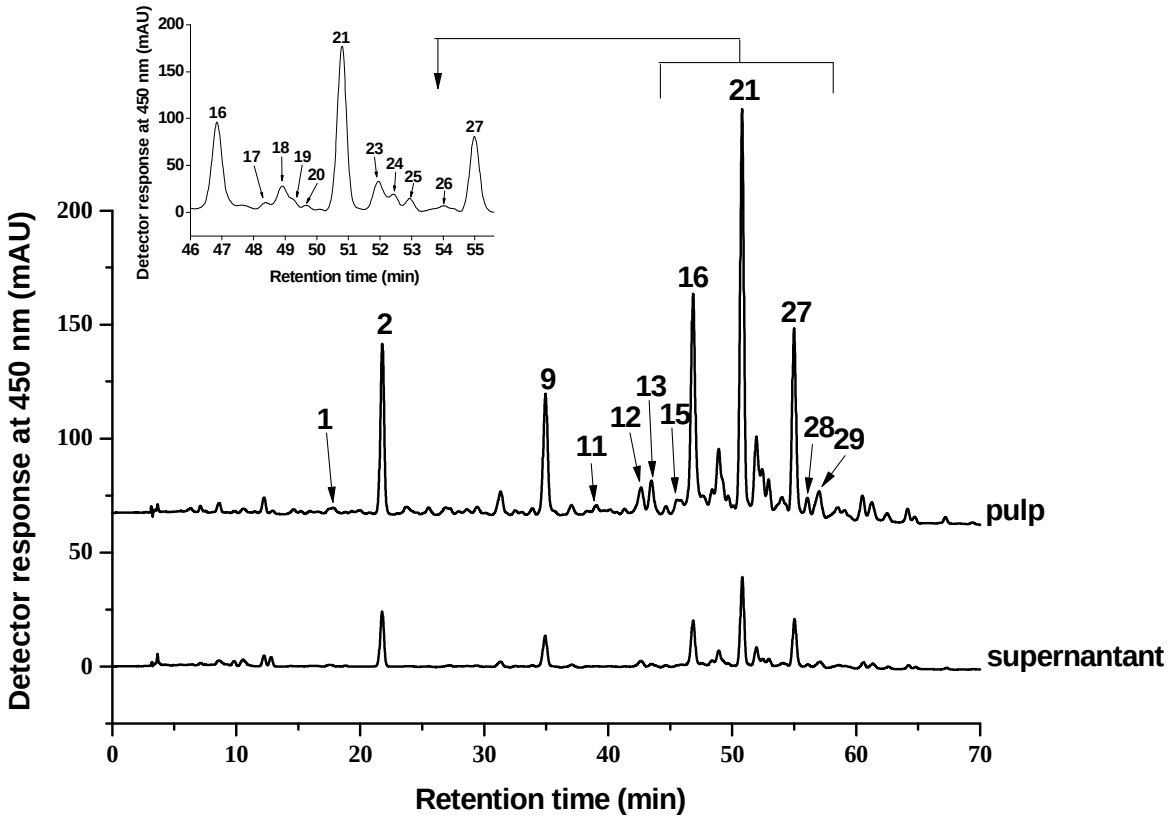

Figure S1. Chromatograms, obtained by HPLC-DAD at 450 nm, of carotenoid extracts from mandarin cv. 'Rio' before and after *in vitro* digestion.

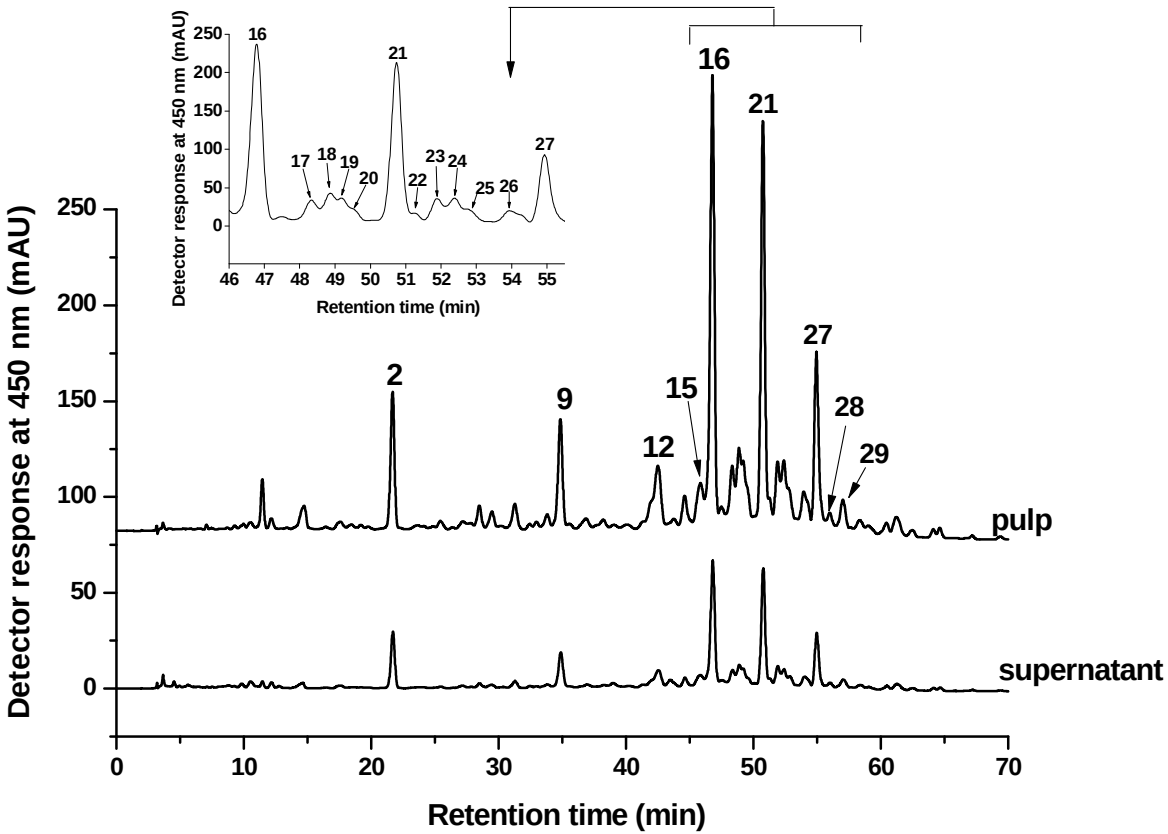

Figure S2. Chromatograms, obtained by HPLC-DAD at 450 nm, of carotenoid extracts from tangor cv. 'Murcott' before and after *in vitro* digestion.