

Appendix A: Supplementary files to this article

Table S1a. Significance of wheat cultivar, cooking duration and their interactions on the concentrations and of all- trans carotenoids (mg/100g dry weight) in undigested and digested refined semolina (RS) Pasta

	AI	ND								
Transcend	FCT	ND								
	OC	ND								

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different ($p>0.05$). ^aCooking duration: AI, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected (< LOD), all-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

Table S1b. Significance of wheat cultivar, cooking duration and their interactions on the concentration of cis- carotenoids (mg/100g dry weight) in undigested and digested refined semolina (RS) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion Stage			Recovery (%)				Bioaccessibility (%)	
				Oral	Oral+Gastric	Oral+Gastric+Intestinal	Oral Stage	Oral+Gastric Stage	Oral+Gastric+intestinal stage	Filtrate		
15-cis lutein	AAC Spitfire	Al	0.05a	0.04b	0.04a	0.06a	81.70abc	79.67a	117.13a	0.07ab	ND	129.85ab
		FCT	0.07a	0.05ab	0.04a	0.05a	69.81bc	58.28a	76.80a	0.06ab	ND	87.65ab
		OC	0.06a	0.05ab	0.03a	0.05a	86.11abc	49.85a	78.23a	0.06ab	ND	102.37ab
	CDC Precision	Al	0.05a	0.05ab	0.04a	0.05a	101.83ab	75.25a	91.99a	0.08ab	ND	152.10ab
		FCT	0.06a	0.04ab	0.03a	0.04a	76.33bc	49.91a	70.27a	0.07ab	ND	116.51ab
		OC	0.06a	0.05ab	0.03a	0.05a	83.47abc	61.02a	87.15a	0.04b	ND	74.56b
	Transcend	Al	0.05a	0.06a	0.03a	0.05a	114.22a	62.80a	104.57a	0.11a	ND	195.23a
		FCT	0.06a	0.05ab	0.04a	0.06a	97.42ab	74.40a	100.11a	0.07ab	ND	122.41ab
		OC	0.07a	0.04b	0.03a	0.04a	57.16c	50.88a	57.83a	0.05b	ND	76.12b
<i>Pooled SE</i>			0.004	0.004	0.006	0.006	0.078	0.097	0.128	0.012		22.121
	<i>V</i>		0.350	0.409	0.872	0.268	0.250	0.996	0.767	0.380		0.397
		<i>CD</i>	0.084	0.046	0.468	0.066	0.003	0.076	0.021	0.005		0.002
	<i>V*CD</i>		0.259	0.002	0.587	0.258	0.003	0.251	0.158	0.219		0.327

			Al	0.06b	0.05b	0.05a	0.08a	83.61ab	80.36a	132.38a	0.09bcd	ND	134.39abc
		AAC Spitfire	FCT	0.08ab	0.06ab	0.05a	0.07a	71.5b	64.60a	85.28a	0.09bcd	ND	103.24bc
			OC	0.08ab	0.07ab	0.05a	0.08a	90.76ab	55.17a	99.87a	0.09bcd	ND	112.61abc
13-cis lutein	CDC Precision	Al	0.08ab	0.07ab	0.05a	0.07a	93.38ab	63.06a	86.74a	0.12a	ND	156.90a	
		FCT	0.09a	0.07ab	0.05a	0.08a	82.43ab	51.28a	85.92a	0.13a	ND	130.16abc	
		OC	0.09a	0.08a	0.06a	0.10a	82.90ab	64.31a	103.18a	0.08cd	ND	86.46c	
	Transcend	Al	0.07ab	0.08a	0.04a	0.07a	117.76a	53.72a	99.27a	0.11abc	ND	146.19ab	
		FCT	0.08ab	0.07ab	0.06a	0.09a	99.92ab	73.53a	116.80a	0.11ab	ND	137.24abc	
		OC	0.09ab	0.05b	0.05a	0.06a	56.94b	55.45a	74.45a	0.08d	ND	87.83c	
<i>Pooled SE P-value</i>	<i>V</i>	<i>0.007</i>	<i>0.005</i>	<i>0.009</i>	<i>0.008</i>	<i>0.097</i>	<i>0.085</i>	<i>0.141</i>	<i>0.005</i>				<i>10.287</i>
		<i>0.090</i>	<i>0.011</i>	<i>0.756</i>	<i>0.590</i>	<i>0.490</i>	<i>0.554</i>	<i>0.483</i>	<i>0.000</i>				<i>0.604</i>
		<i>0.003</i>	<i>0.865</i>	<i>0.522</i>	<i>0.297</i>	<i>0.037</i>	<i>0.562</i>	<i>0.480</i>	<i><.0001</i>				<i><.0001</i>
		<i>0.806</i>	<i>0.000</i>	<i>0.577</i>	<i>0.051</i>	<i>0.011</i>	<i>0.119</i>	<i>0.070</i>	<i>0.002</i>				<i>0.052</i>
	AAC Spitfire	Al	0.04c	0.03b	0.03a	0.05ab	89.83ab	90.44a	144.55a	0.06bc	ND	164.76a	
		FCT	0.05abc	0.04ab	0.04a	0.05b	80.62ab	80.27a	100.28a	0.06c	ND	124.53abc	
		OC	0.05ab	0.05a	0.04a	0.06ab	96.58ab	67.82a	116.81a	0.07abc	ND	126.97abc	
13'-cis lutein	CDC Precision	Al	0.04bc	0.05ab	0.04a	0.04b	109.63ab	80.16a	101.28a	0.07abc	ND	172.00a	
		FCT	0.05ab	0.05a	0.04a	0.05ab	96.37ab	66.99a	102.78a	0.08a	ND	143.14abc	
		OC	0.06a	0.05a	0.05a	0.07a	88.79ab	80.29a	125.38a	0.06bc	ND	105.48c	
	Transcend	Al	0.04bc	0.05a	0.03a	0.04b	124.32a	62.29a	108.58a	0.07abc	ND	158.50ab	
		FCT	0.05abc	0.05a	0.04a	0.06ab	104.54ab	82.71a	128.27a	0.08ab	ND	150.85abc	
		OC	0.05ab	0.04ab	0.04a	0.05ab	67.32b	70.29a	93.21a	0.06bc	ND	111.61bc	
<i>Pooled SE P-value</i>	<i>V</i>	<i>0.003</i>	<i>0.003</i>	<i>0.006</i>	<i>0.005</i>	<i>0.099</i>	<i>0.106</i>	<i>0.149</i>	<i>0.003</i>				<i>10.580</i>
		<i>0.052</i>	<i>0.004</i>	<i>0.678</i>	<i>0.500</i>	<i>0.413</i>	<i>0.675</i>	<i>0.605</i>	<i>0.019</i>				<i>0.980</i>
		<i><.0001</i>	<i>0.563</i>	<i>0.247</i>	<i>0.010</i>	<i>0.024</i>	<i>0.841</i>	<i>0.797</i>	<i>0.015</i>				<i><.0001</i>
		<i>0.971</i>	<i>0.000</i>	<i>0.643</i>	<i>0.036</i>	<i>0.037</i>	<i>0.329</i>	<i>0.104</i>	<i>0.008</i>				<i>0.228</i>
	AAC	Al	0.05b	0.04c	0.04a	0.06a	72.22bcd	86.65a	121.66a	0.06b	ND	118.64abc	
		FCT	0.07ab	0.04bc	0.05a	0.05a	62.85cd	68.18a	79.43a	0.06b	ND	87.60c	

Spitfire												
		OC	0.06ab	0.05abc	0.04a	0.06a	83.37abcd	63.62a	96.34a	0.06b	ND	96.38bc
9-cis lutein	CDC Precision	Al	0.06ab	0.06ab	0.05a	0.05a	94.32abc	74.76a	88.42a	0.09a	ND	146.18a
		FCT	0.07a	0.06ab	0.04a	0.05a	82.28abcd	60.99a	79.56a	0.10a	ND	138.34ab
		OC	0.07a	0.06ab	0.05a	0.07a	78.47abcd	72.74a	97.85a	0.06b	ND	84.71c
	Transcend	Al	0.06ab	0.06a	0.03a	0.05a	109.10a	58.92a	91.71a	0.08a	ND	136.85ab
		FCT	0.06ab	0.06ab	0.05a	0.07a	98.29ab	85.97a	111.35a	0.08a	ND	139.84ab
		OC	0.07a	0.04bc	0.04a	0.05a	56.82d	56.24a	68.16a	0.06b	ND	85.02c
<i>Pooled SE</i> <i>P-value</i>	<i>Pooled SE</i>		0.004	0.004	0.008	0.007	0.072	0.127	0.126	0.004		9.392
	<i>V</i>		0.082	<.0001	0.678	0.736	0.035	0.857	0.555	<.0001		0.018
	<i>CD</i>		0.002	0.746	0.687	0.668	0.012	0.644	0.412	<.0001		<.0001
	<i>V*CD</i>		0.225	0.001	0.679	0.127	0.001	0.317	0.051	0.002		0.020
9'-cis Lutein	AAC Spitfire	Al	0.04c	0.03c	0.04a	0.05a	77.07bcd	91.02a	129.92a	0.05b	ND	124.81bc
		FCT	0.06ab	0.04bc	0.04a	0.05a	65.60cd	69.96a	83.79ab	0.05b	ND	90.18d
		OC	0.06ab	0.05abc	0.04a	0.06a	86.99abcd	66.05a	104.15ab	0.06b	ND	94.51cd
	Transcend	Al	0.05bc	0.05ab	0.04a	0.05a	98.53abc	84.59a	96.88ab	0.09a	ND	161.72a
		FCT	0.06ab	0.05ab	0.04a	0.05a	88.53abcd	68.19a	88.01ab	0.09a	ND	148.17ab
		OC	0.06ab	0.06a	0.05a	0.07a	87.72abcd	76.10a	113.82ab	0.06b	ND	87.52d
<i>Pooled SE</i> <i>P-value</i>	<i>Pooled SE</i>		0.003	0.004	0.008	0.007	0.070	0.149	0.126	0.003		6.915
	<i>V</i>		0.023	0.000	0.596	0.596	0.010	0.964	0.757	<.0001		<.0001
	<i>CD</i>		<.0001	0.889	0.557	0.333	0.006	0.596	0.468	<.0001		<.0001
	<i>V*CD</i>		0.014	0.000	0.839	0.103	0.000	0.385	0.014	<.0001		0.0004

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different ($p>0.05$). ^aCooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected (<LOD).

Table S2a. Significance of wheat cultivar, cooking duration and their interactions on the concentration of all- trans carotenoids (mg/100g dry weight) in undigested and digested whole wheat (WWF) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion Stage			Recovery (%)				Bio-accessibility (%)		
				Oral	Oral+Gastric	Oral + Gastric + Intestinal	Oral Stage	Oral +Gastric Stage	Oral +Gastric + intestinal stage	Filtrate			
All- trans Lutein	AAC Spitfire	Al	0.97a	1.07a	1.03a	1.00a	109.06ab	100.86a	104.78a	0.97ab	0.24b	99.13b	
		FCT	0.84a	0.78a	0.92a	0.92a	94.84ab	108.21a	109.13a	0.77b	0.48ab	96.51b	
		OC	0.83a	0.90a	1.41a	1.05a	112.23ab	175.43a	132.44a	0.95ab	0.33ab	116.45ab	
	CDC Precision	Al	0.82a	0.90a	1.15a	0.87a	109.99ab	138.8a	107.67a	0.81b	0.26ab	97.52b	
		FCT	0.98a	0.67a	1.84a	1.09a	69.78b	198.76a	111.66a	0.87ab	0.41ab	89.17b	
		OC	1.09a	1.03a	1.17a	0.96a	95.72ab	118.64a	91.97a	1.24ab	0.56ab	116.71ab	
	Transcend	Al	0.78a	1.01a	1.39a	0.99a	131.79a	176.74a	129.21a	1.31a	0.47ab	170.43a	
		FCT	0.99a	0.82a	1.29a	1.09a	83.69ab	130.94a	110.45a	1.25ab	0.58ab	126.82ab	
		OC	0.95a	1.10a	1.04a	1.12a	119.57ab	120.86a	122.63a	1.12ab	0.58a	119.92ab	
<i>Pooled SE P-value</i>			0.070	0.104	0.232	0.074	0.130	0.321	0.121	0.101	0.063	12.748	
			V	0.363	0.448	0.371	0.256	0.179	0.662	0.231	0.001	0.010	0.002
			CD	0.137	0.010	0.664	0.272	0.009	0.948	0.864	0.255	0.011	0.211
			V*CD	0.052	0.602	0.116	0.296	0.746	0.128	0.274	0.033	0.199	0.070

			Al	0.02a	0.02ab	0.02a	0.03ab	108.4a	89.76a	135.04a	0.02cd	0.03d	114.82c
	AAC Spitfire	FCT		0.02a	0.02bc	0.02a	0.03ab	93.67a	97.74a	137.62a	0.02d	0.05a	113.85c
		OC		0.02a	0.02abc	0.03a	0.03ab	101.23a	135.97a	158.35a	0.03bcd	ND	135.35abc
All trans Zeaxanthin	CDC Precision	Al	0.02a	0.02bc	0.02a	0.02b	101.6a	120.15a	133.22a	0.02d	ND	120.72bc	
		FCT	0.02a	0.02c	0.03a	0.03ab	91.98a	181.87a	153.55a	0.02cd	0.04bc	130.29abc	
		OC	0.02a	0.02abc	0.02a	0.03ab	96.37a	112.75a	132.06a	0.03abcd	ND	141.79abc	
			Al	0.02a	0.02ab	0.03a	0.03ab	114.24a	146.58a	155.35a	0.03a	0.04cd	184.42ab
	Transcend	FCT		0.02a	0.02abc	0.03a	0.03a	114.19a	177.31a	185.42a	0.03ab	0.04bc	197.94a
		OC		0.02a	0.02a	0.03a	0.03a	129.07a	138.26a	175.86a	0.03abc	0.04ab	176.88abc
Pooled SE			0.001	0.001	0.004	0.002	0.090	0.213	0.136	0.002	0.001	14.593	
P-value	V		0.245	0.001	0.093	0.001	0.012	0.047	0.013	<.0001	0.029	<.0001	
	CD		0.145	0.006	0.480	0.124	0.417	0.172	0.260	0.182	0.000	0.632	
	V*CD		0.335	0.171	0.255	0.440	0.752	0.183	0.560	0.054	0.001	0.638	

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different (p>0.05). [#]Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected (< LOD), all-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

Table S2b. Significance of wheat cultivar, cooking duration and their interactions on the concentration of cis- carotenoids (mg/100g dry weight) in undigested and digested whole wheat (WWF) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion Stage			Recovery (%)			Bioaccessibility (%)			
				Oral	Oral+Gastric	Oral + Gastric + Intestinal	Oral Stage	Oral +Gastric Stage	Oral +Gastric+intestinal stage				
				Filtrate	Residue								
15-cis lutein	AAC Spitfire	Al	0.06a	0.05a	0.05a	0.04ab	94.46ab	87.85a	77.56a	0.05ab	ND	88.56a	
		FCT	0.04a	0.04abc	0.05a	0.04ab	89.88ab	106.86a	86.31a	0.04ab	ND	93.19a	
		OC	0.05a	0.04abc	0.06a	0.05a	86.59ab	112.7a	95.56a	0.04ab	ND	88.54a	
	CDC Precision	Al	0.04a	0.04abc	0.05a	0.03b	98.38ab	106.27a	81.68a	0.04b	ND	88.90a	
		FCT	0.05a	0.03c	0.05a	0.04ab	60.24b	105.98a	75.32a	0.04b	ND	79.33a	
		OC	0.05a	0.03bc	0.04a	0.04ab	69.68ab	83.18a	80.52a	0.04ab	ND	92.11a	
	Transcend	Al	0.04a	0.05ab	0.05a	0.04ab	111.1ab	118.71a	81.37a	0.05a	ND	118.23a	
		FCT	0.04a	0.04abc	0.05a	0.04ab	88.17ab	118.33a	93.56a	0.05ab	ND	118.03a	
		OC	0.04a	0.05ab	0.04a	0.05a	124.04a	98.27a	116.06a	0.05ab	ND	124.19a	
<i>Pooled SE</i>			0.004	0.003	0.007	0.003	0.117	0.160	0.101	0.003		12.850	
<i>P-value</i>		<i>V</i>	0.123	0.000	0.530	0.010	0.010	0.589	0.113	0.001		0.006	
		<i>CD</i>	0.716	0.000	0.724	0.014	0.086	0.647	0.119	0.357		0.900	

		<i>V*CD</i>	0.167	0.654	0.485	0.356	0.274	0.592	0.523	0.127		0.966		
13-cis lutein	AAC Spitfire	Al	0.09a	0.07ab	0.07a	0.08ab	82.74ab	78.25a	93.48a	0.08a	0.05d	95.06a		
		FCT	0.09a	0.07ab	0.08a	0.08ab	74.74ab	92.19a	95.88a	0.08a	0.09a	95.27a		
		OC	0.09a	0.07ab	1.10a	0.10ab	82.15ab	109.79a	112.25a	0.10a	0.06cd	106.78a		
	CDC Precision	Al	0.08a	0.06ab	0.07a	0.08b	72.16ab	90.42a	96.73a	0.08a	0.05d	99.26a		
		FCT	0.09a	0.05b	0.11a	0.10ab	56.48b	137.87a	117.71a	0.09a	0.07cd	98.09a		
		OC	0.11a	0.08ab	0.09a	0.09ab	67.94ab	86.19a	85.80a	0.12a	0.07bc	104.70a		
	Transcend	Al	0.08a	0.08ab	0.10a	0.08ab	97.64ab	120.65a	102.19a	0.11a	0.07bc	133.25a		
		FCT	0.09a	0.07ab	0.10a	0.10ab	85.92ab	143.49a	119.92a	0.11a	0.08abc	134.11a		
		OC	0.09a	0.08a	0.08a	0.10a	113.32a	96.54a	135.18a	0.11a	0.09ab	146.37a		
<i>Pooled SE</i>			0.010	0.006	0.015	0.005	0.112	0.262	0.176	0.008	0.003	18.657		
<i>P-value</i>			<i>V</i>	0.401	0.013	0.755	0.608	0.004	0.469	0.335	0.014	0.001	0.027	
			<i>CD</i>	0.194	0.014	0.408	0.001	0.230	0.353	0.555	0.032	<.0001	0.748	
			<i>V*CD</i>	0.540	0.636	0.392	0.279	0.864	0.649	0.578	0.188	0.001	0.999	
13'-cis lutein	AAC Spitfire	Al	0.05ab	0.03bc	0.04a	0.05cd	65.28abc	70.44a	103.69a	0.05c	0.04de	103.54a		
		FCT	0.05ab	0.04bc	0.04a	0.06bcd	67.43abc	80.44a	108.40a	0.05bc	0.07ab	105.76a		
		OC	0.06ab	0.04ab	0.06a	0.07ab	73.54abc	105.73a	115.51a	0.07abc	0.05cde	114.05a		
	CDC Precision	Al	0.05ab	0.03c	0.04a	0.05d	54.02bc	79.06a	103.8a	0.05c	0.04e	104.40a		
		FCT	0.06ab	0.03c	0.07a	0.07abc	41.33c	105.07a	107.05a	0.06abc	0.05cd	93.53a		
		OC	0.07a	0.04ab	0.06a	0.06abcd	62.38abc	87.14a	94.02a	0.08a	0.05cd	115.89a		
	Transcend	Al	0.05b	0.04abc	0.05a	0.05d	84.56ab	108.18a	107.33a	0.07abc	0.05cd	149.97a		
		FCT	0.06ab	0.04abc	0.07a	0.07abc	68.18abc	129.02a	118.07a	0.08ab	0.06bc	136.03a		
		OC	0.06ab	0.05a	0.05a	0.08a	94.53a	95.42a	147.29a	0.08a	0.08a	159.03a		
<i>Pooled SE</i>			0.005	0.003	0.009	0.003	0.079	0.202	0.149	0.005	0.003	19.399		
<i>P-value</i>			<i>V</i>	0.244	0.001	0.456	0.251	0.000	0.289	0.186	0.001	0.000	0.017	
			<i>CD</i>	0.006	<.0001	0.061	<.0001	0.035	0.528	0.522	0.000	<.0001	0.534	

		<i>V*CD</i>	0.476	0.514	0.291	0.056	0.701	0.637	0.527	0.222	0.001	0.992	
9-cis lutein	AAC Spitfire	Al	0.06a	0.06a	0.06a	0.06a	102.67a	90.36a	94.13a	0.06ab	0.05b	93.40a	
		FCT	0.06a	0.05a	0.06a	0.06a	91.27a	99.21a	100.02a	0.05b	0.06a	91.22a	
		OC	0.06a	0.05a	0.08a	0.07a	98.50a	144.49a	118.6a	0.06ab	ND	103.86a	
	CDC Precision	Al	0.06a	0.05a	0.07a	0.05a	91.26a	116.05a	91.35a	0.05ab	ND	91.40a	
		FCT	0.07a	0.04a	0.10a	0.07a	67.29a	160.8a	103.51a	0.06ab	0.06ab	86.97a	
		OC	0.07a	0.06a	0.07a	0.06a	85.78a	107.11a	92.22a	0.07ab	ND	107.05a	
	Transcend	Al	0.05a	0.06a	0.08a	0.06a	122.39a	154.08a	116.79a	0.08a	0.05ab	146.88a	
		FCT	0.06a	0.05a	0.08a	0.07a	88.00a	133.02a	119.48a	0.07ab	0.06ab	128.46a	
		OC	0.06a	0.06a	0.06a	0.07a	133.25a	125.37a	141.4a	0.07ab	0.07a	149.53a	
<i>Pooled SE</i>			0.005	0.005	0.012	0.004	0.161	0.242	0.146	0.006	0.003	18.917	
<i>P-value</i>		<i>V</i>	0.101	0.303	0.293	0.224	0.058	0.425	0.047	0.002	0.311	0.007	
		<i>CD</i>	0.608	0.028	0.662	0.029	0.139	0.861	0.385	0.284	0.011	0.518	
		<i>V*CD</i>	0.504	0.674	0.129	0.257	0.818	0.232	0.788	0.115	0.132	0.992	
9'-cis Lutein	AAC Spitfire	Al	0.05a	0.05a	0.05a	0.06a	107.26a	90.607a	112.34a	0.05bcd	ND	98.12a	
		FCT	0.05a	0.04a	0.05a	0.05a	93.71a	103.55a	111.44a	0.04d	0.05a	91.93a	
		OC	0.05a	0.05a	0.07a	0.06a	101.06a	144.65a	124.18a	0.05abcd	ND	104.46a	
	CDC Precision	Al	0.05a	0.05a	0.06a	0.05a	98.74a	118.52a	101.88a	0.05cd	ND	91.11a	
		FCT	0.05a	0.04a	0.10a	0.06a	75.55a	183.00a	116.69a	0.05bcd	0.04b	95.06a	
		OC	0.06a	0.05a	0.07a	0.06a	91.99a	124.85a	106.42a	0.07ab	ND	121.26a	
	Transcend	Al	0.05a	0.05a	0.07a	0.06a	115.46a	152.35a	126.77a	0.08a	0.05a	162.61a	
		FCT	0.05a	0.04a	0.07a	0.06a	99.31a	150.38a	140.12a	0.07abc	0.05a	154.00a	
		OC	0.05a	0.06a	0.06a	0.06a	136.15a	127.7a	143.19a	0.06abcd	0.06a	154.14a	
<i>Pooled SE</i>			0.004	0.005	0.011	0.005	0.152	0.267	0.150	0.005	0.001	18.411	
<i>P-value</i>		<i>V</i>	0.120	0.541	0.110	0.345	0.092	0.308	0.073	<.0001	0.006	0.001	
		<i>CD</i>	0.467	0.011	0.566	0.280	0.226	0.525	0.637	0.143	0.056	0.677	
		<i>V*CD</i>	0.776	0.751	0.119	0.526	0.835	0.326	0.946	0.017	-	0.881	

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different ($p>0.05$). ^aCooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected (< LOD).

Table S3a. Significance of wheat cultivar, cooking duration and their interactions on the concentration of total all-trans, total all-cis carotenoids and total carotenoid content (mg/100 g dry weight) in undigested and digested refined semolina (RS) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion stage			
				Oral	Oral+Gastric	Oral + Gastric + Intestinal	Filtrate
Total all-trans	AAC Spitfire	Al	0.60b	0.40c	0.49a	0.88a	0.98d
		FCT	0.85ab	0.48bc	0.52a	0.71a	0.96d
		OC	0.86ab	0.66abc	0.43a	0.92a	0.94d
	CDC Precision	Al	0.74ab	0.66ab	0.52a	0.74a	1.55ab
		FCT	0.91a	0.68ab	0.49a	0.82a	1.60a
		OC	0.96a	0.73ab	0.61a	1.07a	0.84d
	Transcend	Al	0.69ab	0.78a	0.40a	0.79a	1.46abc
		FCT	0.73ab	0.72ab	0.62a	1.00a	1.18bcd
		OC	0.95a	0.47bc	0.50a	0.71a	1.10cd
Total all-Cis	AAC Spitfire	Pooled SE	0.065	0.056	0.1094	0.1053	0.08493
		P-value	V	0.156	0.001	0.796	0.859
		CD	0.000	0.943	0.723	0.530	0.000
	CDC Precision	V*CD	0.403	0.000	0.679	0.062	<.0001
		Al	0.24b	0.19c	0.21a	0.31a	0.33bc
		FCT	0.33ab	0.22bc	0.22a	0.26a	0.33bc
	Transcend	OC	0.32ab	0.27abc	0.19a	0.31a	0.34bc
		Al	0.29ab	0.28abc	0.21a	0.26a	0.45a
		FCT	0.34a	0.28abc	0.20a	0.28a	0.47a
	Pooled SE	OC	0.34a	0.29ab	0.25a	0.36a	0.31c
		P-value	V	0.254	0.004	0.825	0.799
		CD	0.001	0.882	0.731	0.584	<.0001
		V*CD	0.392	0.001	0.672	0.078	0.002

	AAC Spitfire	Al	0.84b	0.59c	0.70a	1.19a	1.31b	0.21c
		FCT	1.18ab	0.70bc	0.73a	0.97a	1.29b	0.26c
		OC	1.18ab	0.93abc	0.62a	1.23a	1.29b	0.28bc
Total carotenoids (HPLC- PDA)	CDC Precision	Al	1.02ab	0.94abc	0.73a	1.00a	2.00a	0.27bc
		FCT	1.25a	0.96ab	0.69a	1.10a	2.08a	0.41a
		OC	1.30a	1.02ab	0.86a	1.42a	1.15b	0.45a
	Transcend	Al	0.96ab	1.09a	0.56a	1.05a	1.92a	0.27c
		FCT	1.02ab	1.01ab	0.85a	1.33a	1.60ab	0.40ab
		OC	1.31a	0.68bc	0.70a	0.96a	1.41b	0.43a
	<i>Pooled SE</i>		0.084	0.074	0.146	0.137	0.097	0.025
	<i>P-value</i>	<i>V</i>	0.174	0.002	0.812	0.855	<.0001	<.0001
		<i>CD</i>	0.000	0.957	0.727	0.541	<.0001	<.0001
		<i>V*CD</i>	0.395	0.000	0.677	0.065	0.001	0.288
	AAC Spitfire	Al	0.40e	0.38e	0.27b	0.72ab	0.79b	0.33de
		FCT	0.50b	0.42de	0.33a	0.71ab	0.81b	0.52a
		OC	0.57a	0.57a	0.25b	0.73ab	0.78b	0.40bc
TCC	CDC Precision	Al	0.46bc	0.54ab	0.19c	0.65cd	0.90a	0.31e
		FCT	0.56a	0.51b	0.22c	0.68bc	0.92a	0.44b
		OC	0.54a	0.41de	0.26b	0.72ab	0.54d	0.42b
	Transcend	Al	0.42de	0.47c	0.12d	0.53e	0.76bc	0.30e
		FCT	0.45cd	0.42d	0.20c	0.62d	0.70c	0.35cde
		OC	0.55a	0.31f	0.28b	0.76a	0.45e	0.37bcd
	<i>Pooled SE</i>		0.008	0.008	0.006	0.011	0.014	0.013
	<i>P-value</i>	<i>V</i>	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		<i>CD</i>	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		<i>V*CD</i>	<.0001	<.0001	<.0001	<.0001	<.0001	0.000

Values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and variable are not significantly different (p>0.05). #Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). TCC; Total carotenoid content. ND; Not detected (<LOD). All-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

Table S3b. Significance of wheat cultivar, cooking duration and their interactions on the concentration of total all-trans, total all-cis carotenoids and total carotenoid content (mg/100 g dry weight) in undigested and digested whole wheat flour (WWF) Pasta.

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion stage				
				Oral	Oral+Gastric	Oral + Gastric + Intestinal	Filtrate	
Total All-trans	AAC Spitfire	Al	0.99a	1.09a	1.05a	1.03a	0.99ab	
		FCT	0.86a	0.80a	0.93a	0.95a	0.79b	
		OC	0.85a	0.91a	1.43a	1.08a	0.97ab	
	CDC Precision	Al	0.84a	0.92a	1.17a	0.89a	0.83b	
		FCT	1.00a	0.69a	1.87a	1.12a	0.89ab	
		OC	1.11a	1.05a	1.19a	0.99a	1.27ab	
	Transcend	Al	0.80a	1.04a	1.42a	1.02a	1.35a	
		FCT	1.00a	0.84a	1.32a	1.13a	1.28ab	
		OC	0.97a	1.12a	1.07a	1.15a	1.15ab	
<i>Pooled SE</i>			0.071	0.105	0.235	0.076	0.1023	
<i>P-value</i>			<i>V</i> 0.381	0.430	0.375	0.233	0.001	
			<i>CD</i> 0.146	0.010	0.662	0.268	0.253	
			<i>V*CD</i> 0.053	0.598	0.118	0.299	0.033	
Total All-Cis	AAC Spitfire	Al	0.31a	0.28ab	0.27a	0.29ab	0.30ab	
		FCT	0.29a	0.23ab	0.27a	0.29ab	0.27b	
		OC	0.31a	0.27ab	0.37a	0.34ab	0.32ab	
	CDC Precision	Al	0.28a	0.23ab	0.29a	0.26b	0.26b	
		FCT	0.32a	0.19b	0.42a	0.33ab	0.29ab	
		OC	0.36a	0.27ab	0.33a	0.32ab	0.39a	
	Transcend	Al	0.27a	0.28ab	0.36a	0.28ab	0.38ab	
		FCT	0.29a	0.25ab	0.37a	0.34ab	0.38ab	
		OC	0.29a	0.30a	0.30a	0.35a	0.37ab	
<i>Pooled SE</i>			0.026	0.021	0.053	0.019	0.025	
<i>P-value</i>			<i>V</i> 0.258	0.029	0.564	0.376	0.001	
			<i>CD</i> 0.225	0.009	0.511	0.002	<.0001	
			<i>V*CD</i> 0.474	0.722	0.272	0.271	<.0001	

		AAC	Al	1.30a	1.37a	1.31a	1.32a	1.29ab	0.35a
		Spitfire	FCT	1.14a	1.03a	1.20a	1.23a	1.06b	0.71a
			OC	1.16a	1.18a	1.80a	1.42a	1.29ab	0.45a
	Total carotenoids (HPLC-PDA)	CDC Precision	Al	1.11a	1.14a	1.46a	1.15a	1.09b	0.37a
			FCT	1.32a	0.88a	2.29a	1.45a	1.18ab	0.48a
			OC	1.48a	1.32a	1.52a	1.31a	1.65ab	0.59a
		Transcend	Al	1.07a	1.32a	1.78a	1.31a	1.73a	0.60a
			FCT	1.30a	1.08a	1.69a	1.46a	1.66ab	0.72a
			OC	1.26a	1.42a	1.37a	1.51a	1.51ab	0.75a
		<i>Pooled SE</i>		0.093	0.126	0.288	0.094	0.127	0.094
		<i>P-value</i>	<i>V</i>	0.329	0.306	0.416	0.255	0.001	0.020
			<i>CD</i>	0.198	0.010	0.648	0.124	0.213	0.039
			<i>V*CD</i>	0.072	0.618	0.141	0.288	0.040	0.336
		AAC	Al	0.78ab	1.04bc	1.15ab	0.65ab	0.83abc	0.49e
		Spitfire	FCT	0.75abc	1.07ab	1.09ab	0.68a	0.76c	1.05a
			OC	0.79a	1.13a	1.09ab	0.65ab	0.92abc	0.62bcd
TCC		CDC Precision	Al	0.64ef	0.96bc	1.02ab	0.64ab	0.74c	0.57cde
			FCT	0.69de	0.96cd	1.16a	0.62ab	0.92abc	0.67bc
			OC	0.73bcd	0.99bcd	0.97b	0.64ab	0.99ab	0.69b
		Transcend	Al	0.59f	0.86e	1.00ab	0.52c	1.02a	0.52de
			FCT	0.66e	0.92de	1.07ab	0.58bc	0.81bc	0.64bc
			OC	0.72cd	0.95d	1.06ab	0.62ab	0.99ab	0.59bcde
		<i>Pooled SE</i>		0.011	0.017	0.035	0.016	0.039	0.02
		<i>P-value</i>	<i>V</i>	<.0001	<.0001	0.066	<.0001	0.025	<.0001
			<i>CD</i>	<.0001	0.000	0.085	0.044	0.002	<.0001
			<i>V*CD</i>	0.000	0.390	0.027	0.019	0.002	<.0001

Values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and variable are not significantly different ($p>0.05$). #Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). TCC; Total carotenoid content. ND; Not detected (<LOD). All-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

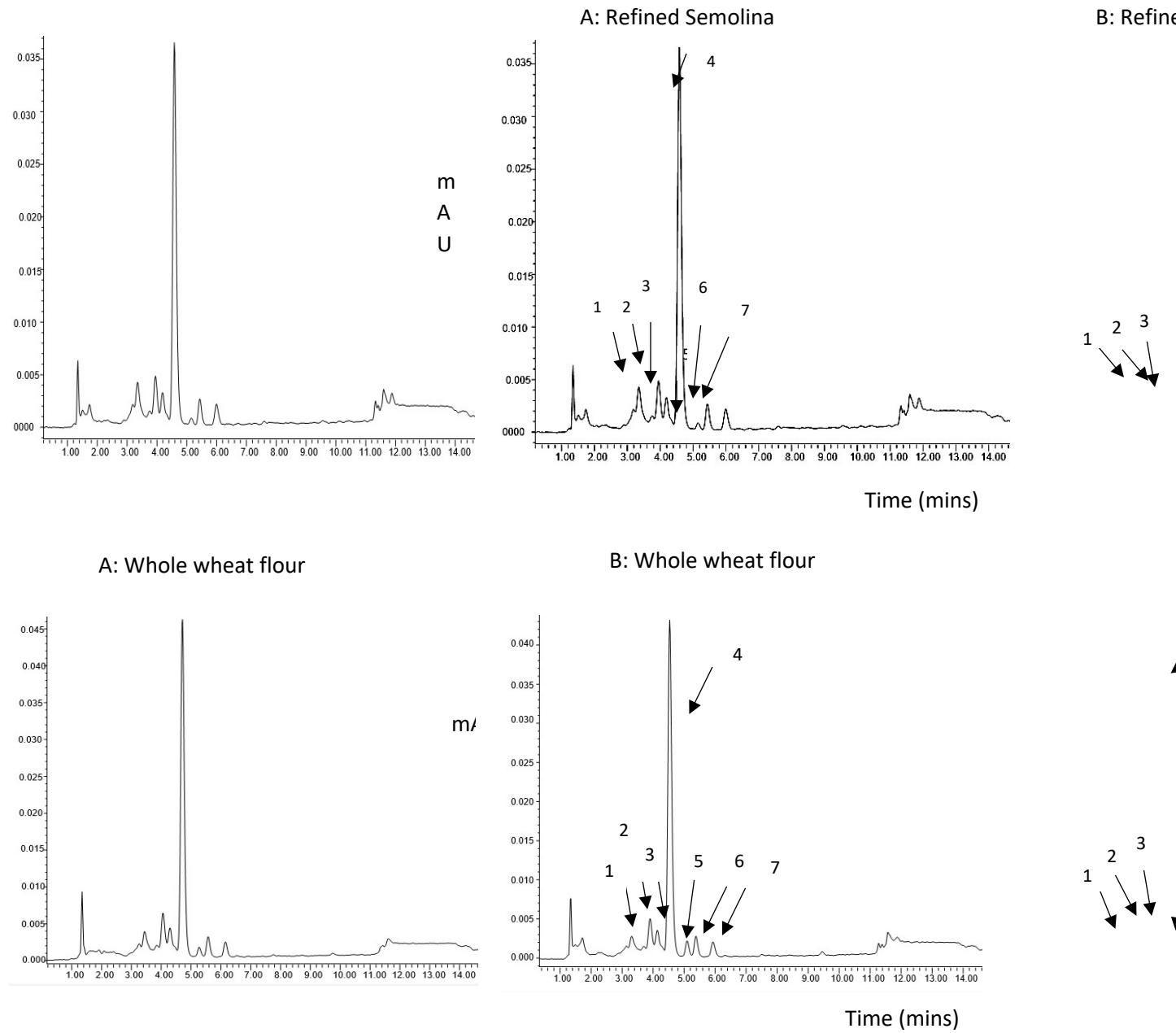


Fig. S1 Sample chromatograms obtained by HPLC -PDA at 450 nm of carotenoid extracts from undigested (A) and digested (B) refined semolina pasta and whole wheat flour pasta cooked to al dente of the Transcend wheat cultivar. Peak assignment: 1- 15-cis lutein, 2- 13-cis lutein, 3- 13'-cis lutein, 4- all-trans lutein, 5- all-trans zeaxanthin, 6- 9-cis lutein, 7- 9'-cis lutein.