



HAL
open science

The zeaxanthin present in a tomato line rich in this carotenoid is as bioavailable as that present in the food sources richest in this xanthophyll

Lisa Morand-Laffargue, Joseph Hirschberg, Charlotte Halimi, Charles Desmarchelier, Patrick Borel

► To cite this version:

Lisa Morand-Laffargue, Joseph Hirschberg, Charlotte Halimi, Charles Desmarchelier, Patrick Borel. The zeaxanthin present in a tomato line rich in this carotenoid is as bioavailable as that present in the food sources richest in this xanthophyll. *Food Research International*, 2023, 168, pp. 112751. 10.1016/j.foodres.2023.112751 . hal-04203812

HAL Id: hal-04203812

<https://hal.inrae.fr/hal-04203812>

Submitted on 11 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 The zeaxanthin present in a tomato line rich in this carotenoid is as bioavailable
2 as that present in the food sources richest in this xanthophyll.

3

4 Lisa MORAND-LAFFARGUE¹, Joseph HIRSCHBERG², Charlotte HALIMI¹, Charles
5 DESMARCHELIER^{1,3}, Patrick BOREL^{1*}

6

7 ¹ C2VN, INRAE, Aix-Marseille Univ, INSERM, Marseille, France.

8 ² Department of Genetics, Alexander Silberman Institute of Life Sciences, The Hebrew
9 University of Jerusalem, Israel.

10 ³ Institut Universitaire de France (IUF).

11

12 * Corresponding author: Patrick.Borel@univ-amu.fr

13 UMR C2VN "Center for CardioVascular and Nutrition Research of Marseille"

14 Faculté de Médecine

15 27, boulevard Jean Moulin

16 13005 Marseille, France

17 Phone: +33 (0)4 91 32 42 77

18 **Abstract**

19

20 It is strongly suspected that, like lutein, zeaxanthin (ZEA) plays a biological role in the
21 human eye. Many studies also suggest that it could reduce the risk of age-related macular
22 degeneration and improve cognition. Unfortunately, it is only present in a very limited
23 number of foods. This is why a new tomato line, named “Xantomato”, whose fruits can
24 synthesize this compound, was generated. However, whether ZEA in Xantomato is
25 bioavailable enough for Xantomato to qualify as a nutritionally relevant ZEA source is not
26 known. The objective was to compare the bioaccessibility and intestinal cell uptake efficiency
27 of ZEA from Xantomato to that present in the richest sources of this compound.
28 Bioaccessibility was assessed using *in vitro* digestions and uptake efficiency using Caco-2
29 cells. Xantomato ZEA bioaccessibility was not statistically different from that of common
30 fruits and vegetables rich in this compound. Xantomato ZEA uptake efficiency (7.8%) was
31 lower ($P<0.05$) than that of orange pepper (10.6%) but not different from that of corn (6.9%).
32 Therefore, the results of the *in vitro* digestion/Caco-2 cell model suggest that Xantomato ZEA
33 could be as bioavailable as that found in common food sources of this compound.

34 **Keywords:** intestinal absorption; bioaccessibility; orange pepper; corn; lutein; freeze-drying.

35 **1) Introduction**

36

37 Zeaxanthin (ZEA) is a plant pigment belonging to the carotenoid xanthophyll family.
38 Although this phytochemical is not synthesized by humans, it is present in our bodies since it
39 is found in certain fruits and vegetables. Its bioavailability is very variable (Bohn et al., 2017;
40 Desmarchelier & Borel, 2017) and it is found in significant concentrations in various tissues,
41 e.g. eye, skin and adipose tissue. Interest for this compound mainly comes from the
42 observation that, along with lutein, another xanthophyll, it accumulates in the central area of
43 the human retina (Bernstein et al., 2001), thereby producing the typical yellow color of the
44 macula lutea. This specific accumulation has led to hypothesize that these molecules could
45 exert a biological role in the eye and since they possess antioxidant properties, it has been
46 suggested that they could participate in the protection of the retina from oxidative damages.
47 This mechanism could, at least in part, explain their beneficial effects on age-related macular
48 degeneration (Ma et al., 2012; Chew et al., 2022). Moreover, as the optical density of the
49 macular pigment, which depends on xanthophyll concentration in the macula, has been
50 recognized as a biomarker of the concentration of these compounds in the brain, recent studies
51 have made it possible to suggest that these xanthophylls also play beneficial role in cognition
52 (Wang et al., 2022).

53 One of the main providers of ZEA in some populations is corn and corn products
54 (Perry et al., 2009). Corn and its derivatives can contain approximately 2 (canned corn) to 25
55 $\mu\text{g/g}$ (bio-fortified varieties) ZEA (Humphries & Khachik, 2003; O'Hare et al., 2015).
56 Another interesting source of ZEA is eggs and egg products because of the high
57 bioavailability of xanthophylls from this food source (Chung et al., 2004). This source can
58 contain about 4 to 13 $\mu\text{g/g}$ of ZEA (Maiani et al., 2009). However, common food sources that
59 provide significant amounts of this phytochemical are scarce (Sommerburg et al., 1998).

60 Therefore, and given the probable importance of this compound in visual function, and
61 possibly in cognition, an increasing number of studies aim at biofortifying fruits and
62 vegetables with ZEA and lutein (Karniel, Koch, Zamir, & Hirschberg, 2020; Wu et al., 2022).
63 To this end, a novel variety of tomato, Xantomato, with >50 µg/g fresh weight of ZEA in
64 fruits, has been developed (Karniel, Koch, Zamir, & Hirschberg, 2020). Regular tomatoes
65 mainly accumulate lycopene but provide only residual amounts of ZEA, e.g., less than 0.1
66 µg/g fresh weight. Xantomato was created through classical genetic breeding, i.e., it is a non-
67 transgenic biofortified variety (Karniel, Koch, Zamir, & Hirschberg, 2020). To assess whether
68 Xantomato can be considered a nutritionally relevant source of ZEA, we first compared ZEA
69 concentration in Xantomato to that in common ZEA food sources. We focused on free ZEA,
70 although some plants such as peppers also contain ZEA esters (Weller & Breithaupt, 2003), as
71 the free form is preferentially absorbed (Chitchumroonchokchai & Failla, 2006) and found in
72 our body (Breithaupt et al., 2004). Next, we compared the bioaccessibility, i.e. the relative
73 amount solubilized in micelles during digestion, of ZEA from Xantomato to that from foods
74 rich in this compound. It is indeed well established that ZEA, like other fat-soluble molecules
75 present in our food, is incorporated into micelles during digestion, and that these vehicles
76 transport the fat-soluble molecules towards the apical membrane of the intestinal cells where
77 they are absorbed (Desmarchelier & Borel, 2017). Finally, we compared ZEA uptake
78 efficiency by intestinal cells when incorporated into micelles resulting from *in vitro* digestion
79 of Xantomato or from foods rich in this compound. The measurement of bioaccessibility,
80 using an *in vitro* digestion model, coupled with that of uptake efficiency by intestinal cells is
81 indeed recognized as a good predictor of lipid phytochemical bioavailability (Reboul et al.,
82 2006) and additionally offers mechanistic insights into their absorption (Reboul et al., 2005).

83 2) Materials and Methods

84

85 2.1. Chemicals

86 Enzymes used in the *in vitro* digestion experiments, i.e. α -amylase from *Bacillus* sp.,
87 pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, and porcine bile
88 extract, were from Sigma-Aldrich (Saint Quentin Fallavier, France). Chemicals used for
89 carotenoid quantification (ethanol, n-hexane, dichloromethane and HPLC grade methanol,
90 methyl tert-butyl ether (MTBE) and water) were from Carlo Erba reagents (Val de Reuil,
91 France). Carotenoid standards, i.e. lutein, ZEA, β -carotene, lycopene and echinenone (HPLC
92 purity > 95%), were from Carotenature GmbH (Müdingen, Switzerland). Cell culture
93 consumables, i.e. DMEM, non-essential amino acids, penicillin and PBS, were from Life
94 Technologies (Villebon sur Yvette, France). Fetal bovine serum (FBS) was from Dutscher
95 (Brumath, France).

96

97 2.2. ZEA-rich food sources and their preparation

98 Commonly consumed ZEA-rich vegetables (**Table 1**) and foods used in the *in vitro*
99 digestion experiments (potatoes, ground beef and olive oil) were purchased from local grocery
100 stores. Fruits of Xantomato (Karniel, Koch, Zamir, & Hirschberg, 2020) and the standard
101 lycopene-producing tomato variety Ailsa Craig (used as a control) were harvested in
102 Jerusalem from greenhouse-grown plants at the “red” stage of ripening. The pericarp,
103 columella, and placenta tissues (i.e. the more solid components of the fruit) were chopped by
104 a kitchen blender to obtain a tomato puree, which was immediately frozen at -80°C. The
105 puree samples were freeze-dried in 50 ml tubes and shipped to France. To compare the results
106 of tomatoes treated as described above with those of the other ZEA-rich vegetables (**Table 1**),
107 the same treatment was applied to these latter, i.e. we reduced them to a puree then we freeze-

108 dried them and finally we rehydrated them before their use, i.e. measurement of their ZEA
109 content and *in vitro* digestion experiments.

110 Since it is well established that cooking foods can have a major effect on carotenoid
111 bioavailability (Desmarchelier & Borel, 2017) and since the ZEA-rich foods studied are
112 commonly consumed cooked, we decided to cook them before freeze-drying. Cooking times
113 were from common French recipes. We chose steam cooking because it is a relatively
114 common culinary preparation method that has the advantage of better preserving
115 micronutrients (Fabbri & Crosby, 2016; Junpatiw et al., 2013). All vegetables were first
116 washed with tap water. Then the non-edible parts were removed. Vegetables were then
117 chopped and blended using a kitchen mixer to form a puree. The puree samples were then
118 freeze-dried until all their water was sublimated. A preliminary experiment allowed us to
119 determine that at least 96 hours were necessary in order for the vegetables to lose all their
120 water (checked by expected theoretical mass loss and by the fact that longer freeze-drying
121 times did not lead to additional mass loss). The puree powders were stored at -80°C until
122 further use. All vegetables were rehydrated as previously described (Qiu, 2019) before *in vitro*
123 digestions, adding a volume of distilled water corresponding to the volume lost upon freeze-
124 drying.

125

126 **2.3. *In vitro* digestions to assess carotenoid bioaccessibility**

127 The *in vitro* digestion model (Reboul et al., 2006) was adapted from a previous model
128 (Garrett, Failla & Sarama, 1999). Samples were prepared as follows: 2 g of freeze-dried
129 sample (rehydrated as described above) of the 6 richest sources of ZEA studied were added to
130 6.7 g boiled potatoes, 1.2 g ground beef (fat content: 5%) and 200 mg olive oil. The rest of the
131 protocol is described in detail in Reboul et al. (Reboul et al., 2006). At the end of the
132 digestion, digestate and micelle samples were frozen at -80°C before liquid-liquid extraction

133 and carotenoid quantification by HPLC (described hereafter). Bioaccessibility was then
134 calculated as follows:

$$135 \text{ Bioaccessibility (\%)} = \frac{\text{Amount of carotenoid recovered in micelles}}{\text{Amount of carotenoid recovered in digestate}}$$

136

137 **2.4. Cell experiments to assess xanthophyll uptake efficiency**

138 2.4.1. Caco-2 cell culture

139 Caco-2 clone TC-7 cells were cultivated in the presence of DMEM supplemented with
140 16% heat-inactivated FBS, 1% non-essential amino acid, and 1% antibiotics (complete
141 medium) as previously described (Reboul et al., 2005). For each experiment, cells were
142 seeded and grown during 15 days on 6-well plates (24 mm diameter, 1 µm-pore-size
143 polycarbonate membrane; Becton Dickinson, Le Pont-de-Claix, France) to obtain confluent
144 and highly differentiated cell monolayers.

145

146 2.4.2. Determination of the dilution to obtain non-cytotoxic micellar solutions

147 Cell viability upon incubation for 4 h at 37 °C with micellar solutions from *in vitro*
148 digestions was determined using a MTT assay adapted from Goncalves et al. (Goncalves et
149 al., 2016). Micellar solutions, diluted in DMEM, were tested until a dilution was found that no
150 longer caused toxicity. Consequently, A 1:5 (v:v) dilution was used for cell uptake efficiency
151 measurements (data not shown).

152

153 2.4.3. Measurement of the uptake efficiency of xanthophylls from the micellar fraction by

154 Caco-2 cells

155 We decided to evaluate intestinal cell uptake efficiency of ZEA and lutein from the
156 micellar fraction of *in vitro* digestions of Xantomato puree, the food source richest in ZEA,
157 i.e. orange pepper, and corn, which is the major dietary ZEA contributor in some population

158 (Perry et al., 2009). Uptake efficiency was studied using Caco-2 cell monolayers with a
159 protocol commonly used in our laboratory (Reboul et al., 2007). The apical side of the cell
160 monolayers received 1 ml of the micellar fraction, which was diluted 5 times (see above) with
161 DMEM. Following incubation for 4 hours at 37°C (Liu et al., 2004), the medium was
162 collected and the cell monolayers were washed twice with 1 ml PBS, scraped and collected in
163 500 µL PBS. Samples were stored at -80°C until analysis.

164

165 **2.5. Xanthophyll extraction**

166 Five hundred µL from the rehydrated vegetable purees, digestate, or micellar fractions
167 were used for xanthophyll extraction, following previously published method (Borel et al.,
168 2021), with only minor modifications for the cell culture samples, i.e. for homogenization
169 (vortex blender, 5 min) and centrifugation (1,257 x g, 5 min, 4°C). For cell culture samples,
170 distilled water was added to reach a final volume of 500 µL.

171

172 **2.6. Xanthophyll quantification by HPLC**

173 The separation and quantification of xanthophylls (free ZEA and lutein) were
174 performed by HPLC as described in detail previously (Borel et al., 2021). Five to 50 µL from
175 extracted samples of rehydrated vegetables, 100 µL from extracted samples of micellar
176 fractions and digestates, and 150 µL from extracted samples of cell experiments, were
177 injected in the HPLC apparatus. A YMC-Pack YMC C30 column (250 x 4.6 mm; 5 µm)
178 (Crawford Scientific Ltd, Strathaven, UK) preceded by a precolumn (10 x 4 mm; 5 µm) set at
179 35°C was used for the HPLC analyses. Carotenoids were detected at a wavelength of 450 nm.

180

181 **2.7. Calculations and statistics**

182 Data were expressed as means \pm SEM. To determine the impact of freeze-drying on
183 carotenoid concentration, a 3-way mixed analysis of variances (ANOVA) was carried out
184 with 2 between-subjects factors, processing and matrix, and 1 within-subject factor,
185 carotenoid species. To assess the normality of our data, Q-Q plots of standardized residuals
186 were used. Homogeneity of variances was assessed by Levene's test. If the main effect was
187 statistically significant, multiple pairwise comparisons using the Bonferroni correction were
188 carried out to determine which group means were different. In the same way, 2-way ANOVA
189 were carried out to estimate the effect of freeze-drying on carotenoid bioaccessibility. To
190 compare ZEA concentration in Xantomato and in ZEA-rich fruits and vegetables, as well as to
191 compare its bioaccessibility, 1-way ANOVA were performed. Homogeneity of variances was
192 checked by Levene's test. In case of data with inhomogeneous variances, a log-transformation
193 was performed before performing the ANOVA. Finally, Tukey-Kramer's test was used as a
194 post hoc test for pairwise comparisons. To estimate the intestinal absorption efficiency of
195 micellar ZEA from in vitro digestion of Xantomato and ZEA-rich fruits and vegetables, a 1-
196 way ANOVA was used as described previously. For the analysis of lutein intestinal uptake
197 efficiency, Student's *t*-test was used with Welch's correction in case of inhomogeneous
198 variances. Differences with $P < 0.05$ were considered significant. All analyses were conducted
199 with R version 1.0.136 for Macintosh (R Core Team, 2020).

200 3) Results

201

202 *Effect of freeze-drying on tomato puree and corn carotenoid content*

203 We chose tomatoes and corn as model plant matrices because they are respectively the
204 matrix of Xantomato and the matrix of one of the major dietary ZEA contributors in certain
205 populations. **Table 2** shows carotenoid concentration in fresh and freeze-dried tomatoes and
206 corn. Lutein and β -carotene concentrations in rehydrated freeze-dried tomato puree were
207 significantly lower than those in fresh tomato puree ($P<0.001$), in contrast to lycopene
208 concentrations in fresh and freeze-dried tomato puree ($P=0.06$). Lutein and ZEA
209 concentrations were lower in freeze-dried corn than in fresh corn ($P<0.05$), which was not the
210 case for β -carotene ($P=0.1$). The average decrease in carotenoid concentration in tomato puree
211 was about 31%, while it was about 18% in corn. There was a significant interaction between
212 the food matrix, the freeze-drying treatment, and the carotenoid species ($P<0.01$). The effect
213 of freeze-drying was different depending on the carotenoid species in corn ($P<0.05$) but not in
214 tomato puree ($P=0.08$). Moreover, the effect of freeze-drying on lutein ($P<0.05$) and β -
215 carotene ($P<0.001$) concentration was not the same depending on the matrix.

216

217 *Effect of freeze-drying on tomato puree and corn carotenoid bioaccessibility*

218 We used again tomatoes and corn as model plant matrices. Results are shown in **Table**
219 **3**. Lutein and ZEA bioaccessibilities from rehydrated freeze-dried corn ($36 \pm 1\%$ and $38 \pm$
220 2% , respectively) were significantly higher (both $P<0.01$) than in fresh corn ($22 \pm 1\%$ and 25
221 $\pm 2\%$, respectively). Concerning tomato, lutein bioaccessibility from rehydrated freeze-dried
222 puree ($43 \pm 3\%$) was significantly higher ($P<0.01$) than in fresh tomato puree ($33 \pm 3\%$). On
223 the contrary, β -carotene bioaccessibility from the freeze-dried puree ($14 \pm 1\%$) was
224 significantly lower than in the fresh puree ($26 \pm 2\%$). Finally, lycopene bioaccessibility from

225 the freeze-dried puree was not significantly different from that from the fresh puree (about
226 2%, $P=0.75$). Freeze-drying did not impact differently lutein bioaccessibility from tomato vs
227 maize ($P=0.5$).

228

229 *ZEA concentrations in Xantomato and in ZEA-rich fruits and vegetables*

230 **Figure 1** shows the ZEA content measured in Xantomato and in ZEA-rich fruits and
231 vegetables. We recall that these measurements were carried out on fruits and vegetables
232 treated in the same way as Xantomato, i.e. reduced to puree, freeze-dried then rehydrated.
233 Orange pepper had the highest ZEA content (22.1 ± 1.0 mg/kg fresh weight), followed by
234 Xantomato (14.6 ± 0.3 mg/kg) and corn (3.2 ± 0.2 mg/kg). ZEA concentration in Xantomato
235 was significantly higher (almost 5 times) than in corn. The four vegetables containing the
236 lowest ZEA content were tomatoes, broccoli, potato and kale. As phytochemical
237 concentrations are affected by different factors, e.g. plant variety and growing conditions, we
238 compared the ZEA concentrations measured in this study to those reported in the literature
239 (Granado et al., 1992; Perry et al., 2009; Murillo et al., 2010). **Table 4** shows that, although
240 concentrations measured in this study are sometimes quite different from the published
241 concentrations, they are of the same order of magnitude. Concerning the lutein concentrations
242 in the foods used in the study (**Supplemental Figure**), two vegetables, namely spinach and
243 kale, can be distinguished from the other vegetables by their very high lutein content ($51.0 \pm$
244 2.4 mg/kg and 48.4 ± 3.2 mg/kg, respectively), which was much higher than that of
245 Xantomato (1.3 ± 0.03 mg/kg).

246

247 *ZEA bioaccessibility from Xantomato and ZEA-rich fruits and vegetables*

248 **Figure 2** shows that ZEA bioaccessibility from corn ($38.1 \pm 1.7\%$) was significantly
249 higher than that from orange pepper ($28.9 \pm 1.1\%$), Xantomato ($26.3 \pm 0.8\%$), spinach ($25.6 \pm$

250 1.1 %), and yellow pepper ($15.5 \pm 1.1\%$). Note that no ZEA was detected in the micelle
251 fractions from digestions of the other fruits and vegetables.

252

253 *Caco-2 cell uptake efficiency of ZEA and lutein present in mixed micelles from in vitro*
254 *digestions*

255 **Table 5** shows the uptake efficiency of ZEA and lutein contained in mixed micelles
256 from *in vitro* digestions of corn, Xantomato and orange pepper. The uptake efficiency of ZEA
257 from Xantomato was not significantly different from that from corn ($P=0.5$), but it was
258 significantly different from that of orange bell pepper ($P<0.01$). Concerning lutein uptake
259 efficiency, it was not significantly different whether it came from corn or Xantomato ($P=0.5$).

260 Discussion

261

262 The aim of this study was to assess the potential of Xantomato as a relevant ZEA food
263 source for humans. To be the case, i) ZEA concentration in Xantomato should be high enough
264 to provide significant ZEA quantities in case of a reasonable consumption, and ii) ZEA in
265 Xantomato should be sufficiently bioavailable. The latter condition is important to verify
266 because Xantomato is biofortified in ZEA and its intracellular localization in membranous
267 structures of chromoplasts (Karniel, Koch, Zamir, & Hirschberg, 2020) may affect its
268 bioaccessibility (Palmero et al., 2013, 2016). Therefore, we first compared Xantomato ZEA
269 content with that of food sources naturally rich in this carotenoid. We then compared ZEA
270 bioaccessibility from Xantomato with that from ZEA-rich fruits and vegetables. Finally, we
271 compared the intestinal cell uptake efficiency of micellarized ZEA from Xantomato with that
272 of micellarized ZEA from common vegetables rich in ZEA. Indeed, measuring the
273 bioaccessibility of a carotenoid and its uptake efficiency by intestinal cells in culture gives a
274 good estimate of its bioavailability (Reboul et al., 2006).

275 Lutein and zeaxanthin are both found in eggs, but unlike lutein, which is present in
276 many fruits and vegetables, ZEA is only found in significant amount in a few vegetables. It is
277 therefore particularly relevant to situate Xantomato in relation to the food sources richest in
278 this pigment. The first way to proceed is to compare with data from literature. However, this
279 method has the disadvantage of comparing values that have not been found under the same
280 experimental conditions (different assay methods, different experimenters, etc.) and that
281 originate from vegetables that have sometimes not been prepared in the same way (raw or
282 cooked with different methods). We therefore decided to measure the concentration of ZEA in
283 Xantomato and in ZEA-rich sources under the same assay conditions. Finally, since we could
284 only freeze-dry Xantomato puree, we measured ZEA content in the other sources also puréed

285 and freeze-dried. This double comparison of ZEA concentrations in Xantomato and in the
286 richest sources of ZEA shows that Xantomato is one of the richest sources of this pigment.
287 Indeed, according to the literature, with 39 mg/kg (Karniel, Koch, Zamir, & Hirschberg,
288 2020), the raw tomatoes of Xantomato rank after the orange pepper (62 mg/kg), but before the
289 scallion (25 mg/kg). Similarly, according to our measurements, freeze-dried Xantomato purée
290 (15 mg/kg) still ranks after the orange pepper (22 mg/kg) but ahead of all the other sources
291 studied. As the losses of lutein and ZEA in corn upon freeze-drying were not significantly
292 different from one another (-17 and -18% respectively), we can reasonably assume a similar
293 behavior for these two xanthophylls in tomato puree. Knowing that the loss of lutein due to
294 freeze-drying in common tomato puree was 33% (**Table 3**), we can therefore estimate that the
295 ZEA content of the Xantomato puree before its freeze-drying was $14.6/0.66 = 22$ mg/kg. In
296 summary, knowing that raw Xantomato tomatoes can contain >50 mg ZEA/kg fresh weight
297 (Karniel, Koch, Zamir, & Hirschberg, 2020) and Xantomato puree 22 mg/kg, we can estimate
298 that 40 g of Xantomato tomatoes or 91 g of Xantomato tomato puree would provide 2 mg of
299 ZEA, which is the daily amount recommended by the US National Eye Institute to reduce the
300 risk of age-related macular degeneration ([https://www.nei.nih.gov/research/clinical-trials/age-](https://www.nei.nih.gov/research/clinical-trials/age-related-eye-disease-studies-aredsareds2/about-areds-and-areds2)
301 [related-eye-disease-studies-aredsareds2/about-areds-and-areds2](https://www.nei.nih.gov/research/clinical-trials/age-related-eye-disease-studies-aredsareds2/about-areds-and-areds2). Accessed 07.11.23)

302 As mentioned at the beginning of the discussion, our second objective was to compare
303 the bioaccessibility of ZEA from Xantomato with that from common vegetable sources of
304 ZEA. The results obtained first show that the bioaccessibilities we measured were in the range
305 of previously observed values, e.g. 38% in corn, which is close to the 35% observed by
306 Hossain & Jayadeep (Hossain & Jayadeep, 2021), and the 43% observed by Zurak et al.
307 (Zurak et al., 2021). This confirms that our *in vitro* digestion results were reliable and
308 therefore usable to compare ZEA bioaccessibility from Xantomato with that from other ZEA-
309 rich food sources. Our ancillary study on the effect of freeze-drying on the bioaccessibility of

310 carotenoids also suggests that the bioaccessibility of lutein is certainly overestimated because
311 of freeze-drying (+30% according to the difference between the bioaccessibility of lutein from
312 freeze-dried tomato puree compared to the non-freeze-dried one). This ancillary study also
313 shows that the effect of freeze-drying on the bioaccessibility of lutein is greater for corn than
314 for tomato puree, which may explain why corn presented the highest bioaccessibility of ZEA.
315 On the whole, this suggests that the bioaccessibility of ZEA from Xantomato is within the
316 range of bioaccessibility values of other fruits and vegetables.

317 Regarding ZEA uptake efficiency by Caco-2 cells, which was our third objective, our
318 results show that it was not significantly different when it was in micelles coming from
319 Xantomato than when it was in micelles coming from corn. The fact that the absorption
320 efficiency of ZEA from orange peppers was significantly higher than that from the other
321 sources of ZEA tested could be due to the fact that orange peppers contain, unlike corn and
322 Xantomato, a very large amount of ZEA esters (Weller & Breithaupt, 2003). We hypothesize
323 that a fraction of these esters was hydrolyzed by Caco-2, so that the amount of free ZEA
324 found in the cells did not only originate from the free ZEA present in the micelles, but also
325 from that derived from the hydrolysis of ZEA esters. ZEA esters hydrolysis by Caco-2 cells
326 was not observed in a previous study (Chitchumroonchokchai & Failla, 2006), although the
327 authors had not designed their experiment to study this very precisely, but such an hydrolysis
328 is quite plausible since it has been observed that Caco-2 cells can hydrolyze a vitamin E ester
329 (Desmarchelier et al., 2013).

330 In summary, this study shows that the ZEA content of Xantomato is very high as
331 compared to all the richest dietary sources of this compound. Indeed, it ranked second either
332 in the literature or in measurements made on a panel of fruits and vegetables rich in ZEA. It is
333 furthermore important to mention that ZEA accumulates in Xantomato as a free molecule,
334 which is the form present in the majority of fruits and vegetables, while some fruits and

335 vegetables, e.g. peppers and goji berry, contain ZEA esters which must be hydrolyzed to be
336 effectively absorbed (Chitchumroonchokchai & Failla, 2006). ZEA from Xantomato also
337 displays a bioaccessibility equivalent to that of ZEA from the majority of the studied sources
338 and its absorption efficiency by enterocytes is equivalent to that of ZEA from corn, which is
339 one the main dietary source of this xanthophyll in some populations. We can therefore
340 conclude that the consumption of a tomato, which is the world's most popular and widely
341 available vegetable, modified in a non-transgenic fashion to accumulate ZEA could provide
342 significant quantities of bioavailable ZEA to groups of the population who consume few or no
343 other sources of this xanthophyll. Of course, a clinical study comparing the ability of
344 Xantomato and a common source of ZEA, e.g. corn, spinach or egg yolk, to increase the
345 blood status of ZEA, or macular pigment optical density, would provide definitive proof of
346 the interest of this tomato rich in ZEA to improve visual function and prevent AMD.

347 **Abbreviation:**

348 ZEA (zeaxanthin).

349

350 **Acknowledgements:**

351 Research in the laboratory of J. Hirschberg was supported by the Israel Science Foundation
352 Grant No. 1930/18”.

353 For the purpose of Open Access, a CC-BY public copyright licence ([Creative Commons —
354 Attribution 4.0 International — CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)) has been applied by the authors to the present
355 document and will be applied to all subsequent versions up to the Author Accepted
356 Manuscript arising from this submission.



357

358

359 **Supporting information:**

360 The costs of this project were covered by the own budget of P. Borel’s research team, which
361 came mainly from INRAE endowments.

362

363 **Credit author statement:**

364 **Lisa Morand-Laffargue:** carotenoid analysis, in vitro digestions, cell culture, analyzed and
365 interpreted data, drafting material and methods and drawing the figures, statistical analysis.

366 **Joseph Hirschberg:** sent the samples of Xantomato, review and editing the manuscript

367 **Charlotte Halimi:** carotenoid analysis supervision. **Charles Desmarchelier:** analyzed and
368 interpreted data, review & editing of the manuscript. **Patrick Borel:** had the idea of the

369 research and has primary responsibility for final manuscript content; designed the protocol,
370 analyzed and interpreted data, project coordination, acquisition of funding, drafting of the
371 manuscript.

372

373 **Conflicts of interest:**

374 No conflicts of interest to declare.

375 **References**

376

377 Bernstein, P. S., Khachik, F., Carvalho, L. S., Muir, G. J., Zhao, D. Y., & Katz, N. B. (2001).

378 Identification and quantitation of carotenoids and their metabolites in the tissues of the
379 human eye. *Experimental Eye Research*, 72(3), 215- 223.

380 <https://doi.org/10.1006/exer.2000.0954>

381 Bohn, T., Desmarchelier, C., Dragsted, L. O., Nielsen, C. S., Stahl, W., Rühl, R., Keijer, J., &

382 Borel, P. (2017). Host-related factors explaining interindividual variability of

383 carotenoid bioavailability and tissue concentrations in humans. *Molecular Nutrition &*

384 *Food Research*, 61(6), 1600685. <https://doi.org/10.1002/mnfr.201600685>

385 Borel, P., Hammaz, F., Morand-Laffargue, L., Creton, B., Halimi, C., Sabatier, D., &

386 Desmarchelier, C. (2021). Using black soldier fly larvae reared on fruits and

387 vegetables waste as a sustainable dietary source of provitamin a carotenoids. *Food*

388 *Chemistry*, 359, 129911. <https://doi.org/10.1016/j.foodchem.2021.129911>

389 Breithaupt, D. E., Weller, P., Wolters, M., & Hahn, A. (2004). Comparison of plasma

390 responses in human subjects after the ingestion of 3R,3R'-zeaxanthin dipalmitate from

391 wolfberry (*Lycium barbarum*) and non-esterified 3R,3R'-zeaxanthin using chiral high-

392 performance liquid chromatography. *British Journal of Nutrition*, 91(5), 707- 713.

393 <https://doi.org/10.1079/BJN20041105>

394 Chew, E. Y., Clemons, T. E., Agrón, E., Domalpally, A., Keenan, T. D. L., Vitale, S., Weber,

395 C., Smith, D. C., Christen, W., & AREDS2 Research Group. (2022). Long-term

396 Outcomes of Adding Lutein/Zeaxanthin and ω -3 Fatty Acids to the AREDS

397 Supplements on Age-Related Macular Degeneration Progression : AREDS2 Report

398 28. *JAMA Ophthalmology*, 140(7), 692- 698.

399 <https://doi.org/10.1001/jamaophthalmol.2022.1640>

400 Chitchumroonchokchai, C., & Failla, M. L. (2006). Hydrolysis of zeaxanthin esters by
401 carboxyl ester lipase during digestion facilitates micellarization and uptake of the
402 xanthophyll by Caco-2 human intestinal cells. *The Journal of Nutrition*, 136(3),
403 588- 594. <https://doi.org/10.1093/jn/136.3.588>

404 Chung, H.-Y., Rasmussen, H. M., & Johnson, E. J. (2004). Lutein Bioavailability Is Higher
405 from Lutein-Enriched Eggs than from Supplements and Spinach in Men. *The Journal*
406 *of Nutrition*, 134(8), 1887- 1893. <https://doi.org/10.1093/jn/134.8.1887>

407 Desmarchelier, C., & Borel, P. (2017). Overview of carotenoid bioavailability determinants :
408 From dietary factors to host genetic variations. *Trends in Food Science & Technology*,
409 69, 270- 280. <https://doi.org/10.1016/j.tifs.2017.03.002>

410 Desmarchelier, C., Tourniaire, F., Prévéraud, D. P., Samson-Kremser, C., Crenon, I., Rosilio,
411 V., & Borel, P. (2013). The distribution and relative hydrolysis of tocopheryl acetate
412 in the different matrices coexisting in the lumen of the small intestine during digestion
413 could explain its low bioavailability. *Molecular Nutrition & Food Research*, 57(7),
414 1237- 1245. <https://doi.org/10.1002/mnfr.201200720>

415 Fabbri, A. D. T., & Crosby, G. A. (2016). A review of the impact of preparation and cooking
416 on the nutritional quality of vegetables and legumes. *International Journal of*
417 *Gastronomy and Food Science*, 3, 2- 11. <https://doi.org/10.1016/j.ijgfs.2015.11.001>

418 Garrett, D. A., Failla, M. L., & Sarama, R. J. (1999). Development of an in vitro digestion
419 method to assess carotenoid bioavailability from meals. *Journal of Agricultural and*
420 *Food Chemistry*, 47(10), 4301- 4309. <https://doi.org/10.1021/jf9903298>

421 Goncalves, A., Margier, M., Tagliaferri, C., Lebecque, P., Georgé, S., Wittrant, Y., Coxam,
422 V., Amiot, M.-J., & Reboul, E. (2016). Pinoresinol of olive oil decreases vitamin D
423 intestinal absorption. *Food Chemistry*, 206, 234- 238.
424 <https://doi.org/10.1016/j.foodchem.2016.03.048>

425 Granado, Fernando., Olmedilla, Begona., Blanco, Inmaculada., & Rojas-Hidalgo, Enrique.
426 (1992). Carotenoid composition in raw and cooked Spanish vegetables. *Journal of*
427 *Agricultural and Food Chemistry*, 40(11), 11. <https://doi.org/10.1021/jf00023a019>

428 Handelman, G. J., Nightingale, Z. D., Lichtenstein, A. H., Schaefer, E. J., & Blumberg, J. B.
429 (1999). Lutein and zeaxanthin concentrations in plasma after dietary supplementation
430 with egg yolk. *The American Journal of Clinical Nutrition*, 70(2), 247- 251.
431 <https://doi.org/10.1093/ajcn.70.2.247>

432 Holden, J. M., Eldridge, A. L., Beecher, G. R., Marilyn Buzzard, I., Bhagwat, S., Davis, C. S.,
433 Douglass, L. W., Gebhardt, S., Haytowitz, D., & Schakel, S. (1999). Carotenoid
434 Content of U.S. Foods : An Update of the Database. *Journal of Food Composition and*
435 *Analysis*, 12(3), 169- 196. <https://doi.org/10.1006/jfca.1999.0827>

436 Hossain, A., & Jayadeep, A. (2021). Infrared heating induced improvement of certain
437 phytochemicals, their bioaccessible contents and bioaccessibility in maize. *LWT*, 142,
438 110912. <https://doi.org/10.1016/j.lwt.2021.110912>

439 Humphries, J. M., & Khachik, F. (2003). Distribution of lutein, zeaxanthin, and related
440 geometrical isomers in fruit, vegetables, wheat, and pasta products. *Journal of*
441 *Agricultural and Food Chemistry*, 51(5), 5. <https://doi.org/10.1021/jf026073e>

442 Junpattiw, A., Lertrat, K., Lomthaisong, K., & Tangwongchai, R. (2013). Effects of steaming,
443 boiling and frozen storage on carotenoid contents of various sweet corn cultivars.
444 *International Food Research Journal*, 2219- 2225.

445 Karniel, U., Koch, A., Zamir, D., & Hirschberg, J. (2020). Development of zeaxanthin- rich
446 tomato fruit through genetic manipulations of carotenoid biosynthesis. *Plant*
447 *Biotechnology Journal*, 18(11), 11. <https://doi.org/10.1111/pbi.13387>

448 Liu, C.-S., Glahn, R. P., & Liu, R. H. (2004). Assessment of carotenoid bioavailability of
449 whole foods using a Caco-2 cell culture model coupled with an in vitro digestion.

450 *Journal of Agricultural and Food Chemistry*, 52(13), 4330- 4337.
451 <https://doi.org/10.1021/jf040028k>

452 Ma, L., Dou, H.-L., Wu, Y.-Q., Huang, Y.-M., Huang, Y.-B., Xu, X.-R., Zou, Z.-Y., & Lin,
453 X.-M. (2012). Lutein and zeaxanthin intake and the risk of age-related macular
454 degeneration : A systematic review and meta-analysis. *British Journal of Nutrition*,
455 107(3), 350- 359. <https://doi.org/10.1017/S0007114511004260>

456 Maiani, G., Periago Castón, M. J., Catasta, G., Toti, E., Cambrodón, I. G., Bysted, A.,
457 Granado-Lorencio, F., Olmedilla-Alonso, B., Knuthsen, P., Valoti, M., Böhm, V.,
458 Mayer-Miebach, E., Behnlian, D., & Schlemmer, U. (2009). Carotenoids : Actual
459 knowledge on food sources, intakes, stability and bioavailability and their protective
460 role in humans. *Molecular Nutrition & Food Research*, 53(S2), S194- S218.
461 <https://doi.org/10.1002/mnfr.200800053>

462 Murillo, E., Meléndez-Martínez, A. J., & Portugal, F. (2010). Screening of vegetables and
463 fruits from Panama for rich sources of lutein and zeaxanthin. *Food Chemistry*, 122(1),
464 1. <https://doi.org/10.1016/j.foodchem.2010.02.034>

465 O'Hare, T. J., Fanning, K. J., & Martin, I. F. (2015). Zeaxanthin biofortification of sweet-corn
466 and factors affecting zeaxanthin accumulation and colour change. *Archives of*
467 *Biochemistry and Biophysics*, 572, 184- 187.
468 <https://doi.org/10.1016/j.abb.2015.01.015>

469 Palmero, P., Lemmens, L., Ribas-Agustí, A., Sosa, C., Met, K., de Dieu Umutoni, J.,
470 Hendrickx, M., & Van Loey, A. (2013). Novel targeted approach to better understand
471 how natural structural barriers govern carotenoid in vitro bioaccessibility in vegetable-
472 based systems. *Food Chemistry*, 141(3), 2036- 2043.
473 <https://doi.org/10.1016/j.foodchem.2013.05.064>

474 Palmero, P., Panozzo, A., Colle, I., Chigwedere, C., Hendrickx, M., & Van Loey, A. (2016).
475 Role of structural barriers for carotenoid bioaccessibility upon high pressure
476 homogenization. *Food Chemistry*, 199, 423- 432.
477 <https://doi.org/10.1016/j.foodchem.2015.12.062>

478 Perry, A., Rasmussen, H., & Johnson, E. J. (2009). Xanthophyll (lutein, zeaxanthin) content
479 in fruits, vegetables and corn and egg products. *Journal of Food Composition and*
480 *Analysis*, 22(1), 1. <https://doi.org/10.1016/j.jfca.2008.07.006>

481 Qiu, J. (2019). A systematic analysis on tomato powder quality prepared by four conductive
482 drying technologies. *Innovative Food Science and Emerging Technologies*, 10.
483 <https://doi.org/10.1016/j.ifset.2019.03.013>

484 Reboul, E., Abou, L., Mikail, C., Ghiringhelli, O., André, M., Portugal, H., Jourdheuil-
485 Rahmani, D., Amiot, M.-J., Lairon, D., & Borel, P. (2005). Lutein transport by Caco-2
486 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class
487 B type I (SR-BI). *The Biochemical Journal*, 387(Pt 2), 455- 461.
488 <https://doi.org/10.1042/BJ20040554>

489 Reboul, E., Richelle, M., Perrot, E., Desmoulins-Malezet, C., Pirisi, V., & Borel, P. (2006).
490 Bioaccessibility of Carotenoids and Vitamin E from Their Main Dietary Sources.
491 *Journal of Agricultural and Food Chemistry*, 54(23), 8749- 8755.
492 <https://doi.org/10.1021/jf061818s>

493 Reboul, E., Thap, S., Tourniaire, F., André, M., Juhel, C., Morange, S., Amiot, M.-J., Lairon,
494 D., & Borel, P. (2007). Differential effect of dietary antioxidant classes (carotenoids,
495 polyphenols, vitamins C and E) on lutein absorption. *British Journal of Nutrition*,
496 97(3), 440- 446. <https://doi.org/10.1017/S0007114507352604>

497 Sa, M. C. de, & Rodriguez-Amaya, D. B. (2003). Carotenoid composition of cooked green
498 vegetables from restaurants. *Food Chemistry*. [https://doi.org/10.1016/S0308-](https://doi.org/10.1016/S0308-8146(03)00227-9)
499 [8146\(03\)00227-9](https://doi.org/10.1016/S0308-8146(03)00227-9)

500 Scott, C. E., & Eldridge, A. L. (2005). Comparison of carotenoid content in fresh, frozen and
501 canned corn. *Journal of Food Composition and Analysis*, *18*(6), 6.
502 <https://doi.org/10.1016/j.jfca.2004.04.001>

503 Sommerburg, O., Keunen, J. E. E., Bird, A. C., & van Kuijk, F. J. G. M. (1998). Fruits and
504 vegetables that are sources for lutein and zeaxanthin : The macular pigment in human
505 eyes. *British Journal of Ophthalmology*, *82*(8), 907- 910.
506 <https://doi.org/10.1136/bjo.82.8.907>

507 Wang, H., Wang, G., Billings, R., Li, D., Haase, S. R., Wheeler, P. F., Vance, D. E., & Li, W.
508 (2022). Can Diet Supplements of Macular Pigment of Lutein, Zeaxanthin, and Meso-
509 zeaxanthin Affect Cognition? *Journal of Alzheimer's Disease: JAD*, *87*(3),
510 1079- 1087. <https://doi.org/10.3233/JAD-215736>

511 Weller, P., & Breithaupt, D. E. (2003). Identification and Quantification of Zeaxanthin Esters
512 in Plants Using Liquid Chromatography–Mass Spectrometry. *Journal of Agricultural
513 and Food Chemistry*, *51*(24), 7044- 7049. <https://doi.org/10.1021/jf034803s>

514 Wu, Y., Yuan, Y., Jiang, W., Zhang, X., Ren, S., Wang, H., Zhang, X., & Zhang, Y. (2022).
515 Enrichment of health-promoting lutein and zeaxanthin in tomato fruit through
516 metabolic engineering. *Synthetic and Systems Biotechnology*, *7*(4), 1159- 1166.
517 <https://doi.org/10.1016/j.synbio.2022.08.005>

518 Zurak, D., Grbeša, D., Duvnjak, M., Kiš, G., Međimurec, T., & Kljak, K. (2021). Carotenoid
519 Content and Bioaccessibility in Commercial Maize Hybrids. *Agriculture*, *11*(7), 7.
520 <https://doi.org/10.3390/agriculture11070586>

521

522

523

524

525 **Figure legends**

526

527 **Figure 1. ZEA concentrations (mg/kg fresh weight) in the ZEA-rich vegetables used in**
528 **this study.** Plants are classified as in Table 4. In order to be able to compare the ZEA contents
529 of Xantomato with those of the other sources of ZEA, these measurements were carried out
530 on fruits and vegetables treated in the same way as Xantomato, i.e. reduced to puree, freeze-
531 dried then rehydrated. Bars represent mean \pm SEM (n=4). Bars with different letters are
532 significantly different ($P<0.05$; ANOVA followed by Tukey's HSD test).

533

534 **Figure 2. ZEA bioaccessibility (%) from the ZEA-rich vegetables.** In order to be able to
535 compare the bioaccessibility of ZEA from Xantomato with that of the other sources of ZEA,
536 the *in vitro* digestions were carried out on fruits and vegetables treated in the same way as
537 Xantomato, i.e. reduced to puree, freeze-dried and then rehydrated. Note that results were
538 presented only for vegetables whose micelles contained a detectable concentration of ZEA.
539 Bars represent mean \pm SEM (n=3). Bars with different letters are significantly different
540 ($P<0.05$; ANOVA followed by Tukey's HSD test).

541 **Table 1. ZEA-rich vegetables investigated in this study and their method of culinary**
 542 **preparation.**

Common name	Scientific name	Form of marketing	Laboratory preparation	Steaming time (min)
Bell pepper, orange	<i>Capsicum annuum</i> <i>var.grossum</i>	Fresh	steamed	18
Bell pepper, red	<i>Capsicum annuum</i> <i>var.grossum</i>	Fresh	steamed	18
Bell pepper, yellow	<i>Capsicum annuum</i> <i>var.grossum</i>	Fresh	steamed	18
Broccoli	<i>Brassica oleracea</i> var. <i>italica</i>	Fresh	steamed	18
Cabbage, Kale	<i>Brassica</i> <i>oleracea</i> var. <i>sabellica</i>	Fresh	steamed	10
Corn	<i>Zea mays</i> var. <i>saccharata</i>	Canned	none	-
Potato	<i>Solanum tuberosum</i> var <i>Gwenne</i>	Fresh	steamed	30
Spinach	<i>Spinacia oleracea</i> L.	Fresh	steamed	14
Tomato	<i>Solanum lycopersicum</i> L.	Fresh	none	-

543

544 **Table 2. Concentrations of carotenoids (mg/kg fresh weight) in fresh and rehydrated freeze-**
 545 **dried tomato puree and corn.**

	Tomato puree			Corn		
	Fresh	Freeze-dried	P-value	Fresh	Freeze-dried	P-value
β -carotene	1.44 \pm 0.03	1.10 \pm 0.03	<0.001	0.16 \pm 0.02	0.13 \pm 0.01	0.145
Lutein	0.24 \pm 0.01	0.16 \pm 0.01	<0.001	4.85 \pm 0.21	4.03 \pm 0.26	0.049
Lycopene	1.83 \pm 0.26	1.18 \pm 0.13	0.062	ND	ND	-
ZEA	ND	ND	-	3.95 \pm 0.12	3.22 \pm 0.24	0.034

546 Values are means \pm SEM (n=4). ND: not detected, i.e. lower than 0.1 mg/kg. The effect of
 547 freeze-drying on carotenoid content was evaluated with a 3-way ANOVA, followed by
 548 pairwise comparisons using T-tests.

549 **Table 3. Bioaccessibility* of carotenoids from fresh and rehydrated freeze-dried tomato**
 550 **puree and corn.**

	Tomato puree			Corn		
	Fresh	Freeze-dried	P-value	Fresh	Freeze-dried	P-value
β -carotene	26.1 \pm 1.9	14.3 \pm 1.2	<0.01	39.1 \pm 1.7	NM	-
Lutein	32.9 \pm 2.8	43.4 \pm 3.1	0.012	22.0 \pm 1.3	35.8 \pm 1.5	<0.001
Lycopene	2.0 \pm 0.3	2.0 \pm 0.1	0.752	NM	NM	-
ZEA	NM	NM	-	24.8 \pm 1.7	38.1 \pm 1.7	<0.01

551 *Bioaccessibility refers to the percentage of food carotenoids that is recovered in the micelle
 552 fraction after *in vitro* digestion.

553 Values are means \pm SEM (n=4). NM: not measured (because the corresponding carotenoid
 554 was below the limit of quantification in the micelle fraction). For each matrix, the effect of
 555 freeze-drying on the bioaccessibility of carotenoids was evaluated with a 2-way ANOVA.
 556 Differences in the bioaccessibility of each carotenoid were then analyzed by pairwise
 557 comparisons using T-tests.

558 **Table 4. ZEA and lutein content of vegetables that are the richest in ZEA according to**
 559 **published data (μ g/100 g fresh weight basis).**

Food*	ZEA	Lutein	References
Bell pepper, orange, raw	1665	208	(Perry et al., 2009)
	6200 \pm 60	790 \pm 60	(Murillo et al., 2010)
Xantomato, raw	3900		(Karniel, Koch, Zamir, & Hirschberg, 2020)
Scallions, cooked	2490		(Perry et al., 2009)
Egg yolk, raw	870	917	(Perry et al., 2009)

	213 ± 85	292 ± 117	(Handelman et al., 1999)
Spinach, cooked	0	13 504	(Perry et al., 2009)
	179		(Holden et al., 1999)
	564 ± 75	6420 ± 1190	(Granado et al., 1992)
Bell pepper, yellow, raw	18	139	(Perry et al., 2009)
	440 ± 60	220 ± 20	(Murillo et al., 2010)
Bell pepper, red, raw	440 ± 40	220 ± 40	(Murillo et al., 2010)
	148 ± 38		(Granado et al., 1992)
Corn, canned	216	200	(Humphries & Khachik, 2003)
	216	336	(Scott & Eldridge, 2005)
Cabbage, Kale, cooked	0	8884	(Perry et al., 2009)
		3150	(Sa & Rodriguez-Amaya, 2003)
	173		(Holden et al., 1999)
Potato, cooked	21 ± 1	44 ± 1	(Granado et al., 1992)

560 Values represent means ± SD * Foods are ranked from the richest to the least rich in ZEA.

561 **Table 5. Uptake efficiency (%) of micellarized xanthophylls from *in vitro* digestions of**
 562 **the Xantomato puree and selected ZEA-rich vegetables.**

	Corn	Orange pepper	Xantomato
Lutein	7.0 ± 0.3 ^b	NM	6.7 ± 0.4 ^b
ZEA	6.9 ± 0.5 ^b	10.6 ± 0.6 ^a	7.8 ± 0.4 ^b

563 Concentrations of carotenoids applied on the cells were as follow: corn (Lutein 23 ± 3 nM and
 564 ZEA 21 ± 2 nM), orange pepper (ZEA 75 ± 10 nM), xantomato (Lutein 13 ± 2 nM and ZEA
 565 130 ± 6 nM). Values are means ± SEM (n=4). For each line, mean values with unlike
 566 superscript letters were significantly different ($P < 0.05$; Student t-test or ANOVA followed by
 567 Tukey's HSD test). NM: not measured (because the corresponding carotenoid was below the
 568 limit of quantification).