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## 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

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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

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6

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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.  $\alpha$ -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,  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -  
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,  $\beta$ -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 ( $22.1 \pm 1.0$  mg/kg fresh weight), followed by  
234 Xantomato ( $14.6 \pm 0.3$  mg/kg) and corn ( $3.2 \pm 0.2$  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  $48.4 \pm 3.2$  mg/kg, respectively), which was much higher than that of  
245 Xantomato ( $1.3 \pm 0.03$  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

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357

358

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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

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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.**

<b>Common name</b>	<b>Scientific name</b>	<b>Form of marketing</b>	<b>Laboratory preparation</b>	<b>Steaming time (min)</b>
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
$\beta$ -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
$\beta$ -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 ( $\mu$ 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)

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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.**

	<b>Corn</b>	<b>Orange pepper</b>	<b>Xantomato</b>
Lutein	7.0 ± 0.3 <sup>b</sup>	NM	6.7 ± 0.4 <sup>b</sup>
ZEA	6.9 ± 0.5 <sup>b</sup>	10.6 ± 0.6 <sup>a</sup>	7.8 ± 0.4 <sup>b</sup>

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).