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Abstract

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

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

1) Introduction

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

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

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

2) Materials and Methods

2.1. Chemicals

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

2.2. ZEA-rich food sources and their preparation

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

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

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

2.3. *In vitro* digestions to assess carotenoid bioaccessibility

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

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

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

2.4. Cell experiments to assess xanthophyll uptake efficiency

2.4.1. Caco-2 cell culture

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

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

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

2.4.3. Measurement of the uptake efficiency of xanthophylls from the micellar fraction by Caco-2 cells

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

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

2.5. Xanthophyll extraction

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

2.6. Xanthophyll quantification by HPLC

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

2.7. Calculations and statistics

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

3) Results

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

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

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

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

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

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

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

ZEA bioaccessibility from Xantomato and ZEA-rich fruits and vegetables

Figure 2 shows that ZEA bioaccessibility from corn (38.1 ± 1.7%) was significantly higher than that from orange pepper (28.9 ± 1.1%), Xantomato (26.3 ± 0.8%), spinach (25.6 ±

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

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

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

Discussion

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

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

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

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

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

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

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

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

Abbreviation:

ZEA (zeaxanthin).

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Credit author statement:

Lisa Morand-Laffargue: carotenoid analysis, in vitro digestions, cell culture, analyzed and interpreted data, drafting material and methods and drawing the figures, statistical analysis. **Joseph Hirschberg:** sent the samples of Xantomato, review and editing the manuscript **Charlotte Halimi:** carotenoid analysis supervision. **Charles Desmarchelier:** analyzed and interpreted data, review & editing of the manuscript. **Patrick Borel:** had the idea of the

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

Conflicts of interest:

No conflicts of interest to declare.

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Figure legends

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

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

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

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

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

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

Values are means ± SEM (n=4). ND: not detected, i.e. lower than 0.1 mg/kg. The effect of freeze-drying on carotenoid content was evaluated with a 3-way ANOVA, followed by 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 ± 1.9	14.3 ± 1.2	<0.01	39.1 ± 1.7	NM	-
Lutein	32.9 ± 2.8	43.4 ± 3.1	0.012	22.0 ± 1.3	35.8 ± 1.5	<0.001
Lycopene	2.0 ± 0.3	2.0 ± 0.1	0.752	NM	NM	-
ZEA	NM	NM	-	24.8 ± 1.7	38.1 ± 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 ± 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 (µg/100 g fresh weight basis).**

Food*	**ZEA**	**Lutein**	**References**
Bell pepper, orange, raw	1665	208	(Perry et al., 2009)
	6200 ± 60	790 ± 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).