Digestive Stability of Xanthophylls Exceeds That of Carotenones As Studied in a Dynamic in Vitro Gastrointestinal System

Stéphanie Blanquet-Diot, Maha Soufi, Mathieu Rambeau, Edmond Rock, and Monique Alric

Abstract

Epidemiological studies have suggested that high consumption of tomato products is associated with a lower risk for chronic diseases. To exert their health effect, the phytochemicals of tomatoes have to be bioavailable and therefore imply their stability through the digestion process. Here, we assessed the digestive stability of the red-pigmented lycopene and other carotenoids brought in nutritional quantity within different food matrices, using the TNO gastrointestinal tract model (TIM). This multicompartmental dynamic system accurately reproduces the main parameters of gastric and small intestinal digestion in human. In vitro digestions of a standard meal containing red tomato (RT), yellow tomato (devoid of lycopene), or lycopene beadlets were performed. Zeaxanthin and lutein were stable throughout artificial digestions, whereas β-carotene and all-trans lycopene were degraded (~30 and 20% loss at the end of digestion, respectively) in the jejunal and ileal compartments. The recovery of β-carotene in the digesta of the RT meal was significantly lower than that in the yellow one, showing a food matrix effect. In the same way, until 180 min of digestion, the recovery percentages of all-trans lycopene from RT were significantly lower than those issued from the supplement. Isomeric conformation also influenced the stability of carotenoids, 5-cis lycopene being the most stable isomer followed by all-trans and 9-cis. No trans-cis isomerization of lycopene occurred in the TIM. By using a relevant dynamic in vitro system, this study allowed us to gain further insight into the parameters influencing the digestive stability of carotenoids, and therefore their bioavailability, in humans. J. Nutr. 139: 876–883, 2009.

Introduction

Numerous epidemiological studies have shown an association between the consumption of tomatoes or tomato-based food products and a reduction in the risk of several human chronic diseases (1,2). Although many scientists have attributed the protective effect of tomatoes to the red-pigmented lycopene, other investigations have shown a higher in vivo effect of whole tomato compared with lycopene alone (3,4). It is thought that synergetic effects of this carotenoid with other phytochemicals of tomato (e.g. other carotenoids, polyphenols, or vitamins C and E) may enhance its benefit. A yellow tomato (YT) variety lacking lycopene could be a useful experimental model to distinguish its effect from that of red tomato (RT), as suggested by Gitenay et al. (5). By comparing the effects of red tomato, YT, or lycopene from a supplement, Gitenay et al. clearly showed a higher potential of tomatoes than lycopene alone to protect against oxidative stress (5) or to module gene expression in a prostate cancer cell line (6).

Bioavailability is a critical feature in the assessment of the role of phytochemicals, such as carotenoids, in human health. In humans, carotenoid bioavailability is often monitored by following changes in plasma concentrations after the ingestion of a carotenoid-rich meal (7). However, this approach failed to indicate the actual amounts that are accessible, absorbed, or metabolized. Some animal models are also available (8) but they are costly and limited in application. Alternatively, simple in vitro digestion models have been developed for assessing carotenoid bioaccessibility (9,10). They are increasingly being used to study, with a high predictive value (16), preabsorptive processes and food-related factors affecting carotenoid bioavailability (11–16). In these in vitro studies, efficiency of micellarization, i.e. the amount of carotenoids transferred from the digested food to the
micellar fraction, is often used as an indicator of bioavailability. Nevertheless, these systems are mono-compartmental and static and are not representative of the continuously changing variables during passage through the stomach and the small intestine. Moreover, they give little information about the digestive stability of carotenoids, especially at the level where degradation occurs throughout digestion, and about their sensitivity to the different digestive secretions (HCl, enzymes, etc.).

TABLE 2 Carotenoid concentrations in test meals

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>YT</th>
<th>RT</th>
<th>LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>β-13-cis carotene</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>0</td>
<td>0.22 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.27 ± 0.01</td>
<td>0.21 ± 0.02</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>Β-Carotene</td>
<td>0.13 ± 0.01</td>
<td>1.23 ± 0.12</td>
<td>0</td>
</tr>
<tr>
<td>5-cis Lycopene</td>
<td>0</td>
<td>3.63 ± 1.28</td>
<td>2.06 ± 0.48</td>
</tr>
<tr>
<td>9-cis Lycopene</td>
<td>0</td>
<td>0.60 ± 0.08</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>All-trans lycopene</td>
<td>0</td>
<td>31.06 ± 2.71</td>
<td>16.41 ± 0.55</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 6.

The TNO gastrointestinal tract model (TIM) system is the in vitro model that presently allows the closest simulation of in vivo dynamic physiological processes occurring within the lumen of the stomach and small intestine of humans (17). This dynamic, computer-controlled system has been designed to accept parameters and data from in vivo studies on human volunteers. The main parameters of digestion, such as pH, body temperature; peristaltic mixing and transport; gastric, biliary, and pancreatic secretions; and passive absorption of small molecules and water are reproduced as accurately as possible. This in vitro system offers accuracy, reproducibility, easy manipulation, and the possibility of collecting samples at any level of the digestive tract and at any time during digestion with no ethical constraints. It has been validated by microbial (18), pharmaceutical (19,20), and nutritional (21,22) studies. The actual limitations of the model include the absence of cellular system and feedback mechanisms.

The aim of the present study was to assess the luminal stability of lycopene and/or other carotenoids from RT, YT, or a lycopene supplement subjected to digestion in the TIM system within a standard meal. The effects of food matrix and digestive secretions were of particular interest.

Materials and Methods

Chemicals and supplies. Lipase was purchased from Amano Pharmaceuticals. Unless otherwise stated, all other chemicals and supplies were purchased from Sigma-Aldrich or Fischer Scientific.
In vitro gastrointestinal system. The gastric-small intestinal system TIM consists of 4 successive compartments simulating the stomach and the 3 segments of the small intestine: the duodenum, jejunum, and ileum (Supplemental Fig. 1). This system has been described elsewhere (17). Briefly, each compartment was composed of glass units with a flexible inside wall. Peristaltic mixing and body temperature were achieved by pumping water at 37°C into the space between the glass jacket and the flexible wall at regular intervals. Mathematical modeling of gastric and ileal deliveries with a power exponential formula (23) was used for the computer control of chyme transit. Chyme transport through TIM was regulated by the peristaltic valves that connect the successive compartments. The volume in each compartment was monitored by a pressure sensor. The pH was computer-monitored and continuously controlled by adding either HCl or NaHCO3. Simulated gastric, biliary, and pancreatic secretions were introduced into the corresponding compartments by computer-controlled pumps. Water and products of digestion were removed from the jejunal and ileal compartments by pumping dialysis liquid through hollow fiber membranes (HG 600, Hospal Cobe).

Preparation of test meals for in vitro digestion. Three different meals were prepared extemporaneously and subjected to digestion into the TIM. The standard meal was designed to contain all the macronutrients present in a typical Western diet but without carotenoid [adapted from Tysandier et al. (24)]. The meal consisted of 60 g of cooked egg white that was previously rinsed with water to completely remove egg yolk, 50 g of cooked pasta, 2.5 g of soy lecithin (Les 3 chênes), and 20 g of sunflower oil. All the ingredients (except soy lecithin) were purchased from a local supermarket. A total of 100 g of double-concentrated puree of RT or YT (Vydac TP54 (250 × 4.6 mm, 5 μm) purchased from Interchim. The mobile phase was an acetonitrile:methanol (containing 50 mmol/L ammonium acetate:dichloromethane:water mixture (70:15:10:5, v:v:v) before injection into an HPLC apparatus. Carotenoids were analyzed by reverse-phase HPLC using a Waters system equipped with a cooled autosampler injector and a UV-visible diode array detector system. Separation was carried out using 2 columns in a series: a Nucleosil C18 (150 × 4.6 mm, 3 μm) followed by a Vydac TP54 (250 × 4.6 mm, 5 μm) purchased from Interchim. The mobile phase was an acetonitrile:methanol (containing 50 mmol/L ammonium acetate:dichloromethane:water mixture (70:15:10:5, v:v:v). The flow rate was isocratic (2 mL/min). Carotenoids were identified and quantified by comparing retention times, spectral profiles, and peak areas with pure standards (zeaxanthin, lutein, β-cryptoxanthin, β-carotene, β-13-cis carotene, and all-trans lycopene). Quantification of cis-lycopene was conducted using calibration curves constructed with all-trans lycopene.

Experimental design. The TIM system was programmed to reproduce the digestion of a solid meal in healthy human adults (Table 1). Because the current work focuses on the luminal stability of carotenoids, passive absorption of digestion products was not reproduced (the hollow fibers and the dialysis pump were disconnected). To prevent photodecomposition and oxidation of the carotenoids, all the digestive compartments and the collection vessels were recovered by tinfoil and maintained under nitrogen flow. The total duration of the digestion was 300 min (n = 3 for each condition). Samples (1 mL) were taken from the meals before introduction into the artificial stomach and were regularly collected from each digestive compartment during digestion. The ideal deliveries were kept on ice and pooled at 60, 60–120, 120–180, 180–240, and 240–300 min. The volumes were measured and aliquots were taken for each period. All samples were stored at −20°C prior to analysis.

Extraction and HPLC analysis of carotenoids. Sample extraction was conducted under subdued (yellow) light, as previously described (24). Echinone (Hoffmann-La Roche) was used as internal standard (0.36 μmol/L). Extracts were dried under nitrogen and dissolved in 200 μL methanol/dichloromethane (65:35, v:v) before injection into an HPLC apparatus. Carotenoids were analyzed by reverse-phase HPLC using a Waters system equipped with a cooled autosampler injector and a UV-visible diode array detector system. Separation was carried out using 2 columns in a series: a Nucleosil C18 (150 × 4.6 mm, 3 μm) followed by a Vydac TP54 (250 × 4.6 mm, 5 μm) purchased from Interchim. The mobile phase was an acetonitrile:methanol (containing 50 mmol/L ammonium acetate:dichloromethane:water mixture (70:15:10:5, v:v:v). The flow rate was isocratic (2 mL/min). Carotenoids were identified and quantified by comparing retention times, spectral profiles, and peak areas with pure standards (zeaxanthin, lutein, β-cryptoxanthin, β-carotene, β-13-cis carotene, and all-trans lycopene). Quantification of cis-lycopene was conducted using calibration curves constructed with all-trans lycopene.

FIGURE 2  Recovery profiles of β-carotene from YT and RT meals in the stomach (A), duodenum (B), jejunum (C), and ileum (D) of TIM. Values are means ± SEM, n = 3. *Different from the marker transit, P < 0.05. *Different from YT, P < 0.05. ND, Nondetectable.
Different from LB, lycopene means 6. The digestive stability of carotenoids in digesta/carotenoids in test meal 3 was evaluated by calculating percentage recovery (carotenoids and that of a nonabsorbable marker, blue dextran, as previously described (17). Values are given as means\(\pm\)SEM (from 3 to 6 independent replicates). Significant differences between treatments were tested by ANOVA with repeated-measure analysis followed by a post hoc test based on a least square difference. The comparison between various times and the initial time (test meal) was made using paired \(t\) test. All analyses were performed using the SAS 9.1 software (SAS Institute). Differences of \(P<0.05\) were considered to be significant.

Results

Carotenoid composition of test meals. The carotenoid concentrations in test meals (YT, RT, and LB) are given in Table 2. Our HPLC method allowed the detection of the main dietary carotenoids (25). \(\beta\)-Cryptoxanthin and \(\beta\)-13-cis carotene were not found in any of the meals. The main carotenoid measured in RT and LB meals was the all-trans lycopene (31.06 ± 2.71 and 16.41 ± 0.55 mg/kg diet, respectively). Two cis isomers of this carotenoid were also detected and identified as 5-cis and 9-cis lycopene by comparing their spectral characteristics with those reported by other authors (26,27). Lycopene was not detected in the YT meal. Small concentrations of lutein (ranging from 0.11 to 0.27 mg/kg diet) were found in all the tested meals. Zeaxanthin was detected in a small quantity only in the RT meal. \(\beta\)-Carotene was found in higher concentrations in the RT than in the YT meal (1.23 vs. 0.13 mg/kg diet).

In vitro digestive stability of carotenoids. Zeaxanthin and lutein were stable during in vitro digestion (Table 3). In the RT experiment, the high variability in their recoveries may be explained by the difficulty of separating lutein from zeaxanthin by HPLC. The stability of \(\beta\)-carotene during digestion depended on the tested meal (Fig. 1). \(\beta\)-Carotene from YT was stable during in vitro digestion, whereas that from RT was degraded, with a recovery percentage of 67.8 ± 9.4% \((n = 3)\) at 300 min. Throughout digestion, the recovery percentages of \(\beta\)-carotene were lower for the RT meal than the YT meal \((P < 0.05)\). The recovery profiles of the carotenoid were compared with that of the transit marker in each digestive compartment of TIM (Fig. 2). In the RT experiment, the curves of \(\beta\)-carotene and that of the marker did not differ in the stomach and duodenum, showing that the degradation of the carotenoid occurred in only the jejunum and ileum. In these compartments, the recovery percentages of \(\beta\)-carotene from RT were lower than that from YT \((P < 0.05)\). All-trans lycopene was not stable during in vitro digestions of RT or LB meals (Fig. 3A). After 300 min of digestion, 83.0 ± 2.4% and 80.6 ± 4.3% \((n = 3)\) of initial all-trans lycopene were recovered for RT and LB experiments, respectively. From the beginning of the experiment to 180 min of digestion, the recovery percentages of all-trans lycopene from RT were lower than that from LB \((P < 0.05)\). As for \(\beta\)-carotene, all-trans lycopene was not degraded in the stomach and duodenum of TIM, but it partly disappeared in the jejunum and ileum (Fig. 4). The recoveries obtained for RT and LB meals significantly differed in the duodenal and jejunal compartments. The behaviors of 5-cis and 9-cis isomers of lycopene were completely different in the simulated gastrointestinal conditions. The results obtained with 5-cis lycopene were highly variable and we did not find significant changes over time or differences between the meals (Fig. 3B). This isomeric form of lycopene was thus considered stable during in vitro digestion. On the contrary, the percentages of 9-cis lycopene fell dramatically from 0 to 120 min of digestion (Fig. 3C). At the end of in vitro digestion, 33.3 ± 13.9 and 60.6 ± 11.0% \((n = 3)\) of initial 9-cis lycopene were recovered in the LB and RT experiments, respectively. From 120 to 300 min digestion, the percent recovery of this carotenoid tended to be lower for the LB than for the RT experiment \((P = 0.19\) at 300 min). 9-cis Lycopene from LB and RT was stable under gastric conditions but was degraded in the small intestine from the duodenal compartment (Fig. 5).

Isomerization of lycopene. We performed further calculations to follow the trans-cis isomerization of lycopene under the sim-
ulated gastrointestinal conditions. In the RT meal, all-trans, 5-cis, and 9-cis isomers represented 88.2, 10.0, and 1.8% of the total amount of lycopene measured, respectively. Similar percentages were found for the LB meal with 87.2, 11.8, and 1%, respectively. In all the digestive compartments, the isomeric distribution of lycopene did not differ regardless of the sampling time and the tested meal (data not shown). Nevertheless, in the gastric compartment during the RT experiment, the mean percentages of all-trans lycopene tended to decrease (79.1% at 120 min), whereas those of 5-cis increased (18.8% at 120 min).

Discussion
Consumption of tomato and tomato-based food contributes to the intake of a wide range of carotenoids, particularly the well-known lycopene. To mediate their health effect, carotenoids should be bioavailable, i.e. absorbed and delivered to the target tissues for utilization or storage. The bioavailability of carotenoids is extremely variable, being influenced by many dietary and physiological factors (28). In vitro digestive systems have been shown to be relevant tools to study the effects of physical properties of carotenoids, such as trans compared with cis isomers (14,15,29) or free compared with esterified forms (13); food source and matrix (12,13,29,30); processing (10,31,32); and interaction with other dietary compounds (10,33–35). The systems that have been utilized to date are static and monocompartmental (9,10,36). Gastric and duodenal digestions are successively simulated in a flask at 37°C under shaking by changing pH and adding the appropriate digestive secretions. Until now, no data, to our knowledge, were available on the stability of carotenoids in the different gastrointestinal compartments and the influence of pH and digestive secretions in dynamic conditions.

In the present study, we assessed the luminal stability of lycopene and other carotenoids (zeaxanthin, lutein, and β-carotene) in the TIM system. Contrary to previously mentioned models, this model reproduces the main dynamic phenomena occurring during gastrointestinal digestion in humans: transit of chyme from one digestive compartment to another, evolution of pH during gastric digestion and from the duodenum to ileum (Table 1), and sequential arrival of digestive secretions. Lycopene and the other carotenoids were supplied by LB, RT, or YT to evaluate the effect of food matrix on their digestive stability. To closely mimic real conditions, they were brought within a standard meal, in a quantity similar to that found in human diet (37).

Zeaxanthin and lutein (regardless of the tested meal) were stable during digestion in TIM (Table 3). No data are available about in vitro digestive stability of zeaxanthin from tomato. Nevertheless, high recovery percentages (from 80 to 100%) were found for zeaxanthin in orange, spinach, or sweet corn (12,13,29). Our results for lutein stability are consistent with those of Granado-Lorencio et al. (12) who found a 100% recovery in tomato paste after simulated gastric or duodenal digestion. This carotenoid seems to be highly resistant to gastrointestinal conditions whatever its food source, because recovery percentages ranging from 80 to 100% were measured in digesta of orange, spinach, or broccoli (11,12,29).

β-Carotene fromYT was stable in the TIM system, whereas a 30% loss was observed when this carotenoid was from RT (Fig. 1). Granado-Lorencio et al. (12) found a recovery of β-carotene from tomato paste of 80% after simulated gastric digestion and of almost 100% after the duodenal phase. This seems to indicate a sensitivity of β-carotene to their gastric conditions (pepsin; pH 1.1;
1 h) that was not found in the present study (Fig. 2A). This may be explained by differences between our 2 experimental protocols (static vs. dynamic conditions). As for the RT meal, previous studies showed a degradation of β-carotene during simulated digestion. Recovery percentages ranging from 50 to 85% were found in digesta of carrot, spinach, broccoli, or mango (11, 12, 29, 35). In the present study, the stability of β-carotene depended on the food matrix. The amount of β-carotene in the tested meals (Table 2) may have influenced its stability during digestion through the efficiency of micellarization, as previously shown for lutein or zeaxanthin (38, 39). Our results showed that the levels of β-carotene from RT significantly decreased in the lowest parts of the small intestine (Fig. 2C, D). It appears that trans-cis isomerization of β-carotene does not occur during digestion either in vitro (15, 30) and in vivo (24, 40). Nevertheless, we suppose that nondetected metabolites/degraded molecules of β-carotene were produced during small intestinal digestion, but no precise data support our hypothesis.

Lycopene is found in most food sources primarily as the all-trans isomer. Similar isomeric distributions were found for lycopene in LB and RT meals, with a predominance of the all-trans isomeric form (87–88%), in accordance with previously published data (27, 41). Whatever the food matrix (RT or LB), all-trans-lycopene was significantly degraded during in vitro digestion in TIM (Fig. 3A). Granado-Lorencio et al. (12) found a recovery of lycopene from tomato paste of 45% and almost 80% after gastric and duodenal digestion, respectively. As previously discussed for β-carotene, such a low recovery in the gastric phase may be imputed to static conditions, because with dynamic ones in TIM, there was no degradation in the artificial stomach (Fig. 4A). Recovery percentages of all-trans lycopene similar to that found here (~80%) were obtained by Failla et al. (14) after gastrointestinal digestion of gac fruit cooked with rice. Until 180 min of digestion, the recovery percentages of all-trans lycopene from RT were lower (P < 0.05) than that from the supplement. As expected, the matrix in which lycopene is located in RT differs from that of the synthetic beadlets and has affected lycopene stability during digestion. Similar in vitro results were obtained by Chitchumroonchokchai et al. (29), who showed that the digestive stability of lutein and zeaxanthin from an oil-based supplement was greater than that from spinach. In humans, lycopene from tomato oleoresin capsules is much more available than that from raw tomatoes (42). Here, it is difficult to conclude that there is a relative advantage of the supplement over RT as a dietary source of lycopene, because at the end of digestion in TIM, similar recovery percentages were found in both cases. 5-cis Lycopene was stable during in vitro digestion (Fig. 3B), whereas 9-cis lycopene was widely degraded (Fig. 3C). Similar results were obtained by Chasse et al. (43) using ab initio molecular modeling.

In our in vitro study, β-carotene and lycopene (belonging to the carotene group) seemed to be more sensitive to digestive conditions than lutein or zeaxanthin (xanthophylls). The hydrophobicity of carotenoid may affect their luminal stability, as has already been observed for their transfer from emulsion lipid droplets to micelles (44). The difference in their localization in the lipid droplets (45) may explain their digestive stability and therefore their availability, as it has already been described in humans for β-carotene and lutein (46, 47). Our results also suggested that the lower plasma status of lycopene compared with β-carotene in humans following tomato product ingestion (48), especially in the elderly (49), may result from postabsorptive events rather than from phenomena occurring in the digestive lumen.

FIGURE 5 Recovery profiles of 9-cis lycopene from RT and LB meals in the stomach (A), duodenum (B), jejunum (C), and ileum (D) of TIM. Values are means ± SEM, n = 3. +Different from the marker transit, P < 0.05. *Different from LB, P < 0.05. ND, Nondetectable.
Although lycopene is present in its all-trans isomeric form in fruits and vegetables, cis-isomers constitute the predominant form in human serum and tissues (41). A trans-cis isomerization has been suspected to occur during digestion (26). In our in vitro conditions, we observed a disappearance of all-trans lycopene in the lowest part of the small intestine (Fig. 4C,D) that could not be directly linked with the formation of 5-cis or 9-cis lycopene that was stable and degraded under digestive conditions, respectively (Fig. 3). We then further analyzed the isomeric distribution of lycopene throughout digestion and showed that trans-cis isomerization occurred in none of the digestive compartments of TIM. Conflicting results have been obtained about trans-cis isomerization of lycopene during digestion. In vitro studies (26,50) have shown that this carotenoid was isomerized as a result of low pH, whereas another study (14) concluded that isomeric profiles of lycopene had no marked change during simulated gastrointestinal digestion. In vivo investigations showed that modest gastric isomerization occurred in the stomach of ferrets (51) but not in that of humans (24). Our results support the hypothesis that isomerization in the digestive lumen does not contribute to the enrichment of cis isomers in the human blood. This enrichment may be rather due to a preferential uptake of cis isomers by enterocytes or a more efficient incorporation into chylomicrons for delivery to tissues (14,41). As suggested above for β-carotene, we could hypothesize that nondetected oxidative metabolites as well as enzyme-catalyzed cleavage products of lycopene (52) were produced during small intestinal digestion in TIM.

In conclusion, we found that the digestive stability of carotenoids depends on their nature, xanthophylls being more stable than carotenoids; their isomeric conformation; the food matrix in which they are included; and the digestive compartment. These data clearly confirm that numerous factors influence the bioavailability of carotenoids in humans and therefore condition their health effects.

**Literature Cited**


38. O’Connell OF, Ryan L, O’Brien NM. Xanthophyll carotenoids are more bioaccessible from fruits than dark green vegetables. Nutr Res. 2007;27:258–64.


