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Kinetic Studies of Lycopene Isomerization in a Tributyrin Model System at Gastric pH

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A semi-preparative HPLC method was developed in order to isolate and purify the 13-*cis*-lycopene isomer in tomato-based materials. The result was compared with the naturally predominant *all-trans*-lycopene isomer, in terms of stability to gastric pH at physiological temperature in a tributyrin model system. Kinetic experiments confirmed that lycopene isomerization is a reversible reaction, and under these conditions the *all-trans* isomer is more stable than the 13-*cis* isomer. In addition, it was found that at gastric pH 13-*cis*-lycopene would predominantly isomerize to the *all-trans* form rather than undergo oxidation/breakdown. A simulation based on the rate constants calculated in the kinetic study indicated that at gastric pH the lycopene isomeric distribution aimed toward an equilibrium characterized by approx 16% 13-*cis*-, 16% 9-*cis*-, and 68% *all-trans*-lycopene. This study suggests that pH-driven isomerization in the stomach is at least partially responsible for the relatively high *cis*-lycopene proportion found *in vivo*.

KEYWORDS: Lycopene; *cis*−*trans* isomerization; kinetics; gastric pH; tributyrin

INTRODUCTION

One of the reasons for the popularity of tomato is its pleasant red color, imparted by lycopene. Lycopene is an acyclic C_{40} nonpolar carotenoid found in tomatoes, watermelon, guava, apricots, and other natural sources. The molecule is highly unsaturated, the 11 conjugated double bonds being responsible for the ruby red color. Of the theoretical 2^{11} geometrical isomer forms, only 72 are thermodynamically possible (*1*). *all-trans*-Lycopene is the predominant lycopene isomer in nature, representing about 80−97% in tomatoes and related products (*2*). In body fluids, however, more that 50% of lycopene was found in various *cis* forms (*3*), leading to the suggestion that the 50:50 ratio between the *cis* and *all-trans* forms may represent an equilibrium under physiological conditions (*4*). Investigators proposed several explanations of this finding, such as the preferential absorption of *cis*-lycopene isomers within the intestines (*5*) or the isomerization of *all-trans*-lycopene in the stomach or intestines (*6*).

The high concentrations of *cis* isomers *in vivo* triggered the hypothesis that they may be more bioavailable and/or more bioactive than the *all-trans* form. Studies of the *cis*:*trans* isomer ratios in various organs and tissues of ferrets fed with a lycopene-rich diet (*2*, *4*) suggested that the *cis* isomers are indeed more bioavailable than the *all-trans* isomer, possibly due to the higher solubility and/or a preferential incorporation of the *cis* isomers into chilomicrons. In terms of bioactivity, Shi and LeMaguer (*7*) pointed out that the bioactive potency of *cis*-lycopene isomers is different from that of the *all-trans*, because of structural differences. Böhm et al. (*8*) indeed found that three unidentified *cis* isomers had a stronger antioxidant activity than the *all-trans* one. For all these reasons, *cis*-lycopene isomers are regarded as having potentially higher health benefits than the *all-trans* isomer.

Lycopene isomerization triggered by the gastric pH is believed to be one of the reasons for the unusually high amounts of *cis* isomers, when compared to the lycopene intake. An *in vitro* study (*6*) found that gastric pH is able to trigger the isomerization of *all-trans*-lycopene to *cis* forms after an exposure as low as 3 min. However, an *in vivo* investigation of the *cis*:*trans*-lycopene ratio in human subjects fed intragastrically (*9*) found that it did not significantly vary in the stomach during digestion, suggesting there is little isomerization, if any. These conflicting results leave open the question whether low-pH-driven isomerization indeed plays a role in the large proportion of *cis*-lycopene isomers found *in vivo*. On the other hand, no information was found on the relative stability of the *cis* forms vs the *all-trans* form under low pH conditions, and the quantification of the relative stability would contribute to the better understanding of the *in vivo* changes. Since the *cis*-lycopene isomers are not commercially available, the comparative investigation of *cis*−*trans*-lycopene isomers in processing or physiological conditions is typically performed by monitoring the changes of *all-trans*-lycopene or mixtures of lycopene isomers. The drawback of this approach is that the system includes several *cis* forms, in very small amounts, which makes the comparison less accurate.

The objectives of this study were to isolate and purify the most abundant *cis*-lycopene isomer from tomato-based sources

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and to compare its stability to the stability of the *all-trans* form, when subjected to gastric pH at physiological temperature in a tributyrin model system. The purified compounds were used to collect kinetic data, enabling the calculation of reaction rate constants for the isomerization reactions undergone by the predominating isomers. Thus, this study attempts to improve the understanding of pH-driven lycopene isomerization *in vivo*, enabling the prediction of the extent of isomerization based on gastric residence time.

MATERIALS AND METHODS

Preparation of the Lycopene Stock. The procedure was derived from procedures reported in the literature (*10*−*12*), and was performed in aluminum foil-wrapped glassware or, when not possible, in dim light. All reagents were purchased from Fisher Scientific (Fairlawn, NJ), and solvents were HPLC grade.

Tomatoes for fresh consumption, purchased from a local supermarket (Shop-Rite, Elizabeth, NJ), were used as the source for preparing *all-trans*-lycopene. A 30-g portion of tomatoes was washed, wiped dry, and then cut in half, and the cores were removed. The material was mixed with 100 mL of extraction solvent (hexane:acetone:ethanol, 2:1:1, v/v/v) in a Nova I blender (Waring Corp., New Hartford, CT). To prevent heating, mixing was performed for 10 min in an iced water bath, in 1-min steps alternating with 3-min breaks. After filtration through Whatman #1 paper (Whatman Inc., Clifton, NJ), the washing and filtration of the retentate with the same extraction solvent mixture was repeated until colorless. Filtrates were pooled, and the nonpolar phase was separated in a separatory funnel. Saponification of the nonpolar phase with 10% KOH in methanol was performed overnight, in the dark. The mixture was then rewashed and the nonpolar phase was again separated in a separatory funnel. After filtration and removal of solvents using a rotary evaporator at 35 °C water bath temperature, the preparation was solubilized in 2 mL of hexane:dichloromethane (4:1, v/v), leading to the crude extract.

Purification was performed using a 300 × 11 mm chromatography column (Kontes, Vineland, NJ), packed with a 1:1 (w/w) mixture of magnesium oxide and Celite and a top layer of anhydrous sodium sulfate of approximately 1-cm height. The sample was loaded onto the column and then eluted stepwise. The first step used 100−150 mL of petroleum ether, aiming to remove the more nonpolar species, including lipid residues. When the coloration of the phase leaving the column returned from yellow to colorless, petroleum ether with 4% acetone was added. This caused a slow movement of the intense red band consisting of lycopene. Elution was stopped when the phase leaving the column was colorless and the intense red band reached the lower third of the column.

The packing of the column was carefully taken out, and the intensely red band was separated. The purified lycopene was obtained by extraction from this area, using the same extraction solvent as above. After solvent removal, lycopene was redissolved in 2 mL of hexane:dichloromethane mixture (4:1, v/v), placed in brown GC vials, slightly flushed with nitrogen, and then sealed and placed immediately in a low-temperature freezer (−65 °C).

Quantification of Lycopene Isomers. The lycopene isomers were quantified by analytical HPLC, using a dedicated 150 × 4.6 mm i.d., 3 μm, Develosil RP-Aqueous C_{30} column (Phenomenex Inc., Torrance, CA), protected by a C_{18} SecurityGuard column, both kept at 25 °C. A 50-μL sample of material was injected, using a loop of 50 μL (Rheodyne L. P., Rohnert Park, CA). The HPLC method used to quantify lycopene isomers was derived from the method reported by Yeum et al. (*14*), modified to improve resolution while decreasing the resolution time. Solvent A was a mixture of methanol:MTBE:water (83:15:2, v/v/v), and solvent B was methanol:MTBE:water (18:80:2, v/v/v). The method duration was 18 min, at a flow rate of 1 mL/min. The solvent delivery scheme consisted of a linear change from 90% solvent A + 10% solvent B at 0 min to 5% solvent A + 95% solvent B at 4 min, followed by a flat profile for the next 12 min and then a linear change back to 90% solvent A + 10% solvent B at 18min. β-8′-Apocarotenal was used as internal standard (*15*), both for building the calibration curve and in the experimental injections.

The HPLC equipment consisted of a 600E solvent delivery system and 991 photodiode array detector (Waters Corp., Milford, MA) and a computer running the dedicated Waters 991 PDA software. For faster processing, the signal at 471 nm, the absorption maxima for lycopene (*13*), was exported via an analogue output channel to a second computer running the Diamir chromatography software (JMBS, Bear, DE).

Preparation of the *cis*-Lycopene Isomer. To improve the *cis* isomers proportion, isomerization of stock lycopene was forced by placing aliquots of the stock (concentration approximately 120 mg/L) in a 1.5-mL brown, septum-closed GC vial (National Scientific, Duluth, GA), and preheating the vial by submersion in an oil bath for 5 min at 85 °C (oil temperature). *all-trans*-Lycopene and the predominating *cis*-lycopene isomer were isolated from the preheated stock by cooling it down and directly injecting the concentrated stock in the HPLC, to increase the concentration of the isomers collected (*16*).

The HPLC system used for the preparation of larger amounts of lycopene isomers was the system described above, except it used a 250 × 10 mm i.d., 5 μm, Develosil RP-Aqueous C30 semi-preparative column protected by a semi-preparative scale C18 SecurityGuard column (both provided by Phenomenex Inc., Torrance, CA), both kept at 25 °C. The injection size was 100 μL. A fraction collector was connected to the outlet of the HPLC and programmed to collect every 0.2 min starting at 13 min. To avoid isomerization caused by temperature or light, fractions were collected in tubes wrapped in aluminum foil and at the end of each injection were transferred into larger, septum-closed, amber vials stored in the freezer. Every 4 h, the similar fractions were pooled together in brown bottles, sealed, and stored in a low-temperature freezer (−65 °C) until further use. For longer storage, solvent was removed by flushing with nitrogen in dim light on ice.

Identification of the Lycopene Isomers Prepared. The fractions carrying lycopene isomers were tentatively identified spectrophotometrically: 1 mL of each fraction was placed in a quartz cuvette (Fisher Scientific, Fairlawn, NJ) and its absorption spectrum was read between 300 and 550 nm, at a rate of 100 nm/min, using a U 3110 UV/vis spectrophotometer (Hitachi Instruments, San Jose, CA). The fractions containing lycopene isomers were further analyzed with the analytical HPLC method, which allowed pooling together neighboring fractions showing the same pattern.

The structural identity of the fractions containing the main two isomers was confirmed using ^{1}H NMR. For this purpose, the solvent was removed by flushing with nitrogen, in the dark and on ice, and then the dried residue was redissolved in deuterated chloroform (*17*). The samples were analyzed using a Varian 400 ^{UNITY}INOVA NMR spectrophotometer with the VNMR version 6.1B (Varian NMR Systems, Palo Alto, CA) software package, at room temperature. The spectra obtained were compared with published NMR spectra of lycopene isomers (*18*), and the identity of isomers was elucidated by comparing the value of the spectroscopic shifts.

Kinetic Studies of Lycopene Stability in a Model System, at Gastric pH. Lycopene is solid and hydrophobic, so a hydrophobic model system able to dissolve lycopene was necessary. Criteria such as liquid state, ability to dissolve lycopene, stability at temperatures above 100 °C, lack of rancidity or oxidation, and relatively low viscosity were used to select the system. The system of choice was tributyrin, a liquid triglyceride having the residues of the saturated butyric acid esterified to glycerol, found in small amounts in butter.

The experiment used a solution of 13-*cis*-lycopene in tributyrin (concentration of 19.8 mg/L) and a solution of *all-trans*-lycopene in tributyrin (concentration of 20.5 mg/L). A 1-mL aliquot of isomer solution was mixed with 2 mL of simulated gastric juice (composition: 0.7% hydrochloric acid, 0.2% sodium chloride, 1.07 ppm pepsin in distilled water; pH 1.5; Fisher Scientific, Fairlawn, NJ) and 5 μL of Tween-20. The emulsion was prepared by sonication on ice in an XL2020 instrument (Heat Systems Inc., Farmingdale, NY) set at level 5, using six 10-s steps alternating with 10-s rest. The simulated gastric juice (SGJ) was preheated at 37 °C, and the temperature of the emulsion after preparation did not exceed 42 °C in any of the samples. An aliquot of the emulsion was stored at room temperature to check the stability and 48 h after preparation did not show any phase separation.

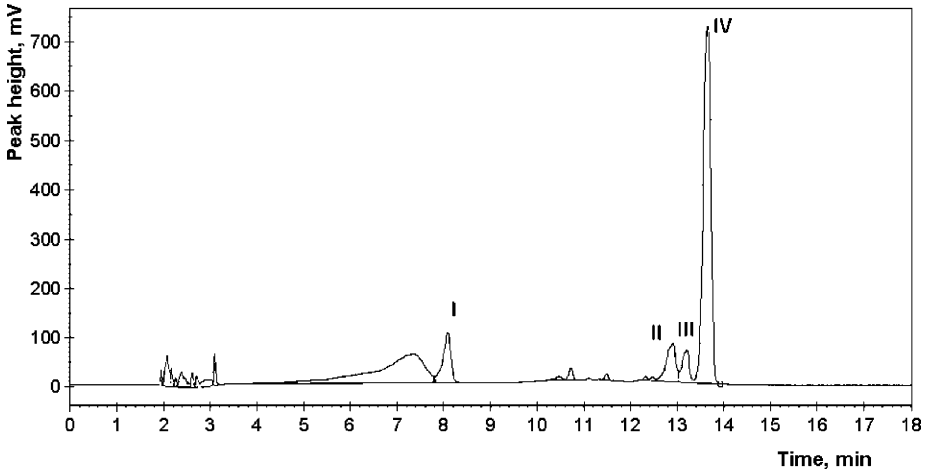


Figure 1. Chromatogram of the lycopene stock. Peaks are as follows: I, internal standard (*β*-8′-apocarotenal); II, 13-*cis*-lycopene; III, 9-*cis*-lycopene; IV, *all-trans*-lycopene.

Aliquots of the emulsions were placed in septum-closed, brown GC vials and immersed in an oil bath preheated at 37 ± 1 °C. Each 30 min, 50 μL of each sample was injected in the HPLC, and the concentration of each lycopene isomer was quantified using the analytical method described above, assuming the same specific absorption coefficient for both isomers (*8*). The experiments were performed in two replicates and lasted for 6 h, after which peaks were below the limit of quantification.

RESULTS AND DISCUSSION

Preparation and Identification of the Lycopene Isomers Prepared. Comparison and spiking tests of the lycopene stock with authentic *all-trans*-lycopene (Sigma-Aldrich, St. Louis, MO) showed that the lycopene stock mostly consisted of *all-trans*-lycopene. HPLC quantification found that the *all-trans* isomer accounted for about 90% of the lycopene stock.

Chromatograms of the lycopene stock showed four main peaks (**Figure 1**). While spiking experiments showed that peak I belongs to the internal standard and peak IV belongs to *all-trans*-lycopene, peaks II and III were tentatively identified as 13-*cis*- and 9-*cis*-lycopene, by comparison with the chromatograms published by Schierle et al. (*19*). The quantification of these *cis* isomers was performed by assuming the same specific absorption coefficient as the one calculated from the calibration curve for *all-trans*-lycopene. This assumption was used previously by Böhm et al. (*8*) and Ishida et al. (*20*). The *Q*-ratios for the tentatively identified *cis*-lycopene isomers were calculated as the ratio between the areas of peaks II or III and the area of peak IV.

The fractions collected using the preparative method were screened spectrophotometrically, and the ones showing higher color intensities were further analyzed by HPLC and ^{1}H NMR. **Figure 2** shows that, while the *all-trans* fraction appeared to be close to purity, the assumed 13-*cis* fraction still had small amounts of *all-trans* and 9-*cis* isomers, and the assumed 9-*cis* fraction was even less pure. Possible causes for these residues could be a partial coelution of *cis*−*trans* isomers (reported also by Böhm et al. (*8*)) or generation of the *all-trans* form as an artifact between separation and storage in the freezer. However, the very short exposure to light and room temperature makes the second possible cause rather improbable. For purity reasons, the fractions collected were the ones corresponding to *all-trans*- and 13-*cis*-lycopene.

UV/vis spectra and chromatograms of collected fractions corresponding to each individual peak are shown in **Figure 2**. The UV/vis spectra of the fractions assumed to correspond to 13-*cis*- and 9-*cis*-lycopene (**Figure 2**, parts **A** and **B**) had a peak around 360 nm. This peak was not found in the spectrum of *all-trans*-lycopene (**Figure 2C**). In addition, the UV/vis spectra had absorption maxima at 471 nm for the *all-trans* form (**Figure 2C**), at 467 nm for the fraction assumed to be 9-*cis*-lycopene (**Figure 2B**), and at 464 nm for the fraction assumed to be 13-*cis*-lycopene (**Figure 2A**). Both the so-called "*cis*-peaks" and the hypsochromic shifts in the absorption maxima were previously reported in the literature as specific for *cis*-carotenoid isomers (*21*).

The ultimate proof was provided by the NMR spectra of the assumed lycopene isomer preparations. The hydrogen atoms bound to the polyene chain carbons have chemical shifts between 5 and 7 ppm (*17*, *18*), so this particular zone of the NMR spectrum can be used to identify the isomer. The chemical shifts found experimentally for the two preparations analyzed showed very good match with the NMR data reported by Hengartner et al. (*18*), for both the *all-trans*- and 13-*cis*-lycopene, which confirmed the identity of the two fractions investigated by NMR.

While the purity of the *cis*-lycopene fractions collected using this method is critical, the yield is also very important for the feasibility of further experiments. To quantify the amount of fractions prepared, about 1 mL of *all-trans* stock preparation (concentration of 323.6 mg/L) was used to prepare *cis* forms as described. On the basis of the assumption of the same specific absorption coefficient, about 48 μg of 13-*cis*- and about 160 μg of *all-trans*-lycopene were prepared. A balance of lycopene found that in the preheating process less than 30% of the initial *all-trans*-lycopene broke down, while 71% was found in the preparations collected.

Kinetic Studies of Lycopene Stability in a Model System, at Gastric pH. The concentrations of each lycopene isomer over time are shown in **Figure 3**. In both cases, the isomer that predominated in the initial material underwent a steady loss with time, while the other isomer accumulated up to a maximum, after which the concentration decreased. This result confirms that the pH-driven isomerization is a reversible reaction: the *all-trans* isomer was lost with formation of the 13-*cis* form (**Figure 3A**), while 13-*cis*-lycopene was lost with formation of the *all-trans* isomer (**Figure 3B**). This result is easier to visualize when looking at the *Q*-ratio (**Figure 4**): the 13-*cis* *Q*-ratio showed an initial large drop when starting from 13-*cis*-lycopene, but the curve flattened rather quickly. When starting from *all-*

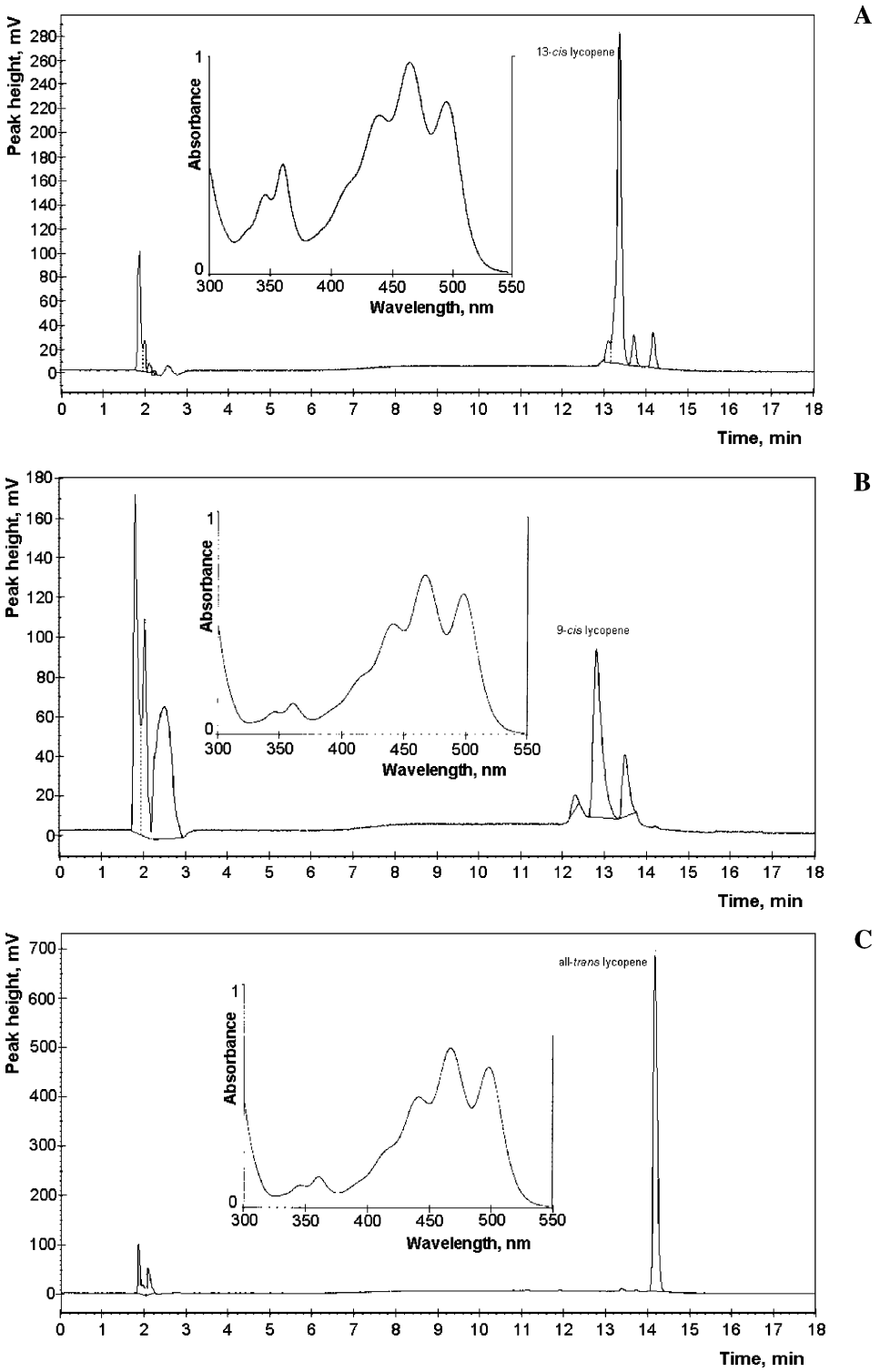


Figure 2. UV/vis spectra and chromatograms of the lycopene fractions collected using the semipreparative HPLC method: **A**, 13-*cis* fraction; **B**, 9-*cis* fraction; **C**, *all-trans* fraction.

trans-lycopene the *Q*-ratio steadily increased, indicating a continuous accumulation of the 13-*cis* isomer.

The total lycopene concentration in **Figure 3** was calculated by summing the concentrations of the *all-trans*, 13-*cis*, and 9-*cis* isomers; the other isomers were probably negligible, as new peaks did not appear in the chromatograms. The curves for total lycopene had similar slopes in **Figure 3A,B**, suggesting that overall lycopene was lost to a similar extent in both cases and confirming that low pH causes not only lycopene isomerization but also breakdown. This was in fact expected, since acids such as nitric or trichloroacetic are known to initiate degradation of carotenoids in foods by isomerization or oxidation: Mortensen and Skibsted (*22*) reported that carotenoids are protonated slowly by such acids, by a mechanism still largely unknown. It was also reported that carotenoids can react with Lewis acids (e.g. I_2), generating species that absorb in the same area as carotenoids radicals (*22*).

When the experiment started with *all-trans*-lycopene (**Figure 3A**), the *all-trans* concentration dropped rather quickly, as shown by the steep slope. The slope of the curve corresponding

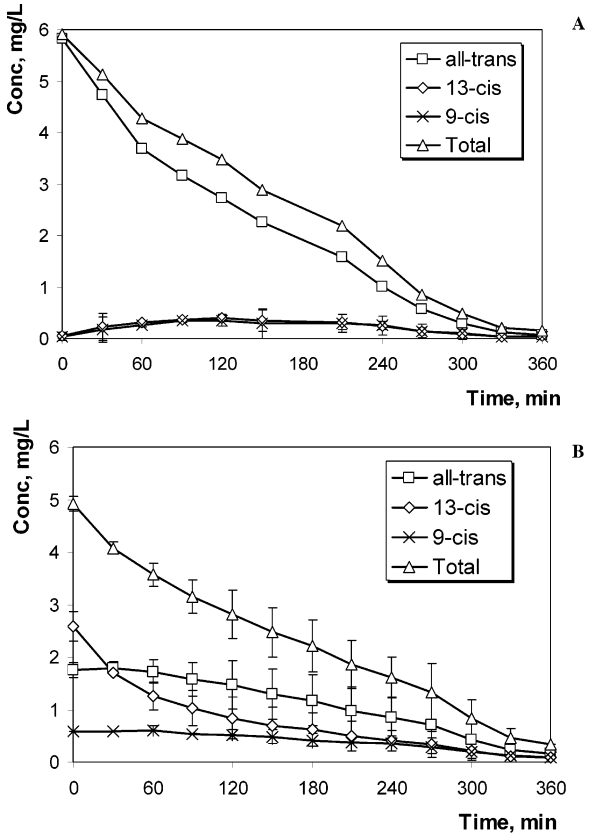


Figure 3. Concentrations of lycopene isomers during exposure of *all-trans*- and 13-*cis*-lycopene to gastric pH for 6 h: **A**, *all-trans* fraction; **B**, 13-*cis* fraction.

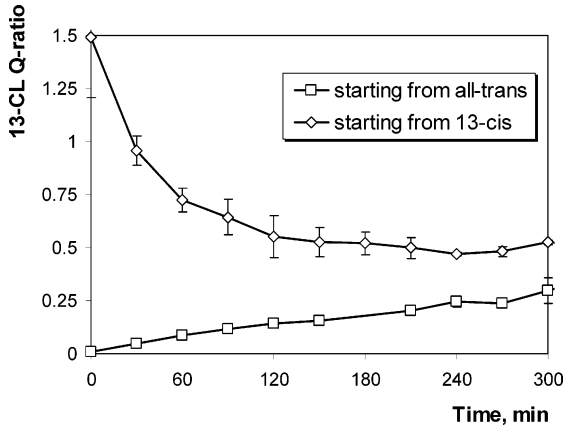


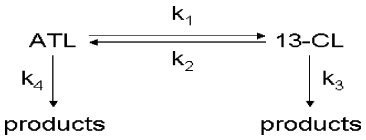
Figure 4. 13-*cis*-Lycopene *Q*-ratios during exposure of *all-trans*- and 13-*cis*-lycopene to gastric pH for 6 h.

to 13-*cis*-lycopene in **Figure 3B** was comparatively less steep, leading to the hypothesis that the 13-*cis* isomer may be more stable than the *all-trans* at low pH. This hypothesis justified the goal of calculating the kinetic reaction rate constants for the isomerization reactions.

The curves of 13-*cis*- and 9-*cis*-lycopene in **Figure 3A** were very similar, indicating that at low pH both isomers were formed to a similar extent. However, their concentrations were low when compared to **Figure 3B**, suggesting that at low pH the predominating reaction for the *all-trans* isomer would be breakdown, not isomerization. Interestingly, when starting from 13-*cis*-lycopene (**Figure 3B**), both *all-trans*- and 9-*cis*-lycopene curves had a rather flat appearance. Since under such conditions the *all-trans* isomer was lost rather quickly (**Figure 3A**), its generation by isomerization of 13-*cis*-lycopene must have been large enough to partially compensate the loss and lead to the small overall decrease in **Figure 3B**. Therefore, at gastric pH, isomerization of 13-*cis*-lycopene to *all-trans* must be quite significant.

In summary, the experimental data suggests that, at gastric pH, 13-*cis*-lycopene was lost to a lower extent than *all-trans*-lycopene, *all-trans*-lycopene had a tendency to breakdown rather than to isomerize, while the isomerization of 13-*cis*-lycopene to *all-trans* was more intense than its breakdown. These hypotheses were verified by performing the kinetic study described above.

Calculation of Reaction Rate Constants for the Isomerization of *all-trans*- and 13-*cis*-Lycopene at Gastric pH. The data acquired in the kinetic study was used to calculate the reaction rate constants for the isomerization of 13-*cis*- and *all-trans*-lycopene. With the exception of the *all-trans* and 13-*cis*, the other lycopene isomers were not individually considered. Since their concentrations were much lower, for this study they were included in the generic term "products", together with breakdown products. Therefore, the pH-driven isomerization process was considered to occur according to the following schematic:



The other assumption was that the reactions followed first-order kinetics. This assumption is based on the numerous studies reporting first-order kinetics for degradation of carotenoids in various environments (*23*−*25*) and was necessary in order to build the mathematical model describing the reactions shown above.

If the reactions in the schematic above followed first-order kinetics, then the concentrations of *all-trans*- and 13-*cis*-lycopene at any given time are given by the equations

$$\frac{\mathrm{d[ATL]}}{\mathrm{d}t} = -k_1[\mathrm{ATL}] - k_4[\mathrm{ATL}] + k_2[\mathrm{13\text{-}CL}] \qquad (1)$$

$$\frac{\mathrm{d[13\text{-}CL]}}{\mathrm{d}t} = k_1[\mathrm{ATL}] - k_2[\mathrm{CL}] - k_3[\mathrm{13\text{-}CL}] \qquad (2)$$

where the notation [X] indicates the concentration of species X. The reaction rate constants k_1 and k_2 characterize the reversible, pH-driven isomerization of *all-trans*-lycopene to 13-*cis*.

The system of eqs 1 and 2 can be used to calculate the reaction rate constants k_1, k_2, k_3, and k_4. When starting from *all-trans*-lycopene the 13-*cis* concentration reached a maximum (**Figure 3A**), and conversely, when starting from 13-*cis*-lycopene, the *all-trans* concentration reached a maximum (**Figure 3B**). At these maximum points, the derivatives of the concentration (left term in eqs 1 and 2) are zero. This observation, also used in a more complex approach (*25*), allows solving the system of eqs 1 and 2 numerically. When starting from *all-trans*-lycopene, after rearrangements, eq 2 becomes

$$\frac{\mathrm{d[13\text{-}}CL]}{\mathrm{d}t} = k_1([\mathrm{ATL}] - K[\mathrm{13\text{-}CL}]) \qquad (3)$$

where $K = [\mathrm{ATL}]_M/[\mathrm{13\text{-}CL}]_M$ is the isomers concentrations ratio at the maximum point. Therefore, the value of k_1 can be

calculated by linear regression, using eq 3. Similarly, when starting from 13-*cis*-lycopene, the value of k_2 can be calculated from the linear regression of the equation

$$\frac{d[ATL]}{dt} = k_2([13\text{-CL}] - K'[ATL]) \quad (4)$$

where $K' = [13\text{-CL}]_M/[ATL]_M$.

The value of each derivative in equations 3 and 4 was approximated with the slope for that particular time interval, taken at the midpoint. For example, for 13-*cis*-lycopene

$$\frac{d[13\text{-CL}]}{dt} \cong \frac{[13\text{-CL}]_{t_2} - [13\text{-CL}]_{t_1}}{t_2 - t_1} \quad (5)$$

where t_1 is the time at the beginning of the interval considered, and $t_2 = t_1 + 30$ min is the time corresponding to the end of the interval. The concentrations of *all-trans*- and 13-*cis*-lycopene at the midpoint of each time interval were calculated with

$$[ATL] = \frac{[ATL]_{t_1} + [ATL]_{t_2}}{2} \text{ and}$$
$$[13\text{-CL}] = \frac{[13\text{-CL}]_{t_1} + [13 - CL]_{t_2}}{2} \quad (6)$$

To improve the accuracy of the result, the calculation used each data point, not the averages (*26*).

The reaction rate constant for *all-trans*-lycopene isomerization with formation of the 13-*cis* isomer, obtained from the linear regression, was 0.0016/min. The coefficient of determination r^2 was about 0.81, indicating a strong correlation. Since the number of experimental points is 22, the number of degrees of liberty for the correlation analysis is 20; therefore, with $\alpha = 0.05$, the critical r value is 0.423 (*27*). This suggests that the linear correlation above is highly significant.

For the isomerization of 13-*cis*-lycopene to *all-trans*, the value of k_2 calculated is 0.0053/min, but with much weaker correlation ($r^2 = 0.19$). However, the critical value of r for $\alpha = 0.05$ and 20 degrees of liberty is 0.423 (*27*), so the correlation is still significant. It should be noted that the value of k_2 (0.0053/min) is higher than the one of k_1 (0.0016/min). A higher k_2 value indicates that the isomerization of 13-*cis*-lycopene back to *all-trans* is a faster reaction that the direct isomerization of *all-trans*-lycopene to 13-*cis*.

Once the reaction rate constants k_1 and k_2 for the isomerization reactions were found, it was possible to calculate the reaction rates k_3 and k_4 for the breakdown reactions. The value of k_3 can be calculated using eq 2, from the linear regression between $k_1[ATL] - k_2[ATL] - d[13\text{-CL}]/dt$ and [13-CL]. The value of k_3 found by this calculation was 0.0022/min, but the very poor correlation coefficient (0.006) indicates it was not significant. The value of k_4 was similarly calculated from eq 1, using the linear regression between $k_2[13\text{-CL}] - k_1[13\text{-CL}] - d[ATL]/dt$ and [ATL]. The calculated value was 0.0004/min, but again with a poor and nonsignificant correlation coefficient (0.06).

The fact that the correlations for calculating k_3 and k_4 are not significant was in fact expected, since the model used to quantify the kinetics of pH-triggered isomerization and breakdown is oversimplified. In fact, there were many other reactions involving both *all-trans*- and 13-*cis*-lycopene, both isomerizations and oxidations with formation of smaller products, and they most likely occurred at various rates and possibly following different orders of kinetics. However, this simplified model allowed us to obtain quantitative information about the kinetics of the reversible isomerization of *all-trans*- to 13-*cis*-lycopene.

The reaction rate constants confirm that *all-trans*-lycopene is more stable than 13-*cis*-lycopene at low pH. The isomerization of *all-trans*-lycopene to 13-*cis* has $k_1 = 0.0016$/min, while the isomerization of 13-*cis*-lycopene to *all-trans* has $k_2 = 0.0053$/min, being 3.5 times faster. The values of k_3 and k_4 were not significant, but if taken as an order of magnitude, then the 13-*cis* isomer undergoes breakdown about 5 times faster than the *all-trans* isomer. For 13-*cis*-lycopene, the isomerization to *all-trans* had a reaction rate $k_2 = 0.0053$/min, while the breakdown reaction constant appeared at least 2 times lower, which indicates that the hypothesis that at low pH 13-*cis*-lycopene would rather isomerize to *all-trans* than breakdown is probably valid.

The question whether at low pH *all-trans*-lycopene would rather isomerize than breakdown is not clearly answered by this study. Since $k_1 = 0.0016$/min, at low pH *all-trans*-lycopene would isomerize easily to 13-*cis*. However, the other breakdown or isomerization processes in which *all-trans*-lycopene participates could not be modeled by a single first-order reaction, so kinetic constants to characterize these other processes could not be generated for comparison. It may be possible to get a clear answer to this question if considering the 9-*cis*-lycopene levels and adding a third equation describing the variation of its concentration, similar to eqs 1 and 2. However, this requires purification of the 9-*cis* isomer (which is difficult, as it elutes much closer to *all-trans*-lycopene) and performing the same study starting with 9-*cis*-lycopene. Besides doubling the amount of experimental work, the mathematical analysis of the model becomes very complex.

An interesting question is, why, when starting from the *all-trans* isomer, a more significant accumulation of *cis* isomers was not found? This is largely an effect of the magnitude of k_1 and k_2: since $k_2 \gg k_1$, any *cis*-lycopene formed would quickly isomerize back to *all-trans*. To test this hypothesis, the model described by the schematic above and reaction rate constants k_1-k_4 were used to simulate the behavior of lycopene, in two scenarios: starting with 100% *all-trans*-lycopene and with 100% 13-*cis*-lycopene, respectively. The analysis was done incrementally using Excel (Microsoft, Redmond, WA), the concentrations of *all-trans*- and 13-*cis*-lycopene being calculated using the equations

$$[ATL]_{i+1} = [ATL]_i - (k_1 + k_4)[ATL]_i\Delta t + k_2[CL]_i\Delta t \quad (7)$$

$$[CL]_{i+1} = [CL]_i + k_1[ATL]_i\Delta t - (k_2 + k_3)[CL]_i\Delta t \quad (8)$$

where the length of the increment was $\Delta t = 5$ min. The result of this simulation is shown in **Figure 5**, and it indicates that when starting from any isomer, the other isomer would reach a maximum. This simulation answers the question above: indeed, when starting with *all-trans*-lycopene a relatively small concentration of 13-*cis* isomer is found, while when starting with 13-*cis*-lycopene a relatively higher *all-trans* isomer concentration is found at the maximum. An interesting result was obtained when plotting the *Q*-ratio for 13-*cis*-lycopene (**Figure 5**, secondary *y* axis): the simulation indicates that in both cases it plateaus to 0.236, so the model suggests that the system aims to reach a dynamic equilibrium. The isomeric distribution of lycopene at this particular point can be calculated by assuming that both 13-*cis*- and 9-*cis*-lycopene, the main isomers detected, are formed to about the same extent (as indicated by **Figure 3A**). In this case, a simple algebraic calculation suggests that the equilibrium isomeric distribution of lycopene at gastric pH

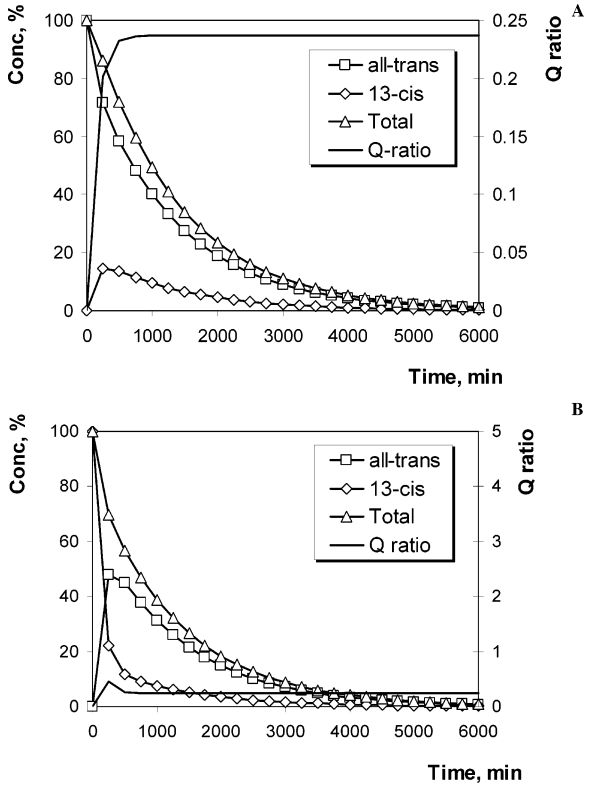


Figure 5. Simulation of the lycopene isomers concentration over time, using the model described, and the calculated reaction rate constants: **A**, starting from 100% *all-trans*-lycopene; **B**, starting from 100% 13-*cis*-lycopene.

consists of approximately 16% 13-*cis*-, 16% 9-*cis*- and 68% *all-trans*-lycopene.

This interesting result may put in a new light the fact that in body fluids more than 50% of lycopene was in *cis* forms (*5*). There is some controversy regarding the extent of lycopene isomerization triggered by gastric pH: Re et al. (*6*), in an *in vitro* study, showed that gastric pH is able to trigger the isomerization of *all-trans*-lycopene to *cis* forms, while Tyssandier et al. (*9*) showed using *in vivo* data that there is no significant *cis*−*trans*-lycopene isomerization in the stomach. The data above suggests that pH-driven isomerization is responsible at least partially for the high *cis*-lycopene proportion found *in vivo*. One could then hypothesize that the reason for the higher *cis* concentration in the body is that *cis* isomers are generated in the stomach and probably better absorbed in the intestines because of the more advantageous shapes/sizes of the molecules (*2*), and the mechanisms of transport from stomach to the various destinations inside the body may prevent a re-formation of *all-trans*-lycopene when reaching more neutral pH levels, possibly due to an association of *cis*-lycopenes with biomolecules involved in the transport mechanisms. Further studies are needed to elucidate this hypothesis, including a simulation of the behavior of 13-*cis*-lycopene in the intestines as well as a comparison of the intestinal absorption of 13-*cis*-lycopene vs *all-trans*-lycopene.

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Supporting Information Available: Comparison of the experimental ^{1}H NMR chemical shifts for the two preparations analyzed to literature data for *all-trans*- and 13-*cis*-lycopene. This material is available free of charge via the Internet at http://pubs.acs.org.

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