

Lycopene Stability in Tomato Powders

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ABSTRACT: The chemical stability of lycopene in 2 commercial tomato powders was evaluated during storage. Liquid chromatography and spectral analysis were used to determine lycopene loss and the formation of *cis* isomers and degradation products. Tomato powder products were stored at 6 and 45 °C or under fluorescent light for up to 6 wk. Several lycopene degradation products were tentatively identified in the initial and stored powders. After 6 wk at 45 °C, 60% of the lycopene was degraded. At lower storage temperatures the losses were about 30% after 6 wk. Mechanisms of loss appear to be both isomerization and oxidation.

Key Words: lycopene, tomato powder, storage stability

Introduction

LYCOPENE IS THE PRINCIPLE CAROTENOID FOUND IN TOMATOES. It is also one of the major carotenoids present in human serum and organs (Stahl and Sies 1996). Epidemiological studies have shown that serum levels of lycopene and dietary intake of lycopene from tomatoes were inversely related to risk of certain types of cancer, such as prostate cancer, digestive-tract cancers, and lung cancer (Giovannucci and others 1995; Franceschi and others 1994; Le Marchand and others 1989). Therefore, the content and stability of lycopene in foods has taken an added importance.

Tomato and tomato products are the major sources of lycopene in the human diet (Stahl and Sies 1996). All-*trans* lycopene is the predominant geometrical isomer in fresh tomatoes (Deuel 1951). Processing and storage of tomato products causes lycopene degradation as reviewed by Nguyen and Schwartz (1999). It was suggested that the first stage of lycopene degradation during drying and storage of tomato powders was the reversible isomerization of all-*trans* lycopene to less colored, more oxidizable *cis* isomers (Boskovic 1979). Autoxidation of all-*trans* lycopene and the *cis* isomers occurred parallel to *trans-cis* isomerization causing a split of the lycopene molecule into smaller fragments, such as volatile aldehydes and ketones developing hay or grassy off-flavors. Some authors suggested that certain storage conditions may favor *cis-trans* reversion and deepening of the color of the powders (Boskovic 1979; Wong and Bohart 1957). In processed tomato products lycopene isomerization and autoxidation cause a decrease of total lycopene content, a decrease in the proportion of all-*trans* lycopene, color loss, and development of grassy off-flavors (Schierle and others 1997; Lovric and others 1970).

In low-moisture products, like tomato powders, carotenoids are readily oxidized causing color loss and off flavors (Lovric and others 1970). The stability of lycopene during oxidation determines how lycopene would influence food quality and shelf life. Environmental factors such as air, light, and temperature may be very important for the isomerization and autoxidation of lycopene in tomato powders. In order to ensure lycopene stability and to minimize color loss and appearance of off flavors, there is a need to understand the influence of the environmental factors on lycopene isomerization and oxidation in tomato powders.

The purpose of this work was to study the stability of lycopene during storage of spray-dried tomato powders and to compare the influence of air, light, and temperature on the isomerization and oxidation of lycopene in spray-dried tomato powders.

Results and Discussion

THE CHARACTERISTIC CHROMATOGRAPHIC PROFILE OF THE EXTRACTS OF TOMATO POWDERS IS SHOWN IN FIG. 1. Peaks were identified on the basis of their HPLC retention times and absorbance spectra (Table 1). All-*trans* lycopene was the predominant carotenoid. Based on spectral characteristics relative to published data, 15-mono-*cis* and 5,5'-di-*cis* lycopene isomers were tentatively identified in both powders. 15- and 5-mono-*cis* lycopene were previously found in processed tomatoes (Schierle and others 1997), but the presence of 5,5'-di-*cis* lycopene has not been previously reported. *Cis*-lycopene isomers, while possibly present at low levels in raw tomatoes, may have increased during spray-drying of the powders. A probable hydroxy derivative of lycopene eluting at about 3.35 min on the chromatograms monitored at 470 nm was found initially in both tomato powders. It was tentatively identified on the basis of its absorbance spectrum as 5,6-dihydroxy-5,6-dihydrolycopene (lycopene-5,6-diol). The amount was about 4% of total lycopenes calculated on the basis of HPLC peak areas at 470 nm. Presence of 5,6-dihydroxy-5,6-dihydrolycopene in raw tomatoes and processed tomato products was reported in previous publications (Khachik and others 1992; Tonucci and others 1995).

Initial total lycopene contents in tomato powder products T1

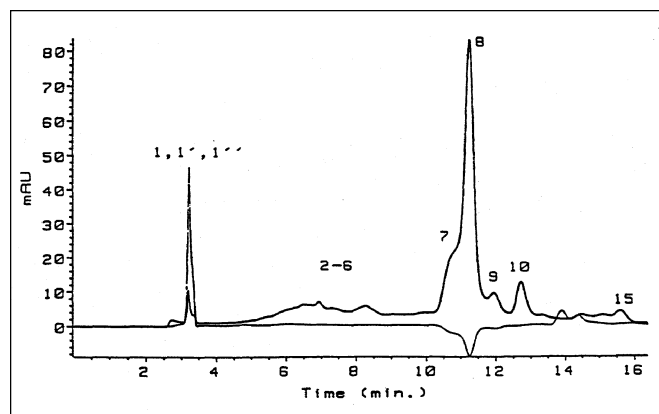


Fig. 1—Typical HPLC profile of tomato powders. Simultaneous measurement at 470 nm (upper curve) and 350 nm (lower curve). Mobile phase acetonitrile: methanol: 2-propanol (44:54:2 % by vol.). Assignment of peak identity is described in Table 1.

and T2 were 821 $\mu\text{g/g}$ dry solids, and 883 $\mu\text{g/g}$ dry solids, respectively, which are lower than the values of 1000 to 1200 $\mu\text{g/g}$ dry solids of tomatoes reported in previous publications (Lovric and others 1970). All-*trans* lycopene, and the presumptive 5,5'-*di-cis* lycopene and 15-mono-*cis*-lycopene comprised, respectively, 94.4%, 4.3%, and 1.3% of total lycopene in tomato powder T1 and 92.6%, 6.2%, and 1.2%, respectively, in tomato powder T2. The content of 15-mono-*cis* lycopene in the powders is in agreement with the reported 1% to 3% 13- and 15-mono-*cis* lycopene expressed as a percent of the total lycopene in tomato paste and other tomato products (Schierle and others 1997).

To compare the importance of isomerization and autoxidation as reaction pathways for the degradation of lycopene in tomato powders, commercial samples T1 and T2 were stored for 6 wk in the dark in the air at 6 and 45 °C and also under fluorescent light at room temperature. Lycopene degradation in tomato powders T1 and T2 during light exposure or storage at 6 °C was accompanied by color fading and release of hay or grassy odors, which is characteristic of the odors due to oxidation products reported in previous publications (Lovric and others 1970). In addition to the release of grassy odors, storage at 45 °C caused darkening of the color to dark brown, likely, due to nonenzymatic browning, and caking of the powders.

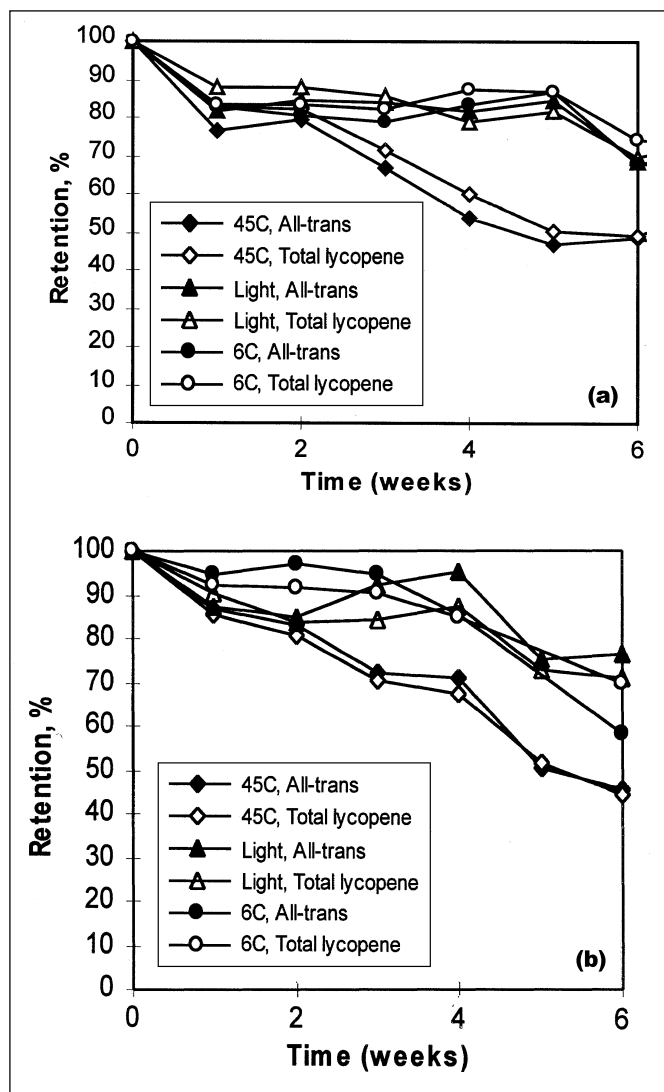


Fig. 2—(a) All-*trans* lycopene and total lycopene degradation during storage of tomato powders T1. (b) All-*trans* lycopene and total lycopene degradation during storage of tomato powders T2.

Table 1—Presumptive identification of HPLC peaks in extracts of tomato powders

HPLC peak	Carotenoid	Absorbance maxima, nm ^{a,b}
(1 + 1' + 1'') ^c	all- <i>trans</i> lutein, <i>cis</i> -luteins and 5,6-dihydroxy-5,6-dihydrolycopene (lycopene-5,6-diol)	473, 446, 424 483, 453, 432
(2, 3, 4, 5, 6) ^c	lycopene epoxides	
7 ^d	<i>cis</i> -lycopenes	
8 ^d	all- <i>trans</i> lycopene	504, 473, 446
9 ^c	5,5'- <i>di-cis</i> -lycopene	504, 473, 446, 360
10 ^c	all- <i>trans</i> β -carotene and <i>cis</i> - β -carotene	480, 454, (430) 480, 454, (430), 343
11 ^c	<i>cis</i> -phytofluene	368, 350, 333, 258
12 ^c	<i>trans</i> -phytofluene	368, 350, 333
13 ^c	ζ -carotene	427, 401, 380
14 ^c	phytoene	(278), 287, (297)
15 ^d	15-mono- <i>cis</i> lycopene	499, 467, 440, 360

^aPhotodiode array detector data (described in the text).

^bValues in parenthesis represent inflection points.

^cTonucci and others 1995;

^dHengartner and others 1992.

Table 2—Contents of presumptive 5,5' di-*cis* lycopene in tomato powders (% of total lycopene in the sample after a given storage period)

Weeks	Tomato Powder T1			Tomato Powder T2		
	Light Exposure	6 °C	45 °C	Light Exposure	6 °C	45 °C
0	4.3	4.3	4.3	6.2	6.2	6.2
1	5.6	4.8	6.5	6.7	6.1	5.9
2	5.8	5.4	5.3	5.2	7.6	6.3
3	7.0	5.5	5.4	5.5	6.4	7.0
4	8.6	9.4	9.1	7.7	6.8	7.8
5	8.1	11.0	12.8	11.8	11.7	12.1
6	14.2	18.4	14.6	14.1	18.2	14.1

Lycopene degradation

The changes of all-*trans* lycopene and total lycopene contents during storage of tomato powders T1 and T2 are shown in Fig. 2a,b. Decreases of all-*trans* lycopene and total lycopene in tomato powders T1 and T2 followed the same pattern. All-*trans* lycopene content and total lycopene content in tomato powders T1 and T2 for the different storage conditions did not differ statistically ($p < 0.05$) from each other. After 6 wk of light exposure at room temperature or storage at 6 °C in the dark, 60% to 70% of all-*trans* lycopene or total lycopene were retained in both tomato powders. Storage at 45 °C affected lycopene stability to a greater extent as only about 40% of all-*trans* lycopene or total lycopene contents were retained after 6 wk.

Decreases of all-*trans* lycopene were accompanied by increases in the contents of the 5,5'-*di-cis* isomer and 5,6-dihydroxy-5,6-dihydrolycopene as a proportion of the total lycopene present in the sample after storage. This suggests that degradation of all-*trans* lycopene proceeded through isomerization and autoxidation.

All-*trans* lycopene isomerization

Isomerization of all-*trans* lycopene at all 3 different storage conditions affected mostly the content of presumptive 5,5'-*di-cis* lycopene. Changes in the content HPLC peak considered to be 5,5'-*di-cis*-lycopene present in tomato powders T1 and T2 during storage are shown in Table 2. Increases from 0 to 6 wk of storage are in the range of 2- to 4-fold. Differences among the storage treatments are not obvious from the data. But the amount of this *cis* isomer as a percent of the total lycopene increased to the 14% to 18% range, regardless of the storage conditions. A possible

reason why the contents of the apparent 5,5'-di-*cis*-isomer in the samples stored at 45 °C or under fluorescent light at room temperature were not higher compared to the samples stored at 6 °C in the dark may be the degradation of the *cis* isomer due to autoxidation. *Cis* isomers were reported to be more susceptible to autoxidation than the all-*trans* isomer (Deuel 1951).

The initial content of 15-mono-*cis* lycopene in both powders was about 1.2% to 1.3% and decreased during the first 4 wk of storage to about 0.3% to 0.8 % and then increased to about the initial content after 6 wk of storage at 6 °C, 45 °C, or light exposure at room temperature. The observed degradation pattern may be connected with the autoxidation of the 15-mono-*cis* isomer which occurred simultaneously with or just after the isomerization of all-*trans* lycopene. This isomer did not accumulate even as all-*trans* lycopene was being lost.

The evidence that peak 9 is 5,5'-di-*cis* lycopene is based on the spectrum shown in Fig. 3. Peak 9 has a nearly identical spectral maxima in the visible range with all-*trans* lycopene and differs from the latter only in its slightly reduced fine structure. This suggests that isomerization of all-*trans* to 5,5'-di-*cis* lycopene would not cause a significant decrease in absorbance of the sample extract in the visible range and was not likely the reason for the observed 30% to 60% decrease of all-*trans* lycopene content of tomato powders during storage. This means that autoxidation of either the all-*trans* or *cis* isomer intermediate was likely the major pathway for lycopene degradation. This conclusion is consistent with our observation that the degradation of all-*trans* lycopene measured by HPLC peak areas was not different from the degradation of total lycopene monitored by spectroscopy at 470 nm ($p < 0.05$) (Fig. 2 a,b).

Lycopene autoxidation

The main autoxidation product of lycopene that shows up in the chromatogram absorbing at 470 nm appears to be 5,6-dihydroxy-5,6-dihydrolycopene. This is based on a spectral match (Table 1). As shown in Table 3, increases with storage time of this oxidized form of lycopene were not obvious except at 45 °C,

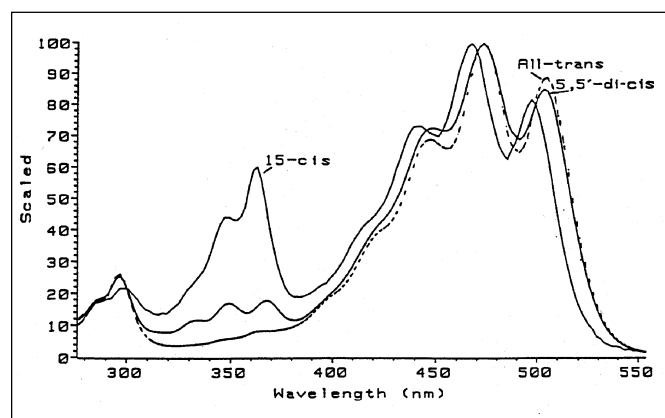


Fig. 3—UV/Visible spectra of all-*trans* lycopene, 5,5'-di-*cis* lycopene and 15-mono-*cis* lycopene in tomato powders.

Materials and Methods

Preparation and storage of samples

Two commercially produced spray-dried tomato powders T1 and T2 were used. Tomato powder T1 was produced by a cold break process, and T2 was produced by a hot break process. Moisture content of powders T1 and T2 were 1.4% and 1.9%, respectively, determined by the Karl Fischer titration

Table 3—Contents of presumptive 5,6-dihydroxy-5,6-dihydrolycopene in tomato powders (% of total lycopene in the sample after a given storage period)

Weeks	Tomato Powder T1			Tomato Powder T2		
	Light Exposure	6 °C	45 °C	Light Exposure	6 °C	45 °C
0	4.3	4.3	4.3	4.1	4.1	4.1
1	3.4	4.2	3.6	4.0	4.2	5.0
2	3.3	5.0	4.9	3.8	4.1	5.5
3	3.6	5.5	6.4	3.6	5.0	6.8
4	3.7	4.9	7.2	3.4	4.3	7.8
5	2.8	4.0	7.9	5.6	5.0	8.2
6	3.6	5.2	8.1	4.2	5.4	8.9

where the concentrations went from an initial 4% of the total lycopene to 8% to 9%. Losses of lycopene under these conditions was substantial and would not be explained by the accumulation of this hydroxylated form of lycopene. Other oxidation products resulting from the cleavage of the molecule may have been formed, but they were not detected by HPLC at 470 and 350 nm.

The increased formation of lycopene-5,6-diol during storage at 45 °C is consistent with the observed increased degradation of all-*trans* lycopene during storage at 45 °C, as compared to storage at 6 °C in the dark or light exposure at room temperature. This means that in the presence of oxygen, increased temperature had the most unfavorable effect on lycopene stability as it increased autoxidation of lycopene. Furthermore, light and increase of storage temperature from 6 °C to room temperature were not important factors for the stability of lycopene in the tomato powders. This suggests a temperature sensitivity for the storage of tomato powders but the apparent nonlinear effect from 6 to 21 to 45 °C needs further investigation.

Conclusion

LYCOPENE DEGRADATION DURING STORAGE OF 2 TYPES OF spray-dried tomato powders, hot break and cold break, proceeded to the same extent. The accumulation of *cis* isomers did not explain the extent of lycopene degradation and color fading, and, therefore, oxidation is the predominant mechanism of all-*trans* lycopene loss.

The main isomerization product was tentatively identified as 5,5'-di-*cis* lycopene based on spectra. It was present at larger amounts in the samples stored for 6 wk, and there was no apparent difference among those stored at 6 °C in the dark as compared to those stored at 45 °C or under fluorescent light at room temperature. A lycopene oxidation product detected by HPLC was tentatively identified as 5,6-dihydroxy-5,6-dihydrolycopene. It was formed in higher amounts in the samples stored for 6 wk at 45 °C in the dark as compared to those stored at 6 °C in the dark or under fluorescent light at room temperature. In tomato powders exposed to air, increased temperature had the most unfavorable effect on lycopene stability as it increased autoxidation of lycopene. Light and increase of storage temperature from 6 °C to room temperature were not important factors for the stability of lycopene in tomato powders.

method (automatic moisture analyzer, Aquatest-IV, Photovolt Corporation, New York, N.Y., U.S.A.). Water activities of tomato powders T1 and T2 were 0.206 and 0.215, respectively, determined by an AquaLab water activity analyzer (Decagon Devices, Inc., Model CX-2, Pullman, Wash., U.S.A.).

The powders were weighed out in 0.2-g portions that were placed in crimped cap 20 ml clear glass vials and stored at 6 and 45 °C in the dark and at room temperature under fluores-

cent light (38,500 lux measured with a Davis light meter). Control samples of powders T1 and T2 were stored at -18°C in the dark in N_2 atmosphere. Two replicate samples were taken weekly for lycopene analysis. Degradation of lycopene was followed over 6 wk of storage.

Lycopene Extraction

With minor modifications, lycopene extraction procedure was similar to a published procedure for carotenoid extraction from vegetables and fruits (Hart and Scott 1995). For every storage condition, the 2 replicate samples of spray dried powders (0.2 g) were reconstituted by addition of 10 ml water, vortexed for 1 min, and transferred into a glass fiber filter (10 to 20 μm) Buchner funnel. Forty ml of tetrahydrofuran and methanol (1:1 v/v THF:MeOH) were added and the suspension filtered under vacuum. When needed for additional removal of color, a second extraction was done with 20 ml THF/MeOH as described to produce a gray/white precipitate. The combined filtrates were transferred to a separatory funnel. Twenty ml of petroleum ether (40 to 60 $^{\circ}\text{C}$ fraction) and 20 ml 10% sodium chloride solution were added and mixed by careful shaking. The lower THF/MeOH/ aqueous phase was drawn off. The upper petroleum ether layer was washed with 100 ml water to remove the water soluble materials, transferred into a 50 ml flask, and evaporated to dryness under nitrogen. The residue was redissolved, by ultrasonic agitation, to a final volume of 4 ml of hexane, filtered (0.45 μm) and analyzed by high-performance liquid chromatography (HPLC). All procedures were performed under reduced light.

Chromatography

Reverse phase HPLC was performed on a C18 (201TP540) analytical column (5 μm , 25 cm \times 4.6 mm; VYDAC, Hesperia, Calif., U.S.A.). A 20- μl loop was used for solvent injection. Solvent delivery was achieved with Spectra Physics SP8800 System at a flow rate of 1 ml/min. An isocratic mobile phase system of acetonitrile:methanol:2-propanol (44:54:2 by vol) was used (Stahl and others 1993). Detection was monitored with a diode array 1040A Hewlett Packard absorbance detector that also stored spectral data over the range of 190 to 600 nm for spectrophotometric peak identification. The chromatograms were simultaneously monitored at 350 and 470 nm. Lycopene, lutein, α -carotene and β -carotene standards were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Peak identification or presumptive identification was based on retention time and published absorbance spectral data.

Spectrophotometry

All-*trans* lycopene in extracts of tomato powders was quantified spectrophotometrically ($\lambda_{\text{max}} = 470 \text{ nm}$; $\epsilon = 187,000 \text{ M}^{-1}\text{cm}^{-1}$) (Hengartner and others 1992), using UV-Visible Spectrophotometer, Beckman Instruments Inc., Irvine, Calif., U.S.A.). Total lycopene content of stored tomato powders was monitored spectrophotometrically by dissolving 100 μl of the 4 ml hexane extracts into 2 ml ethanol. Measurements were done at 470 nm.

Data Analysis

Statistical differences among the values of all-*trans* lycopene and total lycopene content in the extracts of tomato samples T1 and T2 were determined by multiple comparison ($p < 0.05$) (Weisberg 1985).

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