

Lycopene Stability During Food Processing (44274)

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Abstract. Accumulating epidemiological evidence continues to show that lycopene, found in tomatoes, grapefruits and watermelons, is associated with a reduced risk of developing certain chronic diseases and cancers. With respect to lycopene in tomato products, the effect of thermal processing on its stability has not yet been rigorously addressed. This paper assesses the effect of several different heat treatments on lycopene's isomeric distribution in a variety of tomato products, as well as in organic solvent mixtures containing all-*trans* lycopene. Experimental results indicate that in contrast to β -carotene, lycopene remained relatively resistant to heat-induced geometrical conversion during typical food processing of tomatoes and related products. The presence of fat, the change in percentage of solids, and the severity of heat treatment were not contributing factors in the formation of lycopene isomers in tomato products, except at extreme conditions not regularly employed in the food industry or during food preparation. However, lycopene in organic solvent isomerized readily as a function of time even in the absence of light and the presence of antioxidants. These findings suggest that while lycopene is stable in the tomato matrix, sample handling techniques should be carefully evaluated to minimize the formation of lycopene *cis* isomers in organic solutions.

[P.S.E.B.M. 1998, Vol 218]

In recent years, there has been considerable epidemiological evidence that the carotenoid lycopene may reduce the risk of developing cervical, colon, prostate, rectal, and stomach cancers (1, 2, 3, 4). For example, Giovannucci *et al.* (3) concluded that for a cohort of 47,894 male subjects, consumption of fresh tomatoes, tomato sauce, and pizza, which account for the bulk of lycopene intake, is significantly related to a lower incidence of prostate cancer. These findings have prompted considerable interest in understanding the role of lycopene in the diet and its stability in foods.

In fresh tomatoes as well as in other fruits and vegetables, lycopene has been found to occur predominantly in the all-*trans* geometrical configuration (5, 6, 7, 8). In contrast, *cis* lycopene isomers have been detected in plasma and tissue samples at significant levels (9, 10, 11). Clinton *et al.* (11) reported that lycopene in serum and prostate tissues is predominantly in the *cis* isomeric form, comprising about 58%–88% of the total lycopene content.

To date, isomerization of fruit and vegetable carotenoids has been attributed to thermal treatment during food processing and preparation (12, 13, 14, 15). For example, Lessin *et al.* (15) reported that β -carotene *cis* isomer content increases from 12.9% in fresh tomatoes to 31.2% in canned tomatoes. With respect to lycopene, Stahl and Sies (5) reported that heating of tomato juice not only increased its *cis*-lycopene isomer content but also led to an improvement in uptake of overall lycopene in humans. Likewise, Schierle *et al.* (16) found that bench-top preparation of a spaghetti sauce from canned tomatoes increased the level of lycopene *cis* isomers. Another recent study by Gartner *et al.* (17) reiterates the notion that tomato paste, as a processed product, has more bioavailable lycopene than fresh tomatoes when both are consumed in conjunction with corn oil. One of the difficulties in assessing isomerization reactions of lycopene in foods is the ability to separate the complex mixture of the *cis-trans* isomers of this carotenoid. Recently, we have been employing a specialized C₃₀ high performance liquid chromatography (HPLC) column that exhibits excellent resolution of over 10 different lycopene isomers (8). In this report, we apply this HPLC methodology to monitor the extent of lycopene isomerization during typical thermal processing treatments of tomatoes and tomato products. A better understanding of lycopene's thermal stability

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in tomato products may be useful in assessing both its nutritional and physiological implications in the diet.

Materials and Methods

Standards of all-*trans* β -carotene, lycopene, and butylated hydroxytoluene were purchased from Sigma Chemical Co. (St. Louis, MO). All extraction and HPLC solvents (Fisher Scientific Co., Fairlawn, NJ) were certified HPLC or ACS grade. The fruits and vegetables evaluated were purchased from local markets and heat treated according to standardized industrial food processing requirements (18).

Extraction and Saponification. The following extraction procedure was carried out under subdued light to prevent isomerization and photodegradation. Fresh and processed tomatoes were diced, and 10.0 g samples were homogenized in 50 ml methanol with 1.0 g CaCO_3 and 3.0 g Celite. Samples were successively extracted with a mixture of 1:1 acetone/hexane and vacuum-filtered through Whatman paper No. 1 and No. 42. The filtrates were combined in a separatory funnel, and water was added to induce phase separation. The hexane layer was removed and brought up to volume. Extracts of samples containing lipids were saponified with 30% KOH for 60 min. Triplicate 3-ml samples were dried by flushing the nitrogen, and extracts were analyzed in duplicate by HPLC.

Lycopene Isomerization in Organic Solvent.

Three sets of 2-ml samples containing all-*trans* lycopene in 1:1 methanol and methyl-*t*-butyl ether (MTBE) were incubated in the dark at 27°C with and without 0.02% BHT and at 4°C. Samples were analyzed at 0, 60, 120 and 180 min by HPLC.

HPLC Chromatography. The reverse-phase HPLC system used in this study consisted of a Waters (Milford, MA) 2690 separation module. Separations were achieved using analytical (250 $\times$ 4.6 mm I.D.) 3- μm polymeric C_{30} columns that were prepared at the National Institute of Standards and Technology, Gaithersburg, MD by Dr. Lane Sander (19). Guard columns packed with C_{30} stationary phase were used in-line for all separations (YMC, Inc., Wilmington, NC). Lycopene isomer separation was carried out at 1.0 ml/min using a linear gradient of 40%–50% MTBE in methanol for 35 min.

Recovery Determinations. Results of recovery determinations during extraction, saponification, and chromatography procedures were found to be greater than 95%. *Cis*-isomer content of samples with added standards remained the same as control extracts, indicating that isomerization did not occur during the handling and chromatographic procedures.

Peak Identification. Column effluent was monitored via a Waters 996 Photodiode Array Detector at 200–800 nm with a scanning rate of 2 scan/sec and 1.2 nm spectral resolution. The detector was linked to a Digital (Maynard, MA) S133 Venturis computer with Waters Millennium 2010 chromatography software (LC Version 2.15.01). Quantification of lycopene geometric isomers was

achieved using a standard curve of the all-*trans* isomer (Sigma, St. Louis, MO) and its molar absorptivity coefficient (20). This method approximates the *cis*-isomer content, as the molar extinction coefficient values for individual isomers are not known. Chromatographic peak identification was based on comparison to previously reported separations on polymeric C_{30} columns and UV-visible absorption spectral libraries. Peaks that are identified as *cis*-lycopene isomers have been ascertained using electrospray mass spectroscopy (11) to have the same molecular weight as all-*trans* lycopene and thus are not oxygen-addition products. The identities of all-*trans* isomer peaks were assigned based on retention time and co-chromatography of authentic standards.

Results

Reversed-phase chromatography of a typical fresh tomato extract is depicted in Figure 1. In red tomato, the all-*trans* lycopene isomer predominates, comprising 95.4% of the total lycopene content. Analysis of commercial tomato products, such as vegetable juice, tomato juice, tomato sauce, tomato soup, tomato paste, pizza sauce, spray-dried and drum-dried tomato powders, and sun-dried tomatoes in oil indicates that of all the samples tested, none had a total *cis* isomer content greater than 10.1% (Table I). Although all of these products were processed under different thermal conditions, their *cis* isomer contents do not reflect the relative amount of heat treatment that the products are believed to have received. For example, the hot-filled tomato juice (82°C, 30 sec) and tomato paste (104°C, 50 min) samples did not differ significantly in terms of *cis* isomer levels despite the more rigorous thermal treatment of the latter.

A variety of tomato products were produced at the Ohio State University Food Industry Center to further investigate the formation of *cis* isomers as a result of thermal processing. Unlike the commercial tomato products purchased from local markets, the products made at the Food Industry Center allow for overall control and monitoring of the tomato preparation and processing steps. The lycopene content and isomeric distribution are reported in Table II for these products. The results suggest that lye peeling (18% NaOH treatment) of tomato at 82°C for 15 sec does not induce any *cis* isomer formation. This is consistent with previous observations that nonoxygenated carotenoids such as lycopene and other carotenes are stable against basic conditions such as during saponification using 30% NaOH (8). The lycopene level is slightly lower for peeled tomato as the removed peel material is known to have higher lycopene content (21). Tomatoes that were macerated prior to juicing have essentially the same lycopene content and isomer distribution as the resulting tomato juice. Thermal treatment in a holding tube at 82°C for 15 sec to pasteurize the juice did not induce isomer formation. Likewise, as the juice and whole peeled tomatoes in juice were canned and heat-treated at 104°C for 50 min, their lycopene isomeric distributions did not change either.

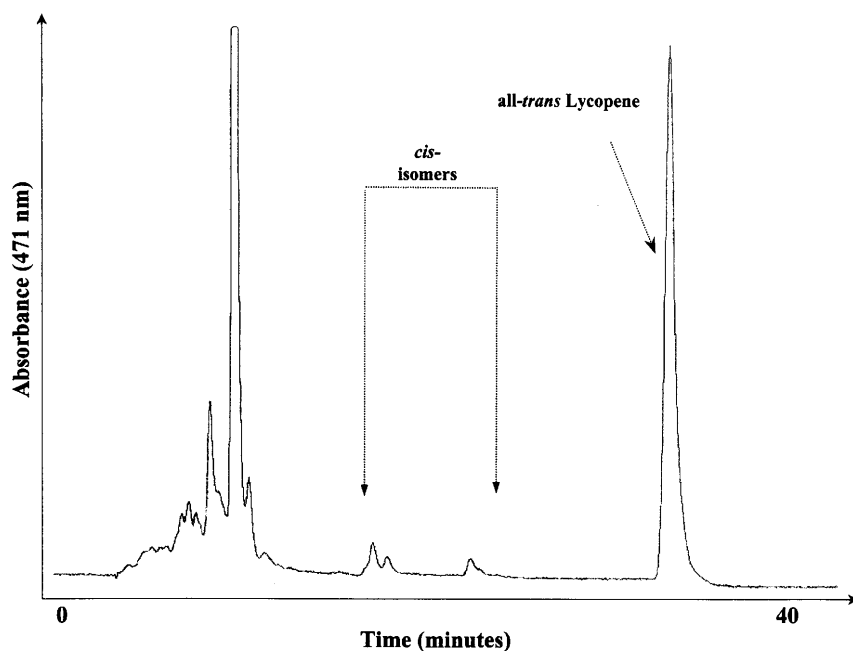


Figure 1. Representative C₃₀ HPLC chromatographic separation of lycopene extracted from fresh tomatoes using a 60:40 to 50:50 (v/v) methanolic MTBE mobile phase gradient.

Further thermal treatment of the remaining pasteurized juice at 85°–95°C for 3 hr to concentrate it into paste did not affect either the formation of lycopene isomers or their relative abundance. Even the tomato soup and sauce, which were made from paste and heat processed at 104°C for 50 min, did not exhibit a significant increases in *cis* lycopene isomer levels. The presence of olive oil at 5% and 15% in the tomato sauce did not affect the isomer content compared to the tomato soup with only 2% fat. The use of glass jars as containers to evaluate light exposure and inertness of contact surfaces did not perceivably impact the formation of *cis* isomer in all processed products tested. Although lycopene did not isomerize under any of the processing conditions above, appreciable levels of β -carotene *cis* isomers did form within the tomato tissue during all of these processing treatments (Table II).

Formation of lycopene *cis* isomers was successfully induced by excessive thermal exposure, such as heating chopped tomato in olive oil at approximately 200°C for 45 min. Similarly, high temperature heating of a thin film of pureed tomato at 200°C for a few seconds to remove moisture rapidly, increased the level of *cis* isomers from 4.2% to 19.1%. However, commercially available drum-dried tomato flakes had a lycopene *cis* isomer level of 6.25%.

In organic solvent, lycopene isomerized readily, and the amount of *cis* isomers increased as a function of time. Solutions of lycopene incubated at 27°C in the absence of light and in organic solvents resulted in a *cis* isomer content of 50% after 3 hr of incubation compared to 4.5% in the initial solution (Fig. 2). A lower incubation temperature of 4°C did slow the formation of isomers. Similarly, the addition of butylated hydroxytoluene (BHT) as an antioxidant also reduced the rate of lycopene isomerization. It is important to note that the changes in incubation temperature and/or the

presence of BHT did not alter the final equilibrium distribution of lycopene isomers.

Discussion

Previous reports have demonstrated isomerization reactions of carotenoids during thermal treatment and food processing of fruits and vegetables (12, 13, 14, 15). In this report, the carotenoid lycopene did not exhibit similar susceptibility to isomerization. Heating of tomatoes and the presence of lipids together may have improved the bioavailability of lycopene, as reported by Sies and Stahl (5) and Gartner *et al.* (17), but these factors were shown to have no influence on the formation of lycopene geometrical isomers. Parker (22) suggests that the improvement in bioavailability may be a result of the destabilization of protein-carotenoid

Table I. Relative Abundance of Lycopene Isomers in Various Commercial Tomato Products

	mg Lycopene/100 g product (wet weight)	<i>Cis</i> -isomer %
Tomato juice	7.83	6.22
Vegetable juice	7.28	4.45
Tomato soup (condensed)	7.99	6.08
Canned whole tomato	11.21	2.49
Canned pizza sauce	12.71	10.13
Sauce from pizza	32.89	4.62
Ketchup	13.44	6.19
Tomato paste	30.07	6.79
Tomato powder (spray-dried)	126.49	5.23
Tomato powder (drum-dried)	112.63	6.25
Sun-dried tomato (in oil)	46.50	1.70
Sigma lycopene standard	N/A	6.61

Table II. Relative Abundance of Lycopene Isomers in Various Thermally-Processed Tomato Products Prepared at the Food Industry Center

	mg Lycopene/100 g product (dry weight using moisture content data)	Lycopene <i>cis</i> -isomer %	β -Carotene <i>cis</i> -isomer %
Tomato (fresh)	152.98	4.16	21.77
Peeled tomato	149.89	5.37	23.83
Tomato juice (hot-break)	161.23	5.98	57.55
Tomato juice (retorted)	180.10	3.56	78.28
Tomato (whole, retorted)	183.49	3.67	62.03
Tomato paste (concentrated)	174.79	5.07	57.82
Tomato paste (retorted)	189.26	4.07	85.85
Tomato soup (retorted)	136.76	4.34	55.57
Tomato sauce (retorted)	73.33	5.13	56.14
Tomato yellow (fresh)	N/A	N/A	11.08
Tomato yellow (cooked)	N/A	N/A	26.56
Tomato in oil (heated)	N/A	10.67	N/A

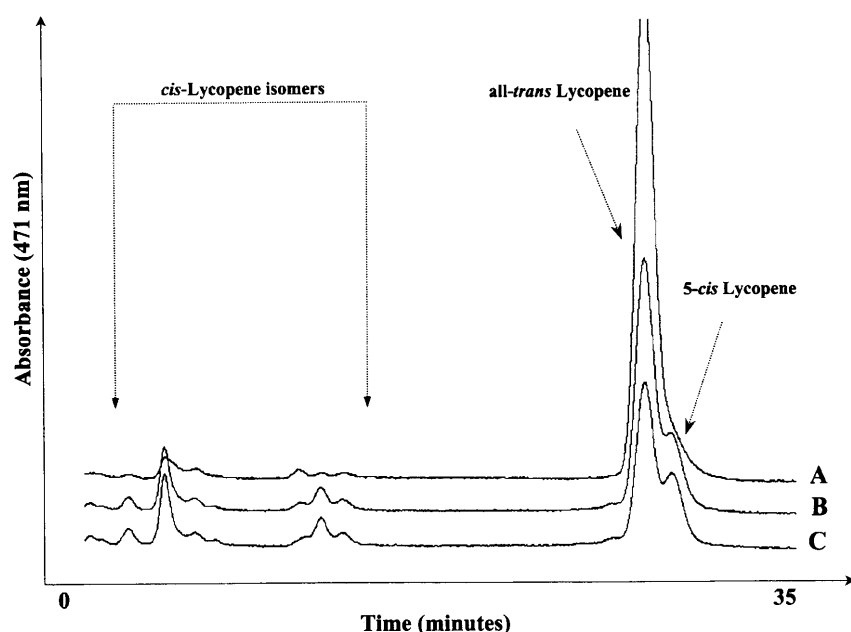


Figure 2. Relative abundance of lycopene isomers of all *trans* lycopene solutions before (A. 0 min at 27°C) and after incubation (B. 180 min at 27°C with BHT, and C. 180 min at 27°C without BHT).

complexes or the dissolution and dispersion of crystalline carotenoid aggregates.

According to our results, heat and shear during typical industrial food processing operations did not initiate lycopene isomerization. The large difference between levels of *cis* lycopene isomers in tomato products or other processed foods and organic solvent mixtures indicates that thermal treatments did not free lycopene from the tomato matrix into solution to undergo geometrical conversion. Furthermore, the fact that β -carotene readily isomerizes during the processing of tomato products whereas lycopene remains relatively stable suggests that these carotenoids have different bond energies and kinetics for isomerization reactions (23). Since the rate of isomer formation was reduced in the presence of the antioxidant BHT, geometric conversion of lycopene may occur *via* the free radical formation pathway suggested by Gao *et al.* (24).

From this study, we conclude that thermal treatment of tomato products during usual food preparation or commercial production processes does not appear to result in a significant shift in the distribution of *cis*-lycopene isomers. These findings are in contrast to β -carotene, which was observed to isomerize readily during typical processing of tomatoes. Further studies are in progress to investigate whether the presence of various *cis* lycopene geometrical isomers in human serum and biological tissues has dietary origin.

The authors thank Dr. Winston Bash and Gary Wenneker of the Food Industries Center at The Ohio State University for their technical support.

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