and similar food products. At this time, only the beta-carotene content of plant foods is used to calculate the vitamin A values for food labels (12), which may be an underestimation of the concentration of this important vitamin in tomatoes and tomato products.

The carotenoids in Table III are listed in order of their biosynthetic sequence (13). Each enzymatic step from phytoene to lycopene adds one double bond to the molecule resulting in lycopene, which is a symmetrical molecule containing 13 double bonds. The steps after lycopene involve enzymatic cyclization of the end groups that result in gamma-carotene (one beta ring) and beta-carotene (two beta rings). The concentration of each carotenoid in this sequence (Table III) provides some clues as to which enzymes may be rate-limiting in this cascade. The very high concentration of lycopene suggests that red tomatoes lack sufficient enzyme activity to convert lycopene to gamma-carotene (insufficient cyclase activity). Furthermore, the high ratio of the concentration of gamma-carotene to beta-carotene indicates that there also is a lack of sufficient enzyme activity to cyclize the second beta ring of gamma-carotene to form beta-carotene. Mechanisms that control these rate-limiting enzymes currently are an active area of research in tomato breeding and production.

Tomatoes contain several additional nutrients and phytonutrients that have been shown to have positive health effects. These include vitamin E (0.32 mg/100 g) and quercetin (0.80 mg/100 g), one of the flavonoids that has very high antioxidant activity relative to alpha-tocopherol (5, 14). A search of one of the phytochemical databases available on the worldwide web (15) indicates that tomatoes also contain several trace elements, phenypropanoids (phenolic acids), phytosterols, and water-soluble vitamins as well as naringenin (Flavonoid). Unfortunately, quantitative values are not associated with entries in this database, therefore, it is difficult to assess the importance that tomatoes and tomato products might have in supplying these components to consumers.

In summary, during the last half-century, the fruit of the cultivated tomato (*Lycopersicon esculentum*) has become a very popular and highly consumed vegetable in the United States. Production of tomatoes in the United States ranks second only to potatoes and is three times greater than sweet corn, the next most highly produced vegetable. Tomatoes provide substantial amounts of several nutrients, inclu folate, potassium, and vitamins A and C to the die American. Currently, tomato-based foods are best kr for their rich source of lycopene, a nonvitamin A–a carotenoid that has high oxygen radical scavenging quenching capacity. In addition, these foods supply se other carotenoids, nutrients, and phytonutrients that m: beneficial to human health.

1. Rick CM. Tomato: *Lycopersicon esculentum* (Solanaceae). In monds NW, ed. Evolution of Crops. New York: Longman, pp 273, 1975.
2. Rick CM. The tomato. Scientific American **257**(8):76–87, 197
3. McCue GA. The history of the use of the tomato: An annotate liography. Ann Missouri Bot Gdn **39**:289–348, 1952.
4. U.S. Department of Agriculture. Agricultural Statistics 19 Washington, DC: United States Government Printing Office, 1
5. U.S. Department of Agriculture, Agricultural Research Service. nutrient database for standard reference. http://www.nal.usda.gc foodcomp/ (Release 11–1), 1997.
6. Block G, Dresser CM, Hartman AM, Carroll MD. Nutrient sou the American diet: Quantitative data from the NHANES II Su Vitamins and minerals. Am J Epidemiol **122**:13–26, 1985.
7. Tonucci LH, Holden JM, Beecher GR, Khahick F, Davis CS, kozi G. Carotenoid content of thermally processed tomato-base products. J Agric Food Chem **43**:579–586, 1995.
8. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. enoid content of fruits and vegetables: An evaluation of analyt J Am Diet Assoc **93**:284–296, 1993.
9. Chug-Ahuja JK, Holden JM, Forman MR, Mangels AR, Beech Lanza E. The development and application of a carotenoid datal fruits, vegetables, and selected multicomponent foods. J Am D soc **93**:318–323, 1993.
10. Stahl W, Sies H. Lycopene: A biologically important caroter humans? Arch Biochem Biophys **336**:1–9, 1996.
11. Food and Nutrition Board, National Research Council. Recom Dietary Allowances. Washington, DC: National Academy of S 1974.
12. Waysek EH. Fat soluble vitamins. In: Sullivan DM, Carpen Eds. Methods of Analysis for Nutrition Labeling. Arlingto AOAC International, pp 123–129, 1993.
13. Goodwin TW. The Biochemistry of the Carotenoids. Volume I New York: Chapman and Hall, 1980.
14. Hertog MGL, Hollman PCH, Katan MB. Content of potentia carcinogenic flavonoids of 28 vegetables and fruits commo sumed in The Netherlands. J Agric Food Chem **40**:2379–238
15. Beckstrom-Sternberg SM, Duke JA. The phytochemical d http://www.probe.nalusda.gov:8300/cgi-bin/browse/phytoc (ACEDB version 4.3-data version July 1994).

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 C_{30} 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 × 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* iso (Sigma, St. Louis, MO) and its molar absorptivity co cient (20). This method approximates the *cis*-isomer tent, as the molar extinction coefficient values for indivi isomers are not known. Chromatographic peak identi tion was based on comparison to previously reported s rations on polymeric C_{30} columns and UV-visible abs tion spectral libraries. Peaks that are identified as lycopene isomers have been ascertained using electros mass spectroscopy (11) to have the same molecular we as all-*trans* lycopene and thus are not oxygen-addition p ucts. The identities of all-*trans* isomer peaks were assi based on retention time and co-chromatography of auth standards.

Results

Reversed-phase chromatography of a typical fres mato extract is depicted in Figure 1. In red tomato all-*trans* lycopene isomer predominates, comprising 9 of the total lycopene content. Analysis of commercia mato products, such as vegetable juice, tomato juice, to sauce, tomato soup, tomato paste, pizza sauce, spray- and drum-dried tomato powders, and sun-dried tomato oil indicates that of all the samples tested, none had a *cis* isomer content greater than 10.1% (Table I). Alth all of these products were processed under different the conditions, their *cis* isomer contents do not reflect the tive amount of heat treatment that the products are beli to have received. For example, the hot-filled tomato (82°C, 30 sec) and tomato paste (104°C, 50 min) sar did not differ significantly in terms of *cis* isomer l despite the more rigorous thermal treatment of the lat

A variety of tomato products were produced at the State University Food Industry Center to further invest the formation of *cis* isomers as a result of thermal pro ing. Unlike the commercial tomato products purchased local markets, the products made at the Food Industry ter allow for overall control and monitoring of the to preparation and processing steps. The lycopene conten isomeric distribution are reported in Table II for these ucts. The results suggest that lye peeling (18% NaOH ment) of tomato at 82°C for 15 sec does not induce ar isomer formation. This is consistent with previous obs tions that nonoxygenated carotenoids such as lycopen other carotenes are stable against basic conditions su during saponification using 30% NaOH (8). The lyc level is slightly lower for peeled tomato as the remove material is known to have higher lycopene content Tomatoes that were macerated prior to juicing have e tially the same lycopene content and isomer distributi the resulting tomato juice. Thermal treatment in a ho tube at 82°C for 15 sec to pasteurize the juice did not i isomer formation. Likewise, as the juice and whole p tomatoes in juice were canned and heat-treated at 104° 50 min, their lycopene isomeric distributions did not cl either.
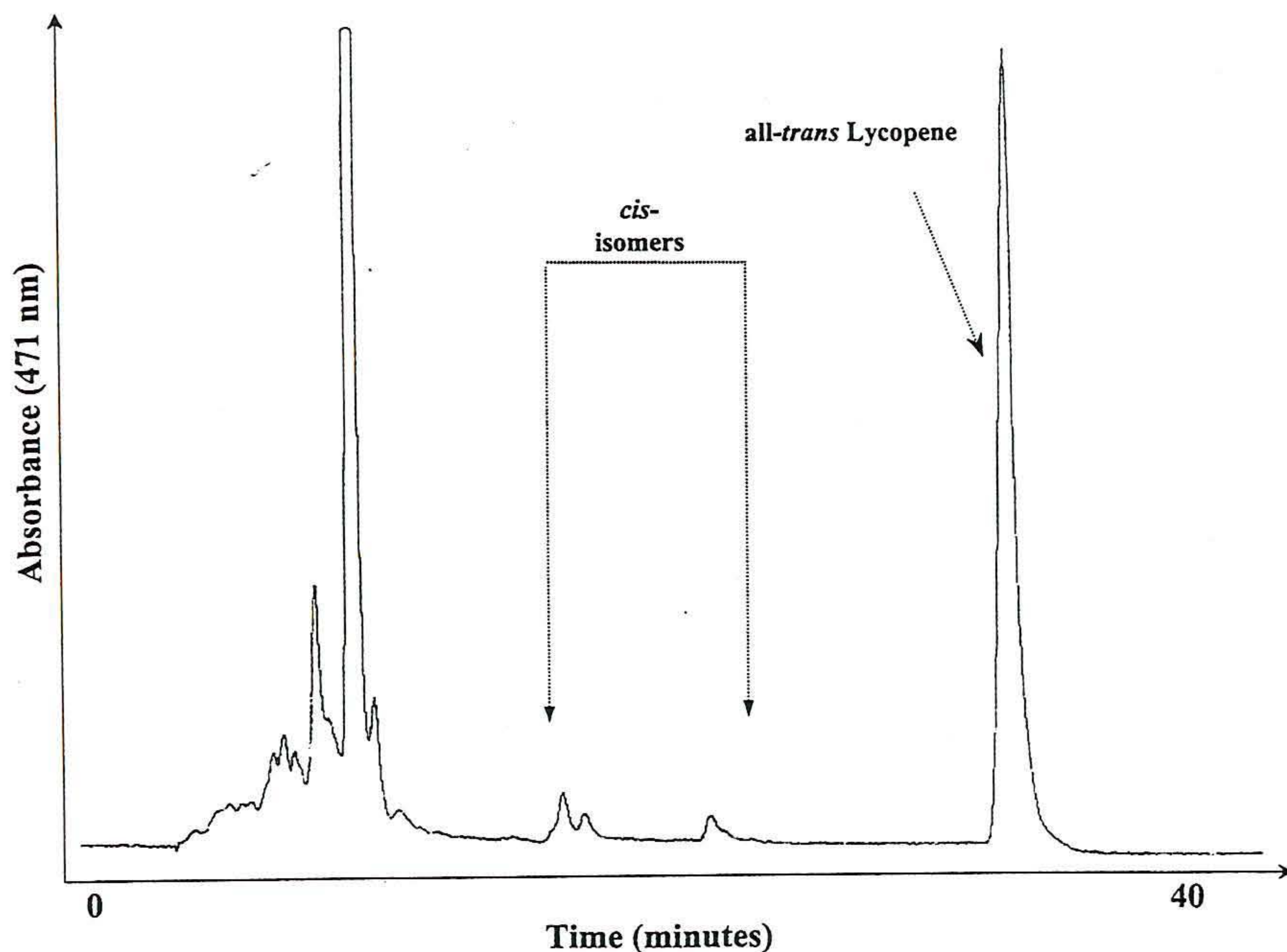
Figure 1. Representative C_{30} HPLC chromatographic separation of lycopene extracted from fresh tomatoes using a 60:40 to 50:50 (v/v) methanolic MTBE mobile phase gradient.

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 hydroxyltoluene (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)	*Cis*-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 cis-isomer %	β-Carotene cis-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
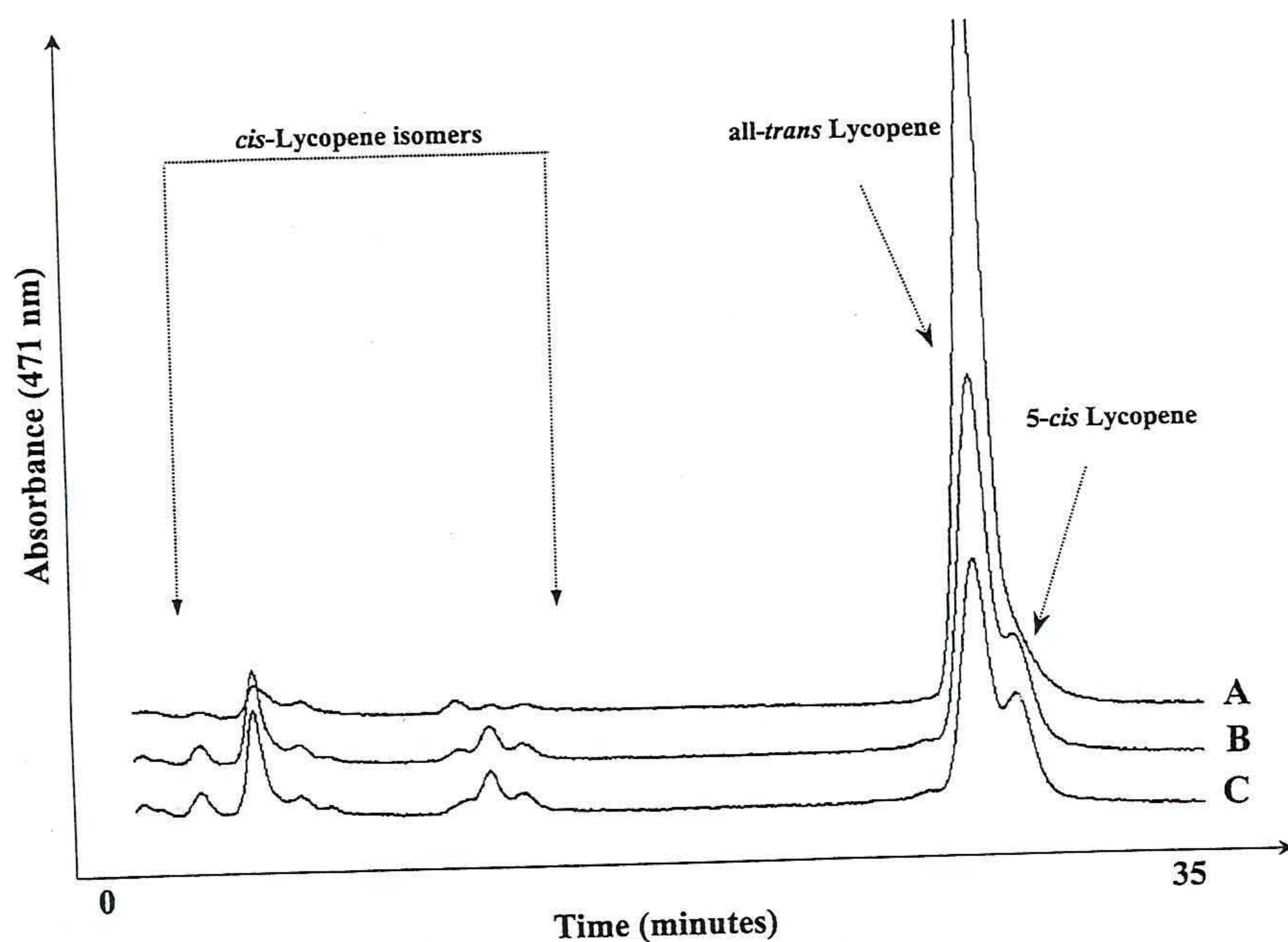
Figure 2. Relative abundance of lycopen[e] isomers of all trans lycopene solutions b[e]fore (A. 0 min at 27°C) and after incubati[on] (B. 180 min at 27°C with BHT, and C. 1[80] min at 27°C without BHT).

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 relativity stable suggests that these carotenes 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 treatme[nt] tomato products during usual food preparation or com[mer]cial production processes does not appear to result [in] significant shift in the distribution of cis-lycopene iso[mers] These findings are in contrast to β-carotene, which [was] observed to isomerize readily during typical processi[ng] tomatoes. Further studies are in progress to invest[igate] whether the presence of various cis lycopene geome[tric] isomers in human serum and biological tissues has d[ifferent] origin.

1. Batieha AM, Armenian HK, Norkus EP, Morris JS, Spate VE stock GW. Serum micronutrients and subsequent risk of cervi[cal]

cer in a population-based nested case-control study. Cancer Epidemiol Biomarkers Prev **2**:335–339, 1993.

2. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr Cancer **18**:1–29, 1992.

3. Giovannucci EL, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in relationship to risk of prostate cancer. J Natl Cancer Inst **87**:1767–1776, 1995.

4. Snowdon D. Antioxidants and reduced functional capacity in the elderly: Findings from the Nun study. J Gerontol **51A**:M10–M16, 1996.

5. Stahl W, Sies H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *J Nutr* **122**:2161–2166, 1992.

6. Khachik F, Goli MB, Beecher GR, Holden J, Lusby WR, Tenorio MD, Barrera MR. Effect of food preparation on qualitative and quantitative distribution of major carotenoids constituents of tomatoes and several green vegetables. J Agric Food Chem **40**:390–398, 1992.

7. Wilberg VC, Rodriguez-Amaya DB. HPLC Quantitation of major carotenoids of fresh and processed guava, mango, and papaya. Lebensmdiel-Wissenschaft und Technologie **28**:474–480, 1995.

8. Emenhiser C, Sander LC, Schwartz SJ. Capability of a polymeric C_{30} stationary phase to resolve *cis-trans* carotenoid isomers in reversed phase liquid chromatography. J Chromatogr A **707**:205–216, 1995.

9. Khachik F, Beecher GR, Goli NB, Lusby WR, Smith JC. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma. Anal Chem **64**:2111–2122, 1992b.

10. Stahl W, Sundquist AR, Hanusch M, Schwarz W, Sies H. Separation of β-carotene and lycopene geometrical isomers in biological samples. Clin Chem **39**(5):810–814, 1993.

11. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, Erdman JW. *Cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev **5**:823–833, 1996.

12. Chandler LA, Schwartz SJ. Isomerization and losses of *trans* β-carotene in sweet potatoes as affected by processing treatments. J Agric Food Chem **36**:129–133, 1988.

13. Quackenbush FW. Reverse-phase HPLC separation of *cis* and *trans*-carotenoids and its application to food materials. J Liq Chromatogr **10**:643–653, 1987.

14. Sweeney JP, Marsh AC. Effect of processing on provitamin A in vegetables. J Am Diet Assoc **59**:238–243, 1971.

15. Lessin WJ, Catigani GL, Schwartz SJ. Quantification of *cis-trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J Agri Food Chem **45**:3728–3732, 1997.

16. Schierle J, Bretzel W, Buhler I, Faccin N, Hess D, Steiner K, Schuep W. Content and isomeric ratio of lycopene in food and human blood plasma. J Agric Food Chem **96**:459–465, 1997.

17. Gartner C, Stahl W, Sies H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. Am J Clin Nutr **66**:116–122, 1997.

18. National Canner's Association, 11th Ed., National Canner's Association: Washington, DC, 1976.

19. Sander LC, Sharpless EK, Craft NE, Wise SA. Development of engineered stationary phases for separations of carotenoid isomers. Anal Chem **66**:1667–1674, 1994.

20. Britton G. Carotenoids: Spectroscopy. Boston: Birkhauser Verlag, Vol **1B**: p57, 1995.

21. Sharma SK, Le Maguer M. Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. Food Research International **29**:309–315, 1996.

22. Parker RS. Carotenoid and tocopherol composition in human adipose tissue. Am J Clin Nutr **47**:33–36, 1988.

23. Henry LK, Catignani GL, Schwartz SJ. Oxidative degradation kinetics of lycopene, lutein, 9-*cis* and all-*trans* β-carotene. J Am Oil Chemist's Soc (in press).

24. Gao G, Wei CC, Jeevarajan AS, Kispert LD. Geometrical isomerization of carotenoids mediated by radical/cation formation. J Phys Chem **100**:5362–5366, 1996.