Thermal Stability and Isomerization of Lycopene in Tomato Oleoresins from Different Varieties

M.M. HACKETT, J.H. LEE, D. FRANCIS, AND S.J. SCHWARTZ

ABSTRACT: Lycopene, a tomato carotenoid, has been associated with the inhibition of certain chronic diseases including prostate cancer. Tomato oleoresin is a lipid-rich material resulting from successive solvent extraction of the tomato fruit. Thermal stability and isomerization of lycopene in oleoresins prepared from 3 different tomato varieties, Roma, High Lycopene, and Tangerine, and tomato peel waste, were studied at 25 °C, 50 °C, 75 °C, and 100 °C in the dark. Thermally degraded lycopene compounds and isomers of lycopene were analyzed by a combination of C_{30} reversed-phase high-performance liquid chromatograph with a photodiode array detector, UV-visible spectrometer, or mass spectrometer. Effects of antioxidants on lycopene were also studied at 50 °C. As the storage temperature increased from 25 °C to 100 °C, the degradation of total lycopene in oleoresin from all samples increased significantly ($P < 0.05$). Lycopene at 25 °C and 50 °C may degrade mainly through oxidation without isomerization. Isomerization of lycopene in tomato oleoresins increased at 75 °C and 100 °C. Tetra-*cis* lycopene in Tangerine tomato varieties followed different degradation and isomerization pathways compared with all-*trans* lycopene in other tomato varieties. Addition of α-tocopherol or butylated hydroxytoluene slowed the rate of degradation of lycopene in oleoresin.

Keywords: lycopene, tomato oleoresin, thermal stability, isomerization, antioxidant

Introduction

The consumption of tomatoes and tomato products has several health benefits including decreasing the development of cervical, colon, prostate, rectal, stomach, and other types of cancers (Ramon and others 1993; Giovannucci and others 1995; Boileau and others 2003). Tomatoes and tomato products are the main source of lycopene in the diet, and many researchers have shown that lycopene consumption from tomatoes and tomato products is associated with lower cancer incidence (Giovannucci and Clinton 1998; Shi 2002; Hadley and others 2003).

Naturally occurring geometrical isomers of lycopene are primarily in an all-*trans* configuration (Zechmeister and others 1941). The main exception is the Tangerine variety tomato, where lycopene is predominately in a tetra-*cis* configuration (Clough and Pattenden 1979). In dietary studies, ingested lycopene exists (about 95%) in the all-*trans* form. However, our laboratory (Clinton and others 1996; Hadley and others 2003) has shown that *cis*-isomers of lycopene represent approximately 50% of total lycopene in blood and up to 80% in prostate tissues. It has been suggested that *cis*-isomers of lycopene are more bioavailable than all-*trans*–isomers, most likely because of the greater solubility of *cis*-isomers in the bile acid micelles, a shorter chain length to fit into micelles, and lower tendency to aggregate (Boileau and others 1999, 2002). The mechanisms explaining the isomerization of all-*trans* to *cis*-lycopene isomers in vivo after food consumption and the physiological importance of *cis*-lycopene is not fully understood (Nguyen and Schwartz 1999; Boileau and others 2002).

MS 20030703 Submitted 12/2/03, Revised 1/30/04, Accepted 4/5/04. *Authors Hackett and Schwartz are with Dept. of Food Science and Technology, The Ohio State Univ., Parker Food Science and Technology Bldg., 2015 Fyffe Rd., Columbus, OH 43210-1007. Author Lee is with Dept. of Food Science and Technology, Seoul Natl. Univ. of Technology, Seoul, Korea. Author Francis is with Dept. of Horticulture & Crop Science, The Ohio State Univ., Columbus, Ohio. Direct inquiries to author Schwartz (E-mail: Schwartz.177@osu.edu).*

Thermal stability and isomerization of lycopene have been studied in different tomato varieties (Nguyen and others 2001) and in lycopene model systems (Lee and Chen 2002). Nguyen and others (2001) reported that all-*trans* lycopene does not isomerize thermally from *trans* to *cis* form during typical processing conditions, while all-*trans*-β-carotene and all-*trans* lutein isomerize. Lycopene appears to be quite stable within the plant matrix but may quickly degrade and isomerize when solubilized in oil or organic solvents. This geometrical thermal stability of lycopene may be because of the structural specificity of lycopene being present in a crystalline state and stored within the plant cell (Nguyen and others 2001). Lee and Chen (2002) reported that standard lycopene compounds underwent isomerization at 50 °C; however, degradation was the dominant mechanism at 100 °C .

Oleoresin is the material that remains after solvent extraction of a plant material followed by removal of the solvent. Oleoresin is a mixture of flavor compounds, pigments, fats, fatty acids, and sterols (Moyler 1999). Tomato oleoresin is a semisolid mixture of a resin and essential oil that can be obtained from tomatoes and tomato pomace. Tomato pomace is a byproduct from the tomato processing industry, consisting of 5% to 10% of the original weight of tomatoes (Fondevila and others 1994). Tomato oleoresin is a lycopene-rich material that has potential for use in foods and supplements to enhance the nutritional value, functionality, color, and flavor. The chemopreventive properties of tomato oleoresin to inhibit lung tumorigenesis has been studied (Hecht and others 1999). Rao and Agarwal (1998) reported that lycopene from tomato oleoresin was readily absorbed and may act as an in vivo antioxidant. Lipid extracts containing lycopene from tomatoes are available commercially for use in foods and nutritional supplements. However, few studies have been reported on the thermal stability and isomerization of lycopene in tomato oleoresin.

The objectives of this study were to determine the thermal stability and isomerization of lycopene in tomato oleoresins from dif-

ferent tomato varieties. In addition, the use of added antioxidants such as α-tocopherol and butylated hydroxytoluene (BHT) were studied to minimize the oxidation and isomerization reaction of lycopene in oleoresins during storage.

Materials and Methods

Materials

Three varieties of tomatoes (*Lycopersicon* esculentum Mill), Roma, High Lycopene, and Tangerine tomatoes, were used. Roma tomatoes were purchased from a local grocery (Columbus, Ohio, U.S.A.). The high Lycopene variety was Ohio FG98-218, an experimental line derived from a single back cross selection from the cross Ohio 9242 × (Ohio 9242 × T4099). Tangerine fruit were pooled from 3 sibling lines (Ohio FG99-130, Ohio FG99-71, and Ohio FG99-77) originally derived from a single back cross selection in the pedigree Ohio 9242 × (Ohio 9242 × LA351). These lines are homozygous for the tangerine gene (*t*) and the crimson gene (*ogc*). Reagent-grade solvents, α-tocopherol, and BHT were purchased from Fisher Scientific Co. (Fairlawn, N.J., U.S.A.). All-*trans* lycopene was purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Lycopene measurement in tomato

The lycopene content of each variety was determined from the harvested fruits following the procedure described in Nguyen and Schwartz (1998). The total solids in all the tomato varieties were determined by a gravimetric method modified from Sharma and Le Maguer (1996).

Oleoresin production

Approximately 6 kg of each variety was used to prepare each oleoresin. Roma tomatoes were also used to generate peel waste for the oleoresin. The tomatoes were diced and pureed using a blender to form a uniform mixture. All procedures were performed in subdued light to minimize isomerization and photodegradation. Five hundred grams of each sample were placed in 4-L beakers and 500 mL of ethanol was added, stirred, and allowed to stabilize for 1.5 min. The mixture was then homogenized for 1 min. The mixture was filtered through Whatman filter papers (Whatman Inc., Clifton, N.J., U.S.A.) nr 42 and 1 and cheesecloth. The filtrate was mixed with 250 mL of acetone/hexane solution (50:50, v/v) and homogenized for 1 min. The mixture was filtered a 2nd time as described previously, and extraction with acetone/hexane was repeated to increase the extraction yield. A separatory funnel was used to separate the nonpolar hexane layer containing lipid materials from the water-soluble fraction, and solvents were removed by reduced pressure at 40 °C. Tocopherol-stripped corn oil was prepared as described by Lee and Min (1988) and added to prepare each oleoresin to approximatly 2% in lycopene concentration.

Sample storage

Two hundred milligrams of tomato oleoresin samples were placed in 300-μL microvials and sealed with approximately 150 μL headspace volume. Samples were kept at 25 °C, 50 °C, 75 °C, and 100 °C in the dark. Samples were prepared in duplicate for each sample analysis.

Oleoresin from Roma, High Lycopene, and Roma peel waste (RPW) stored at 25 °C were analyzed at 0, 1, 2, 3, 4, 5, 6, and 7 wk. The 50 °C stored samples were analyzed at 0, 3, 7, 10, 14, 17, 21, and 24 d. Samples held at 75 °C were removed at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 d, and those at 100 °C were analyzed at 0, 1, 2, 3, 4, 5, 9, 13, 17, 21, and 24 h.

Samples of Tangerine oleoresin were kept at 25 °C for 0, 4, 7, 11, 14, 17, 21, and 24 d. Samples stored at 50 °C were analyzed at 0, 1, 2, 4, 6, 7, and 8 d. Samples stored at 75 °C were analyzed at 1, 2, 4, 6, 8, 24, and 29 h, and those at 100 °C were kept at 0.25, 0.5, 0.75, 1.0, and 1.5 h. Heating conditions for Tangerine oleoresin were shorter than other tomato varieties because of the more unstable tetra-*cis* isomers of lycopene in this variety. After thermal treatment, oleoresin samples were quantitatively removed with hexane, and 3-mL aliquots were dried under nitrogen before high-performance liquid chromatography (HPLC) analysis.

Lycopene isomerization

Lycopene was isomerized according to the method of Zechmeister (1944) and Nguyen and others (2001) to identify the lycopene isomers that developed during storage. All-*trans* lycopene standard was dissolved in hexane, and 20 μL of 1.0% iodine in hexane solution was added. The mixture was exposed to light for 30 min. Water was added to the vial and the epilayer dried over sodium sulfate and analyzed by HPLC to separate *cis* isomers.

HPLC analysis

Waters 2690 reversed-phase (RP)-HPLC equipped with a photodiode array detector (Waters Assoc., Milford, Mass., U.S.A.) was used. Separation was achieved using a 5-μm polymeric C_{30} RP-HPLC column (250 mm × 4.6-mm inner dia) from YMC, Inc. (Wilmington, N.C., U.S.A.) connected with a C_{18} stationary phase guard column, and a pre-column filter (0.5 μm) from Vydac (Hesperia, Calif., U.S.A.).

A mixture of methanol and methyl-*tert*-butyl ether (MTBE) (2:1, v/v) was used to dissolve (3 mL) the samples for injection. The samples were passed through a 200-μm filter before HPLC analysis. Elution was performed by a gradient method at room temperature (25 ± 5 °C) with a binary mobile phase solvent of MTBE (50% to 60%) and methanol (40% to 50%) for all samples except the Tangerine oleoresin samples. From 0 to 30 min, 50% methanol was used and held for 5 min. From 35 to 40 min, the solvent mixture contained 60% methanol and then held for 20 min. The sample injection volume was 50 μL at a flow rate of 1.0 mL/min. Spectra were collected from 250 to 550 nm.

Tangerine oleoresin samples were analyzed using a 3 μm C_{30} column (250 mm × 4.6-mm inner dia) instead of 5 μm size for better separation/resolution. The gradient mobile phases for the tetra-*cis* lycopene and isomer detection were applied as follows: from 0 to 40 min, a ratio of methanol, MTBE, and water changed from 90:2.5:7.5 to 30:70:0 (v/v/v); from 40 to 50 min, the ratio changed to 20:80:0 (v/v/v); and from 50 to 60 min, to 95:5:0 (v/v/v). Chromatograms were monitored at 472 and 438 nm and spectral information gathered from 250 to 550 nm.

Commercially available all-*trans* lycopene was used to generate a standard curve. Tetra-*cis* lycopene was collected from a preparatory HPLC system (Spectra-Physics, Mountain View, Calif, U.S.A.) with a 5-μm polymeric C_{18} RP-HPLC column (250 mm × 4.6-mm inner dia) using binary solvents, 85% methanol and 15% MTBE, and a flow rate of 6.0 mL/min. The concentration of lycopene in the samples was calculated based on the peak areas of the lycopene from HPLC chromatograms and response curves of standards.

Mass spectrometry

All experiments were performed on a Micromass Q-Tof™ II (Micromass, Wythenshawe, U.K.) mass spectrometer equipped with an orthogonal electrospray source (Z-spray) operated in positive ion mode. Lycopene samples were prepared in a solution containing MTBE infused into the electrospray source at a rate of 5 to 10 μL/min. Optimal electrospray ionization (ESI) conditions were capillary

Table 1—Lycopene and solids content in tomato oleoresin from Roma, Roma peel waste (RPW), High Lycopene, and Tangerine varieties

Tomato source	Solids (%)	Mg lycopene/ 100 g tomato fruit	G lycopene/ 100 g of pure oleoresin	Mg lycopene/ 100 g of standardized oleoresin
Roma	5.3	3.7	4.4	1387
Roma peel waste	5.5	18.0	23.6	2140
High Lycopene	8.2	13.3	20.7	3931
Tangerine	7.2	2.1	2.7	757

voltage 3000 V, source temperature 110 ° C, and a cone voltage of 60 V. The ESI gas was nitrogen. Q1 was set to optimally pass ions from m/z 100 to 2000, and all ions transmitted into the pusher region of the TOF analyzer were scanned over the m/z 400 to 950 range with a 1-s integration time.

Antioxidant addition effects on the lycopene stability

To study the effect of antioxidants on the stability of lycopene in tomato oleoresin, 0.02% α-tocopherol or 0.02% BHT was added directly to Roma oleoresin, stored at 50 °C in the dark, and analyzed at 0, 1, 2, 7, 9, 11, 14, 17, and 20 d. Roma oleoresin without the addition of antioxidants was designated as the control. Each antioxidant was tested separately, and samples were prepared in triplicate.

Statistical analysis

All data were analyzed using StatView 5.0 software (SAS Inst., Cary, N.C., U.S.A.). Fisher's protected least significant difference test was used to evaluate the possible pairwise comparisons for the tomato oleoresin varieties at each individual temperature studied. A P value of 0.05 or less was considered significant.

Results and Discussion

Lycopene content in tomato oleoresin

Lycopene content in tomato oleoresin from Roma, RPW, High Lycopene, and Tangerine varieties are shown in Table 1. The solids content of High Lycopene tomatoes was the highest with 8.2% and that of Roma tomatoes was lowest with 5.3%. RPW had the highest lycopene content with 18 mg/100 g. Lycopene content in oleoresin from the RPW, High Lycopene, Roma, and Tangerine tomatoes was 23.6, 20.7, 4.4, and 2.7 g/100 g of oleoresin, respectively (Table 1). Peel waste, a byproduct of tomato processing, contains significantly high amounts of lycopene ($P < 0.05$).

Lycopene content in tomato juice, tomato sauce, tomato ketchup, and tomato paste is 11.6, 15.6, 11.4, and 34.3 mg/100 g food, respectively (Rao and others 1998; Nguyen and Schwartz 2000). Lycopene content in tomato oleoresin is approximately 80 to 2000 times higher than other processed tomato products (Rao and others 1998; Nguyen and Schwartz 2000). Tomato waste has been used as supplement in the diet of animals (Fondevila and others 1994), and dried tomato pulp has been used to increase the pigmentation of egg yolk (Dotas and others 1999). Oleoresin from tomato waste, because of the high content of lycopene, may find application in food and/or supplement uses.

Lycopene degradation in oleoresin

The thermal stability of lycopene in oleoresin from RPW stored at 25 °C, 50 °C, 75 °C, and 100 °C are shown in Figure 1. Total lycopene in oleoresin from the RPW degraded as storage temperature increased from 25 °C to 50 °C, 75 °C, and 100 °C. The degradation rates, half life (t), and activation energy for the degradation of ly-

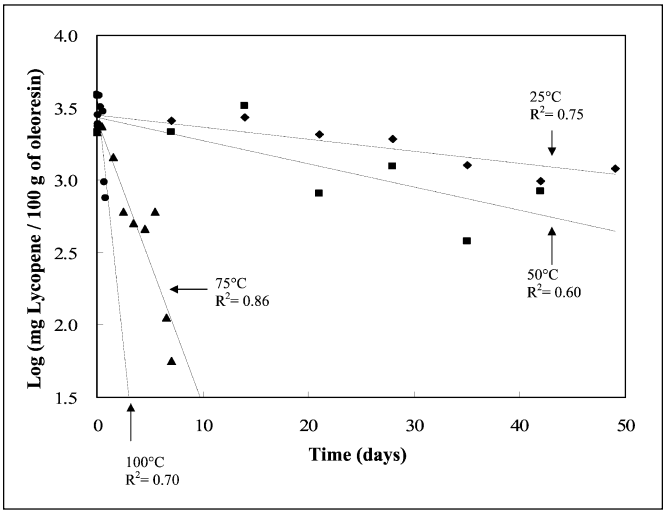

Figure 1—The effect of temperature on the stability of total lycopene in oleoresin from Roma peel waste. ◆ = 25 °C; ■ = 50 °C; ▲ = 75 °C; ● = 100 °C. See text for experimental protocols.

Table 2—First-order degradation rate constants, half-life (t), and activation energy for lycopene degradation from different varieties of tomato oleoresin[a]

	Temperature				
	25 °C	50 °C	75 °C	100 °C	
Tomato	Degradation rate (milligrams of lycopene/100 g of oleoresin/d) and half-life (t), mean ± SD				Activation energy (kcal/mol)
Roma	17.8a ± 4.1	51.8a ± 5.8	138a ± 33	848a ± 169	11.5a ± 7.2%
t (day)	39	13.4	5	0.8	
Roma peel waste	34.9a ± 9.6	82.0a ± 15.6	289b ± 52	1763a ± 3239	12.1a ± 7.4%
t (day)	30.7	13.1	3.7	0.6	
High Lycopene	46.5b ± 14.0	83.5a ± 18.1	374b ± 50	2840a ± 841	11.7a ± 9.3%
t (day)	42.3	23.5	5.3	0.7	
Tangerine	22.3a ± 4.5	64.6a ± 18.3	291b ± 75	9030b ± 3009	15.0b ± 6.7%
t (day)	17	5.9	1.3	0.1	

[a]Values followed by different letters were significantly different in column ($P < 0.05$). SD = standard deviation. Each data point represents an average of 3 replicates.

copene in oleoresin are shown in Table 2. Apparent 1st-order rate constants were determined from the data plots, and activation energies for the degradation of lycopene were obtained according to the Arrhenius equation. As expected, the rate of degradation of lycopene in oleoresin increased with the increase of temperature. The degradation rates of lycopene in oleoresin from RPW, High Lycopene, Roma, and Tangerine varieties were not significantly different at 50 °C ($P > 0.05$) (Table 2). Lycopene in Tangerine oleoresin, tetra-*cis* lycopene, showed significantly more rapid reaction rates of degradation at 100 °C ($P < 0.05$) (Table 2), most likely because of its different isomer structure. The half life (t) of lycopene in oleoresin from Roma, High Lycopene, RPW, and Tangerine ranged from 17.0 to 42.3 d at 25 °C, 5.9 to 23.5 d at 50 °C, 1.3 to 5.3 d at 75 °C, and 0.1 to 0.8 h at 100 °C (Table 2). The lycopene half life present in tangerine tomatoes was much lower because of the increased degradation rate at higher temperatures. The half life of lycopene in tangerine oleoresin was shorter than the other samples at all storage temperatures.

The activation energies of degradation of lycopene in oleoresin from Roma, High Lycopene, RPW, and Tangerine were 11.5, 11.7, 12.1, and 15.0 kcal/mol, respectively (Table 2). The activation energy of degradation of lycopene in oleoresin from Roma, High Lycopene, and RPW was not significantly different ($P > 0.05$), whereas that from Tangerine was significantly higher ($P < 0.05$). The *trans* forms of carotenoids are reported to be more thermodynamically stable than the *cis* forms (Nguyen and Schwartz 2000). Tetra-*cis* lycopene in Tangerine oleoresin would be more reactive and should have lower activation energy compared with the other oleoresin containing the stable *trans* lycopene. Tetra-*cis* lycopene in Tangerine oleoresin may proceed through different degradation and isomerization mechanisms than the all-*trans* isomer of lycopene, which results in the higher activation energy of lycopene degradation than other varieties of oleoresin.

The activation energy of all-*trans* lycopene degradation was reported as 14.5 kcal/mol (Lee and Chen 2002), 5.0 kcal/mol in oil-in-water emulsion (Ax and others 2003), and 19.8 kcal/mol in safflower oil (Henry and other 1998). The activation energy of lycopene degradation in oleoresin from Roma, High Lycopene, and RPW was lower than that of pure lycopene (14.5 kcal/mol) and higher than that in oil-in-water emulsion (5.0 kcal/mol), which may be because of other components present in oleoresin, differences in sample matrices and temperature ranges used to generate the degradation rates. The degree of unsaturated fatty acids, high solubility of oxygen in lipid, the presence of free radicals, mono- and diglycerides, and the possible presence of trace metals in oleoresin acting as prooxidants (Min 1998) can accelerate the degradation of lycopene. The higher activation energy of lycopene degradation (19.8 kcal/mol) in safflower oil may be because of the presence of high tocopherol content within the oil. The lower activation energy of lycopene degradation (5.0 kcal/mol) in oil-in-water emulsion system may arise from differences in the sample matrices and processing effects such as heating oil up to 165 °C. These high temperatures could generate free radicals of fatty acids accelerating the degradation of lycopene.

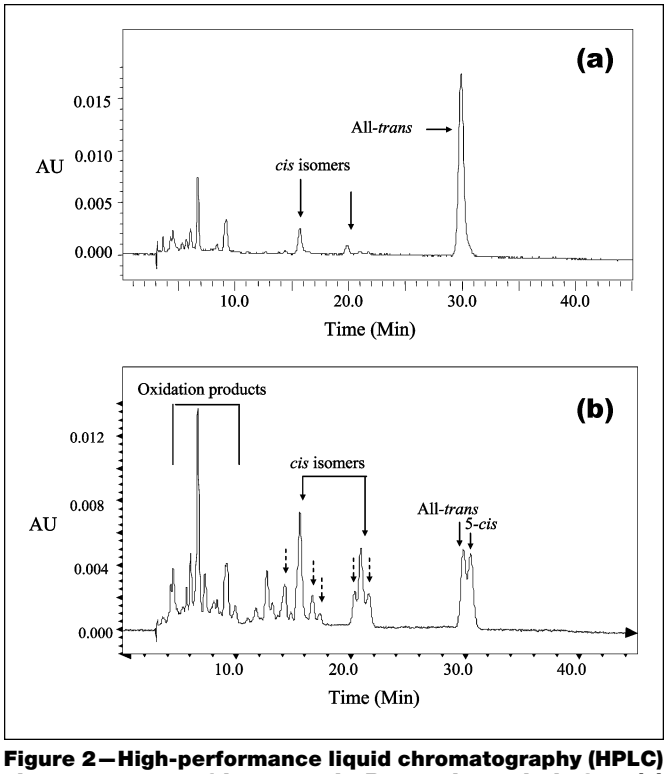

Figure 2—High-performance liquid chromatography (HPLC) chromatograms of lycopene in Roma oleoresin before (a) and after (b) heat treatment of 100 °C. Newly formed *cis*-lycopene isomers are indicated by broken arrows. See text for experimental protocols.

Lycopene isomerization in oleoresin

HPLC chromatograms of lycopene from Roma oleoresin before and after heat treatment are shown in Figure 2. All-*trans* lycopene from Roma oleoresin was the major peak eluting with a retention time of 30.0 min before heat treatment. Two *cis* isomers of lycopene were detected at 15 and 20 min of elution time from the Roma tomato oleoresin (Figure 2a).

At the storage temperatures of 25 °C and 50 °C, the degradation products of lycopene eluted from the column within the first 10 min of the chromatogram, and as the storage time increased, lycopene degradation products increased. Lycopene degradation products may arise from oxidation mechanisms because these more polar components were not present before the heat treatment, and they eluted in a more polar region. Lycopene is highly susceptive to oxidative degradation because of its highly conjugated polyene structure (Nguyen and Schwartz 2000). Anguelova and Warthesen (2000) reported on lycopene degradation compounds formed by oxidative mechanisms that increased in tomato powder stored below 50 °C for 6 wk. The structure and identification of the hypothesized lycopene oxidation products were not determined in this study.

When the storage temperature was 75 °C and 100 °C, *cis* isomers of lycopene were formed from all-*trans* lycopene and increased. Isomerization of all-*trans* lycopene in oleoresin led to the formation of numerous *cis* lycopene isomers (Figure 2b). As storage time increased at 75 °C and 100 °C, the relative concentration of all-*trans* lycopene in oleoresin decreased, new *cis* isomers appeared, and the existing *cis* isomers and the lycopene degradation products increased. The degradation mechanism of lycopene in oleoresin seems to depend on the storage temperature. At 25 °C and 50 °C, lycopene in oleoresin degrades predominantly through oxidation, whereas isomerization of lycopene in oleoresin increases at 75 °C and 100 °C. Results of this study agree with previous report of Ax and others (2003), who investigated the thermal isomerization of lycopene in oil-in-water emulsion systems at 25 °C and 90 °C. Thermal treatment at 90 °C caused the decrease of all-*trans* lycopene and increase of the 9-*cis* isomer within 10 h, whereas at 25 °C, increase in isomerization of lycopene was not observed for 24 h (Ax and others 2003).

Lycopene from Roma oleoresin stored at 100 °C produced 8 peaks of lycopene geometrical isomers, tentatively identified using UV-

Vis spectra; 14.4 min (433, 460, 488 nm), 15.5 min (439.2, 464.5, 494.8 nm), 17 min (433, 458.5, 487.5 nm), 18 min (433, 459, 487.5 nm), 20.5 min (439.2, 465.8, 497.2 nm), 20.8 min (439.2, 465.8, 497.2 nm), 30 min (444, 471.8, 503.3 nm), and 30.9 min (444, 471.8, 503.3 nm) (Figure 2b). The degradation and isomerization of lycopene in oleoresin from RPW and High Lycopene showed similar patterns compared with those from Roma oleoresin (not shown).

HPLC chromatograms of lycopene from Tangerine tomato oleoresin are shown in Figure 3. Tangerine tomato oleoresin contains predominately tetra-*cis* lycopene (Figure 3a). At 25 °C and 50 °C storage conditions, lycopene from Tangerine tomato oleoresin degraded without an increase in isomerization (data not shown). When the storage temperature was 75 °C and 100 °C, tetra-*cis* lycopene isomerized into other *cis* and *trans* forms of lycopene, along with their degradation products (Figure 3b). It appeared that the tetra *cis* lycopene was converted into tri-*cis* and perhaps some di- and mono-*cis* as well as all-*trans* lycopene.There were several unidentified peaks formed from Tangerine tomato oleoresin stored at 100 °C. Small peaks of phytoene (λ = 275, 286, 297nm), phytofluene (λ = 331, 348, 368nm), and zeta-carotene (λ = 378, 398, 424nm) eluted at 27.6, 28.0, and 30.3 min, respectively. Zeta-carotene coeluted with tetra-*cis* lycopene. The UV-Vis spectral data observed in this study for phytofluene, zeta-carotene, and phytoene agreed with previous data (Britton 1995).

The conversion of the all-*trans* lycopene into *cis* forms resulted in a color change. The red color of tomato oleoresin began to lighten into an orange/yellow color as the storage time increased. This may be because of the decrease in the λ_{max} of 472 nm of all-*trans* lycopene while increasing in the λ_{max} of 460-nm range of *cis* isomers. However, with Tangerine oleoresin, as the thermal storage time increased, the orange-colored Tangerine oleoresin turned into a more intense red color because of the increase of *trans* isomers and other *cis* isomers.

Mass spectrometry and UV-Vis spectra were used to confirm the identity of lycopene and its *cis* isomers. Mass spectra of tetra-*cis* lycopene and all-*trans* lycopene in tomato oleoresin are shown in Figure 4. Tetra-*cis* lycopene and all-*trans* lycopene have a base peak at 536.4 (m/z, mass/charge), which is the molecular weight, and a protonated peak at 537.4 (m/z). Mass spectrometry using electrospray ionization has been commonly used to analyze carotenoids because of the production of abundant molecular cations with little fragmentation, the efficiency of the solvent removal in the liquid chromatography mass spectrometry (LC-MS) interface, and the compatibility with a wide range of HPLC flow rates. More information on structural isomers can be obtained from fragmentation patterns provided by collision-induced dissociation with ESI (van Breemen 2001).

Effects of antioxidant addition on the lycopene stability

Degradation rates and half-life of lycopene in Roma tomato oleoresin with or without 0.02% α-tocopherol or 0.02% BHT at 50 °C are shown in Table 3. The lycopene stability in Roma tomato oleoresin

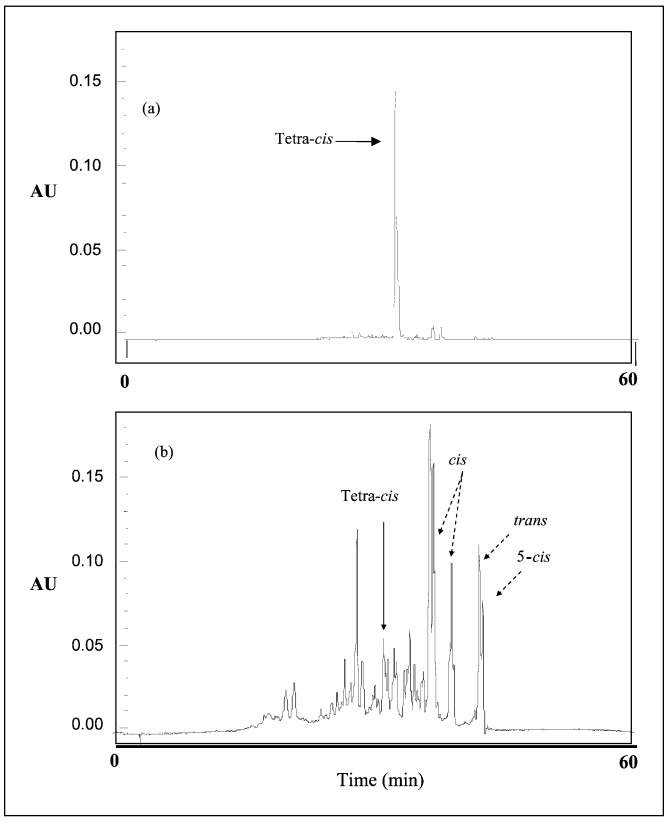

Figure 3—High-performance liquid chromatography (HPLC) chromatograms from Tangerine oleoresin before (a) and after (b) heat treatment at 100 °C. Newly formed lycopene isomers are indicated by broken arrows. See text for experimental protocols.

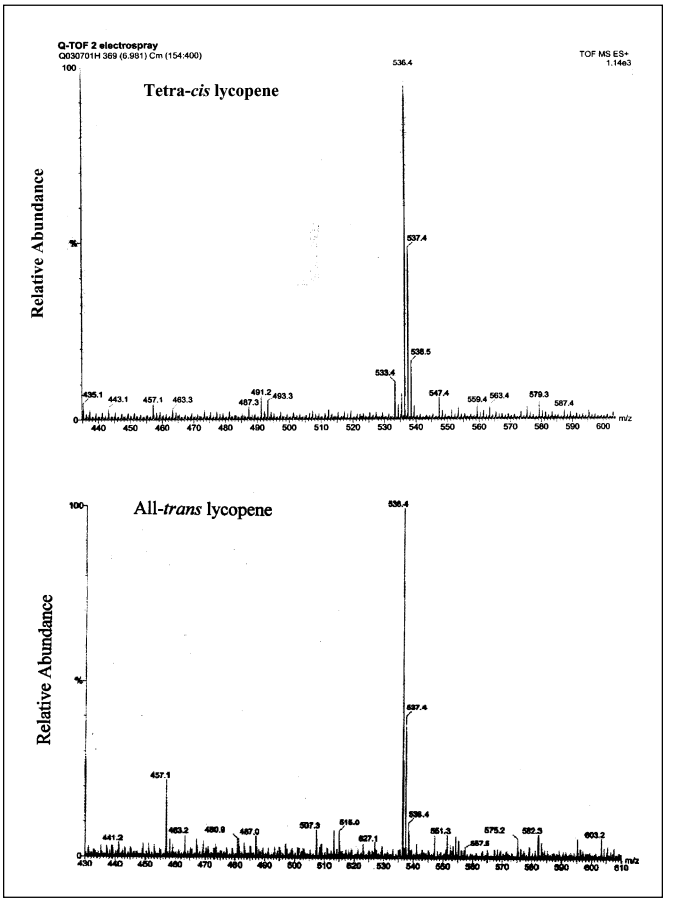

Figure 4—Mass spectra of tetra-*cis* lycopene and all-*trans* lycopene by electrospray ionization (ESI) mass spectrometry. See text for experimental protocols.

Table 3—First-order degradation rate constants and half-life ($t_{1/2}$) of lycopene degradation from Roma oleoresin with or without addition of 0.02% α-tocopherol or 0.02% BHT at 50 °C in the dark[a]

Tomato	Degradation rate (mg Lycopene/ 100 g oleoresin/d), mean ± SD	Half life ($t_{1/2}$) (d)
Roma oleoresin without antioxidants	$105.7^{a} \pm 0.3$	6.1^{a}
Roma oleoresin with α-tocopherol	$52.1^{b} \pm 8.8$	12.5^{b}
Roma oleoresin with BHT	$53.0^{b} \pm 2.9$	12.3^{b}

[a]Different superscripts were significantly different in column ($P < 0.05$). SD = standard deviation. Each data point represents an average of 3 replicates.

with 0.02% α-tocopherol or BHT significantly increased compared to the control samples without the addition of antioxidants ($P < 0.05$) (Table 3). Half-life of lycopene degradation of control, oleoresin with α-tocopherol, or BHT was 6.1, 12.5, and 12.3 d, respectively. The antioxidant effects between α-tocopherol and BHT on the stability of lycopene degradation in oleoresin were not significant ($P > 0.05$). Storage at 100 °C showed that antioxidants, especially BHT, slowed the degradation rate but the degree of isomerization remained unaffected.Tomato oleoresin consists of mainly lycopene, phospholipids, sterols, mono-, and diglycerides (Nir and others 1993). The stability of lycopene in tomato oleoresin and its degradation may depend on the composition and components of oleoresin and treatment conditions. Prooxidants such as trace metals or various free radicals in oleoresin may induce the degradation and isomerization of lycopene. Addition of antioxidants can enhance the stability of lycopene and minimize the degradation of lycopene in tomato oleoresin.

Conclusions

The degradation and isomerization of lycopene in tomato oleoresin from different varieties were determined. Isomerization of lycopene in tomato oleoresin depends on the storage temperature. Lycopene in oleoresin may degrade mainly through an oxidation mechanism at 25 °C and 50 °C. As the storage temperature increased to 75 °C and 100 °C, the extent of lycopene isomerization was enhanced. Addition of antioxidants such as α-tocopherol or BHT can minimize the degradation of lycopene in oleoresin. Tetra-*cis* lycopene present in oleoresin from the Tangerine variety of tomatoes degraded more rapidly in comparison to lycopene (all-*trans*) present in oleoresin prepared from other tomato varieties. Oleoresin, with unique carotenoid composition and structure, can be considered for use as food additives, functional foods, and nutritional supplements.

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