DOI:10.1021/jf1012665

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Carotenoid Stability during Production and Storage of Tomato Juice Made from Tomatoes with Diverse Pigment Profiles Measured by Infrared Spectroscopy

Daniel E. Rubio-Diaz,[†] Alejandra Santos,[†] David M. Francis,[‡] and Luis E. Rodriguez-Saona*,[†]

[†]Department of Food Science and Technology, 110 Parker Food Science and Technology Building, The Ohio State University, 2015 Fyffe Road, Columbus, Ohio 43210, and [‡]Department of Horticulture and Crop Science, The Ohio State University, 1680 Madison Avenue, Wooster, Ohio 44691

Chemical changes in carotenoids and lipids were studied during production and storage of canned tomato juice using ATR infrared spectroscopy and HPLC. Samples from 10 groups of tomatoes with different carotenoid profiles were analyzed fresh, after hot-break and screening, after canning, and at five different time points during 1 year of storage. An apparent increase of carotenoids was observed after hot-break due to improved extraction efficiency. This increase was accompanied by some degree of lipid oxidation and carotenoid isomerization. Canning produced the most intense changes in the lipid profile with breakdown of triglycerides (~1743 cm^{-1}), formation of fatty acids (~1712 cm^{-1}), and degradation and isomerization of *trans*-carotenoids (~960 and ~3006 cm^{-1}). Isomerization was corroborated by the relative increase of HPLC areas corresponding to carotenoid *cis* isomers. Canning reduced *trans*-lycopene, *trans*-δ-carotene, *trans*-β-carotene, and *trans*-lutein by 30, 34, 43, and 67%, respectively. HPLC data indicate that canning causes a drastic reduction of *tetra-cis*-lycopene and promotes its isomerization to other geometric forms, including *all-trans*-lycopene. Infrared spectra of tomato juice lipid fractions correlated well with the number of days in storage (SECV < 11 days, r values > 0.99), demonstrating continuous degradation of lipids. Results demonstrated that individual carotenoids and their isomeric forms behave differently during production and storage of canned tomato juice. Information collected by infrared spectroscopy complemented well that of HPLC, providing marker bands to further the understanding of chemical changes taking place during processing and storage of tomato juice.

KEYWORDS: Carotenoids; stability; thermal treatment; storage; tomato juice; tomato; HPLC; infrared spectroscopy; PLSR; isomerization; ATR; lycopene

INTRODUCTION

A growing number of studies support the association between the dietary intake of carotenoids and specific health benefits (*1*). Lycopene, for instance, has been linked to decreased risks of prostate cancer and oxidative stress mediated conditions, such as cardiovascular diseases and osteoporosis (*1*). Carotenoids can exist in many geometrical configurations due to the presence of conjugated carbon−carbon double bonds in their molecular structure. Each double bond could, in theory, be present in *cis* or *trans* form, producing a large number of possible combinations. With 11 conjugated double bonds, lycopene could theoretically be present in 2048 configurations. However, the existence of many of these configurations is limited by steric hindrance, and only a small number are structurally favorable (~72 in the case of lycopene with 7 stereochemically effective double bonds in a symmetrical chain) (*2*).

*Corresponding author [phone (614) 292-3339; fax (614) 292-0218; e-mail rodriguez-saona.1@osu.edu].

Research in humans suggests that carotenoid bioavailability can be affected by their isomeric configuration (*3*−*6*). Results from these studies showed that lycopene from tomatoes may be better absorbed in *cis* forms, which could result in higher efficacy to deliver the potential health benefits. Carotenoid isomeric diversity in tomato products can be traced to the plant material itself or derive from the application of food-processing technologies (*7*−*11*). Red tomatoes contain lycopene in the *all-trans* conformation. Natural genetic variation exists, which modifies the concentration of lycopene as well as the abundance of lycopene in the *tetra-cis* configuration. Several studies have consistently reported higher lycopene bioavailability in processed tomato products than in fresh tomatoes (*3*, *6*, *12*), suggesting that disrupting the cellular matrix enhances uptake in the diet. In addition, processing conditions can modify the proportion of *cis*-lycopene (*3*, *4*, *6*, *12*). Therefore, there is an increased interest in understanding the effect of food processing and storage conditions on carotenoid content and isomeric conversion and in developing new plant varieties with carotenoid content and structure optimized for human health.

 Published on Web 07/14/2010

To achieve the goals of monitoring carotenoid structure during processing and selecting plant varieties with specific content and profile, the food industry requires tools to efficiently monitor content, stability, and isomerization of carotenoids during food production and storage. HPLC, the standard technique in laboratories for the analysis of carotenoids, requires highly trained personnel and expensive equipment and supplies that may hinder its application in routine analyses. Infrared spectroscopy represents an excellent alternative because it is a simple, cost-effective technique that has already won a place in the food industry. Several studies have demonstrated the capability of this technique to quantitate lycopene and β-carotene in tomato and tomato products on the basis of the specific interaction of infrared light with their chemical structure (*13*, *14*). Infrared spectroscopy has also been used for profiling carotenoids in genetically diverse tomatoes with a variety of carotenoid contents and isomeric distributions (*15*). The specific absorptions of *trans* and *cis* chemical structures in the mid-infrared spectrum make this technology a powerful tool to evaluate lipid isomerization during food processing and storage (*16*).

The objective of this research was to study chemical changes of carotenoids during processing and storage in tomato juice from 10 varieties with distinct pigment profiles and to evaluate the ability of infrared spectroscopy to provide fast and accurate information about these chemical changes.

MATERIALS AND METHODS

Plant Material. Ten varieties used in this study were grown and harvested from The Ohio State University North Central Agricultural Research Station in Fremont, OH (2008). These varieties were developed by selective breeding to combine specific naturally occurring alleles of genes affecting the biosynthesis of carotenoids. Specifically, three alleles of the fruit-specific β-cyclase, which converts lycopene into β-carotene, were used: the normal, or "wild type", allele; the *old gold crimson* (og^c) allele, which accumulates lycopene due to a mutation in the gene; and the *Beta* allele, which accumulates more enzyme, leading to higher provitamin A. In addition, the *dark green* (*dg*) allele of the *high pigment-2* gene was used to increase total carotenoids. The *Delta* allele of the epsilon cyclase was used to increase levels of δ-carotene at the expense of lycopene and β-carotene. Finally, *tangerine* (*t*) alleles of the lycopene isomerase were used to increase *tetra-cis*-lycopene at the expense of *trans*-lycopene. Varieties used include normal, NO (OH8245, "wild type"); midhigh *all-trans*-lycopene, ML (OH2641, og^c); high *all-trans*-lycopene, HL (FG99-218, *dg* and og^c); β-carotene, BE (97L97, *Beta* in the OH8245 genetic background); high β-carotene, HB (OH08-3123, *Beta*, *dg*); δ-carotene, DE (OH02-8505, *Delta*); high δ-carotene, HD (OH08-3082, *Delta*, *dg*); high *cis*-lycopene, HC (OH08-3075, *t*, *dg*); *tetra-cis*-lycopene, T3 (FGH04-163); and *tetra-cis*-lycopene, T9 (FGH04-169).

Tomato Juice. Tomato juices were produced from each of the 10 tomato varieties previously described under Plant Material at the pilot plant of The Ohio State University Food Industries Center. Processing steps included washing, crushing, hot-breaking (93 °C for 5 min), screening, filling (can dimensions: diameter = 76.2 mm; height = 112.7 mm), sealing, heating (110 °C for 30 min), and cooling to room temperature with tap water. Approximately 30 cans per variety were processed. The pH values of the 10 tomato juices were similar and ranged from 4.04 to 4.31. Samples from fresh tomatoes (blended and frozen at −35 °C), tomato juice after hot-breaking and screening, after canning, and at five different time points during storage (30, 75, 135, 272, and 365 days) were analyzed in triplicate (three independent cans per point) by infrared spectroscopy and HPLC.

Extraction of Paste and Lipid Fraction from Tomato and Tomato Juice. Blended tomato or tomato juice (5 g) was mixed with methanol (5 mL) and centrifuged for 12 min at 5100 rpm (3400*g*). Approximately 2 g of tomato solids (paste) was collected after the supernatant (mainly water and methanol) had been discarded. The carotenoid-containing lipid fraction was extracted from the paste in a dimmed light environment to reduce isomerization and photodegradation. Extraction was carried out as described by Nguyen and Schwartz (*10*) with some modifications (recovery level > 95%). Paste (~1 g) was mixed with 20 mL of acetone/ethanol/hexane 1:1:2 (v/v/v) and continuously shaken for 15 min (Rotamix model RK DYNAL, Dynal Biotech Inc., Lake Success, NY) at 30 rpm. Then, 3 mL of deionized distilled water was added to induce separation, and the sample was shaken for an additional 5 min (30 rpm). Finally, the sample was left to rest for 5 min, and two aliquots (3 mL each) of the hexane top layer were dried under nitrogen flow in amber glass tubes. One aliquot was analyzed by HPLC and the other by infrared spectroscopy.

Infrared Spectroscopy. Paste and lipid fraction spectra (three per type) were collected from three independent extractions of fresh and hot-break samples. For the evaluation of canned tomato juice, three spectra were collected from the extraction of a single can, and three cans were analyzed per time point. Therefore, nine spectra of both types (paste and lipid fraction) were collected from each variety at different processing stages (fresh, hot-break, canned) and storage times (30, 75, 135, 272, 365 days). Infrared spectroscopic analysis was done as described by Rubio-Diaz et al. (*15*) using an FTS 3500GX Fourier-transform infrared spectrometer (Varian, Palo Alto, CA) with a potassium bromide beam splitter and deuterated triglycine sulfate (DTGS) detector. The instrument was continuously purged with CO_2-free dry air from a CO_2RP140 dryer (Dominick Hunter, Charlotte, NC). Spectra were collected in the 4000−700 cm^{-1} infrared region at 8 cm^{-1} resolution using attenuated total reflectance crystals. Sixty-four scans were co-added followed by Beer−Norton apodization. Paste spectra were collected using a three-bounce diamond crystal plate and a high-pressure clamp (Pike Technologies, Madison, WI) to increase contact. Lipid fractions were resuspended in 20 μL of chloroform and applied onto a ZnSe crystal plate. Spectra were collected after 1 min to allow for chloroform evaporation.

Multivariate Analysis: SIMCA and PLSR. The multivariate statistics program Pirouette for Windows Chemometrics Modeling Software, version 3.11 (Infometrix, Inc. Bothell, WA), was used for spectral analysis. Spectra were transformed by using vector length normalization and a 15-point polynomial-fit Savitzky−Golay second-derivative function. In spectroscopy each wavenumber is considered to be a variable and, therefore, multivariate statistical tools are commonly used for the analysis of these large data sets. Two multivariate techniques were applied to analyze spectral changes in this study, soft independent modeling of class analogy (SIMCA) and partial least-squares regression (PLSR). SIMCA is a supervised classification technique based on principal component analysis used to reduce the dimensionality of multivariate data sets. SIMCA helped to analyze similarities or differences among groups of spectra (classes), building a distinct confidence region (95%) around each class (*17*, *18*). Results from SIMCA analysis include the interclass distance, which is an indicator of how similar two classes are; the discrimination power, which describes how well a variable (wavenumber) helps to classify (differentiate) the samples; and the SIMCA class projection, which allows visualization of clustering among samples (sample patterns, groupings or outliers) using the first three principal components. This technique has been successfully used for the analysis of tomato lipids in genetically diverse tomatoes (*15*). PLSR was used to produce calibration models that correlate lipid fraction spectra of canned tomato juice with time of storage. Models were cross-validated using a leave-one-out approach. Performance was evaluated by the number of latent variables, coefficient of determination (*r* value), and the standard error of cross-validation (SECV), which estimates the expected error of predicting an unknown with the PLSR model.

High-Performance Liquid Chromatography Analysis. Carotenoids were determined by HPLC using an HP1050 system equipped with a photodiode array detector (Agilent Tech, Palo Alto, CA). Lipid fraction aliquots were redissolved in a 3 mL mixture of 1:1 methanol/MTBE solvent. One hundred microliters of sample was injected into the HPLC system for carotenoid analysis. A C_{30} YMC column (5 μm particle size, 250 × 4.6 mm) (Waters Corp., Milford, MA) and a guard column C_{30} YMC (5 μm particle size, 20 × 4.0 mm) (Waters Corp.) were used in-line for all separations. Elution solvents were (A) 81% methanol, 15% MTBE, and 4% water; and (B) 90% MTBE, 7% methanol, and 3% water. Separations were carried with a 75 min linear gradient (0−88% B). The flow rate was 1.0 mL/min with detections at 348 nm (phytofluene, ζ-carotene), 450 nm (*all-trans*-β-carotene, 13-*cis*-β-carotene, neurosporene, *tetra-cis*-lycopene, *all-trans*-δ-carotene, *all-trans*-lutein), and 470 nm (*all-trans*-lycopene). Carotenoids were tentatively identified using retention

times and UV−vis absorption spectra. Typical retention times were 14.0, 25.5, 30.0, 36.0, 37.0, 37.4, 37.5, 48.0, and 69.5 min for *all-trans*-lutein, phytofluene, 13-*cis*-β-carotene, *all-trans*-β-carotene, ζ-carotene, neurosporene, *tetra-cis*-lycopene, *all-trans*-δ-carotene, and *all-trans*-lycopene, respectively. Commercial samples of *all-trans*-lycopene and *all-trans*-β-carotene (Sigma-Aldrich, St. Louis, MO) were used as external standards to validate constant chromatographic conditions during this study. The average percent relative standard deviation of replicates (precision), including extraction and chromatography procedure, was 5%.

RESULTS AND DISCUSSION

Previous research has demonstrated the capability of infrared spectroscopy to classify genetically diverse tomatoes on the basis of their lipid and carotenoid profile (*15*). In this study we evaluated the use of IR spectra to characterize changes in tomato juice at different processing stages: fresh tomato, after hot-break and screening (referred to as "hot-break" for simplification), after canning, and during storage for 1 year. Two types of spectra were collected from tomato juice samples, one from the paste (tomato solids separated after mixing the juice with methanol and centrifugation) and the other from the lipid fraction (extracted from the paste using hexane). Ten tomato varieties with different carotenoid profiles (NO, ML, HL, HC, T3, T9, DE, HD, BE, and HB) were evaluated by infrared spectroscopy and HPLC (**Figure 1**).

SIMCA classification models constructed with infrared spectra collected from the paste discriminated fresh tomato from processed samples (hot-break, canned, 1-year-old canned) with interclass distances higher than 7 for all tomato varieties (**Table 1**). Interclass distances higher than 3 are considered to be significant (*19*). The large interclass distances indicated that composition and chemical changes during processing modified the infrared spectra of fresh tomatoes to a large extent. Infrared bands in the regions 920−970 cm^{-1} (*trans* C═C double bonds), 1210−1240 cm^{-1} (C—O stretching), and 1750 cm^{-1} (C═O in esters) had the highest discrimination power in spectra classification. Modifications of these bands are commonly associated with lipid structures, suggesting that thermal processing and storage cause important changes in the lipid components of tomato. In general, paste spectra from samples after hot-break and after canning did not produce significant SIMCA interclass distances (**Table 1**). The paste spectrum contains information from many other components (water, carbohydrates, etc.) that could mask unique signals associated with compositional changes during tomato juice processing. Interestingly, discrimination between hot-break and 1-year-old canned samples was significant (IC > 3) in tomato juices with high concentrations of carotenoids (HL, HB, HC, HD) and the NO variety. This suggests chemical changes during storage and corroborates the role of lipids as important factors to differentiate spectra at different stages (**Table 1**). **Figure 2** presents SIMCA tridimensional projections illustrating the discrimination of paste spectra collected at the four processing steps for the T3 variety.

SIMCA models constructed with the spectra collected from lipid fractions showed improved discrimination capabilities. The improved performance derives from the removal of interference produced by other tomato constituents (*15*). As opposed to models based on paste spectra, lipid fraction models were able to discriminate hot-break juice from freshly canned tomato juice and freshly canned tomato juice from 1-year-old canned tomato juice (**Table 1**; **Figure 2**). This provided explicit evidence of lipid chemical changes during production and storage of tomato juice.

Some spectral changes in lipid fractions were common to all tomato juice varieties at the four processing stages (fresh, hot-break, canned, 1-year-old canned). The intensity of bands in regions

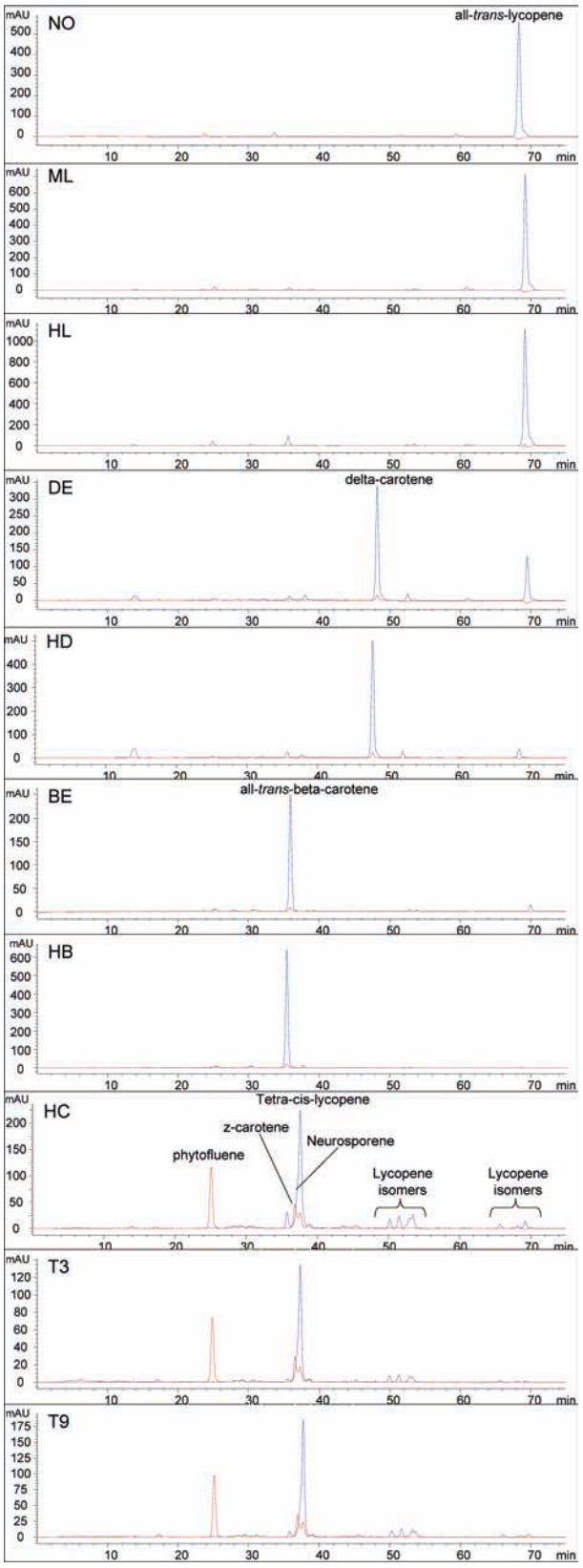

Figure 1. HPLC chromatograms at 450 nm (blue) and 348 nm (red) of tomato varieties used in this study.

1750−1710 cm^{-1} (carbonyl groups) and 940−980 cm^{-1} (*trans* C═C double bonds) noticeably changed after hot-break and canning and during storage. Bands at 1743 (C═O esters) and 1712 cm^{-1} (COOH) decreased and increased, respectively, throughout the four stages, suggesting breakdown of triglycerides and

Table 1. SIMCA Interclass Distances of Five Selected Varieties (NO, BE, DE, HC, HL) Using Spectra from Paste and Lipid Fractions of Tomato Juice at Four Different Processing Stages[a]

	fresh tomato					after hot-break					after canning				
	NO	BE	DE	HC	HL	NO	BE	DE	HC	HL	NO	BE	DE	HC	HL
Paste															
after hot-break	10.2	10.7	10.7	13.4	9.7						2.9	2.6	1.4	1.9	9.9
after canning	10.9	15.3	11.3	12.5	16.4	2.9	2.6	1.4	1.9	9.9					
1 year after canning	11.0	14.1	12.2	14.0	15.5	4.2	2.4	1.6	4.0	8.0	1.6	2.9	1.4	2.2	1.3
Lipid Fraction															
after hot-break	21.2	9.4	7.3	14.0	25.2						7.9	7.8	6.3	26.7	11.2
after canning	18.2	7.2	10.2	17.3	37.1	7.9	7.8	6.3	26.7	11.2					
1 year after canning	31.6	20.0	17.1	21.2	28.9	22.3	20.8	18.7	20.7	19.8	12.2	11.7	11.6	10.2	18.9

[a] Models based on the 750−1800 and 2750−3050 cm^{-1} infrared regions; 2−4 principal components per class. Tomato varieties: NO, normal; BE, β-carotene; DE, δ-carotene; HC, high *cis*-lycopene; HL, high *trans*-lycopene.

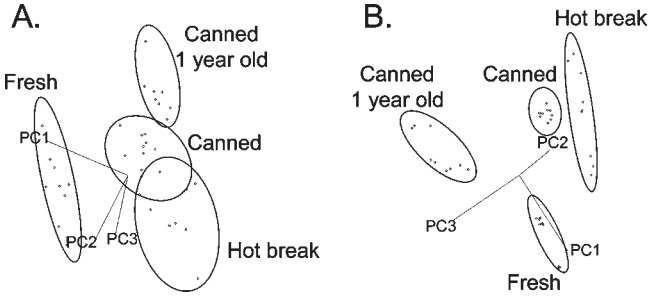

Figure 2. SIMCA class projections of (**A**) paste and (**B**) lipid fraction spectra collected from T3 (Tangerine) samples. Models are based on the 750−1800 and 2750−3050 cm^{-1} infrared regions. PC stands for principal component.

formation of free fatty acids. The band at ~960 cm^{-1} increased after hot-break, decreased after canning, and increased again during storage (**Figure 3**). The increase of this band after hot-break and screening may be explained by the improved extraction efficiency of carotenoids. Previous work has attributed the improved extraction efficiency to the disruptive effect of heat on cell walls and carotene−protein complexes, which facilitate the release of carotenoids during hexane extraction (*7*, *20*). Spectral changes agreed with the apparent increase of carotenoids in tomato juice after hot-break and screening as determined by HPLC (**Figure 4**). After canning, the band at ~960 cm^{-1} decreased, probably due to thermally induced degradation and isomerization reactions. HPLC analysis corroborated the reduction of carotenoids in the *trans* geometric form after canning (**Figure 4**). In most cases, the intensity of the band at ~960 cm^{-1} increased during storage, indicating the formation of C=C *trans* double bonds. These changes are probably caused by oxidation reactions and a certain degree of *cis*−*trans* isomerization.

Besides the general modifications previously mentioned, some specific trends were observed in juice from each tomato variety. In *all-trans*-lycopene-rich samples (HL, ML, NO) the unsaturated *trans* double bond is centered at 957 cm^{-1}, demonstrating the important contribution of this carotenoid to the spectra of lipid fractions (*15*). HPLC and infrared spectroscopy described similar trends for *all-trans*-lycopene during processing and storage of these varieties: (1) apparent increase of *trans*-lycopene after hot-break (attributed to a better extraction), (2) a clear decrease after canning (attributed to degradation and isomerization) ,and (3) stability during storage (**Figure 5**). SIMCA models of NO, ML, and HL revealed strong modification of bands at 1161, 1454, 1712, and 1743 cm^{-1} during storage. This suggests that, although *all-trans*-lycopene is relatively stable, other lipids suffered

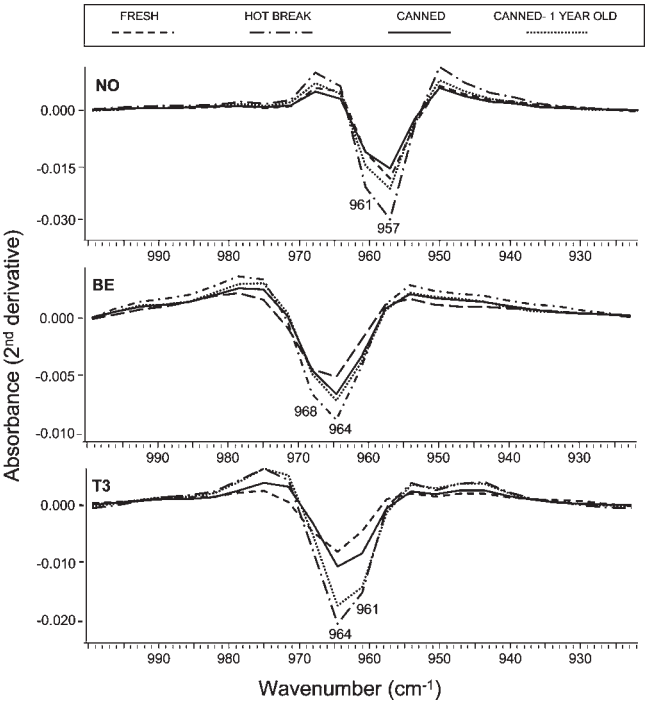

Figure 3. Infrared second-derivative spectra of selected tomato juice varieties at four different processing stages. Infrared region corresponds to C=C *trans* double bonds.

modifications that account for the spectral differences. Intensity changes, small shifts of the band at 957^{-1} toward 960 cm^{-1}, and changes in 1654 cm^{-1} (*cis* −C=C−) band indicate lipid isomerization during storage.

Similar trends were observed for *all-trans*-lycopene in all tomato varieties with an average reduction of 30% after canning (**Figure 4**) except for varieties with high contents of *tetra-cis*-lycopene (HC, T3, T9), which showed an average increase of 24% after caning (**Figure 5**). The conversion of *tetra-cis*-isomers to other isomeric forms during heat treatment, including *all-trans*-lycopene, could explain this difference.

HPLC chromatograms from tomato juices prepared with high *cis*-lycopene tomatoes (T3, T9, HC) showed a strong reduction of the main peak (minute ~37, 450 nm) after thermal treatment and during storage (**Figure 6**). This peak is composed by at least two carotenoids as demonstrated by the shoulder at the left side of the peak. On the basis of retention times and UV−vis absorbance, these compounds were tentatively identified as *tetra-cis*-lycopene

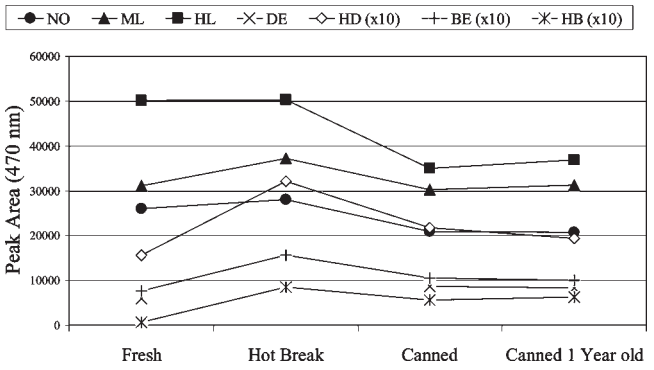

Figure 4. Evolution of *all-trans*-lycopene in tomato juice from selected varieties. All relative standard deviations were <10%, except fresh HB (18%) and canned HL (17%).

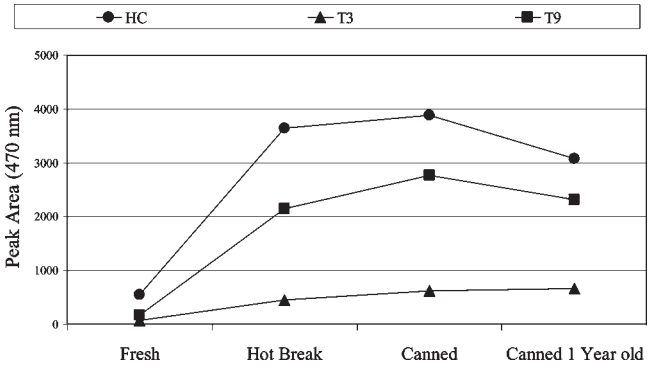

Figure 5. Evolution of *all-trans*-lycopene in tomato juice with high contents of *tetra-cis*-lycopene. All relative standard deviations were <10%, except hot-break T3 (30%) and 1-year-old canned T9 (30%).

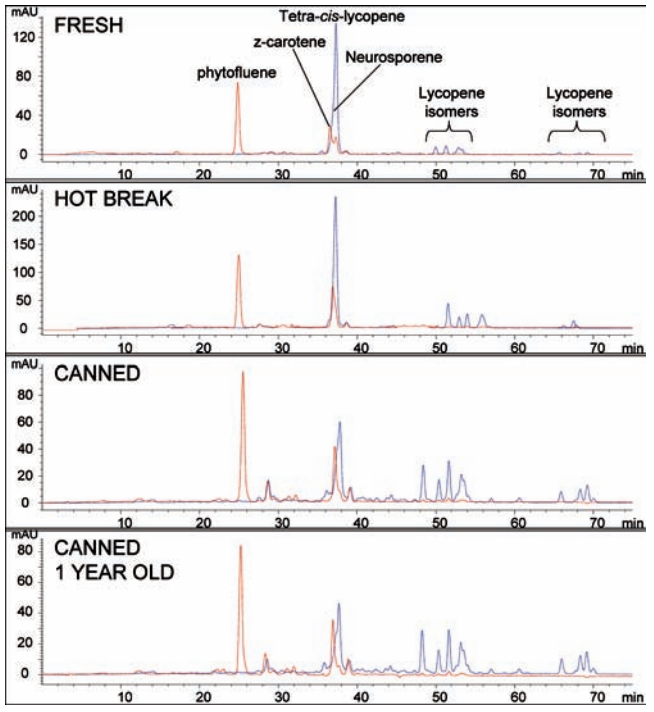

Figure 6. Evolution of HPLC chromatograms at 450 nm (blue) and 348 nm (red) of tomato variety T3 (tangerine) at different processing stages.

and neurosporene. Changes observed in the three tomato juices suggest strong reduction of *tetra-cis*-lycopene during canning and further decrease during storage. This decline can be explained in part by isomerization reactions, which are supported by the increase of peaks corresponding to other isomeric forms of lycopene. **Figure 6** shows chromatographic changes of the T3 tomato juice including the strong reduction of the *tetra-cis* peak. This observation agrees well with results from Unlu et al. (*21*) and Ishida et al. (*22*). Heat-induced degradation of *tetra-cis*-lycopene during canning may also contribute to peak reduction.

Table 2. HPLC Peak Area of 13-*cis*-β-Carotene Expressed as Percentage of the *all-trans*-β-Carotene Peak Area in the BE and HB Varieties[a]

	BE (%)	HB (%)
fresh	1.40	1.62
hot-break	3.65	3.57
canned	11.45	9.73
canned, 1-year-old	3.82	3.72

[a] Mean of three independent samples. Standard deviations <0.4%. Tomato varieties: BE, β-carotene; HB, high β-carotene.

Infrared spectroscopic analysis of the HC, T3, and T9 varieties provided clear evidence of isomerization by showing intensity and shifting in regions associated with *trans* (964 cm^{-1} (−C═C−), 3026 cm^{-1} (═C—H)) and *cis* (1654 cm^{-1} (−C═C−), 3006 cm^{-1} (═C—H)) double bonds. Variables with most discrimination in SIMCA models for tangerine tomato juices were also associated with changes in lipids (1743, 1215, 1160, 1442, 987 cm^{-1}), demonstrating the predominant role of these components in the evolution of the spectra.

Infrared absorption at 964 and 961 cm^{-1} was similar in fresh samples rich in β-caroetene (BE, HB), but showed a trend toward a more predominant 964 cm^{-1} band after canning and after 1 year of storage (**Figure 3**). These changes indicate variations in the quantity and proportion of single and conjugated *trans* double bonds in the lipid fraction. Shifting of the band at 3026 cm^{-1} (C−H *cis* bonds) also pointed toward isomerization during storage. **Table 2** shows the evolution of the HPLC peak corresponding to 13-*cis*-β-carotene (~30 min, 450 nm) as a percentage of the *all-trans*-β-carotene peak. Results suggest that the relative content of 13-*cis* as compared to the *all-trans* isomer increased due to thermal treatment and decreased with time of storage. Significant formation of β-carotene *cis* isomers has been reported during thermal processing of tomato products (*23*). Results obtained from HPLC also showed an important reduction of *all-trans*-β-carotene after canning (−43%, average reduction of BE, HB, NO, ML, HL, and HD varieties) and a slight incremental increase after 1 year of storage (~8%). The increase of *all-trans*-β-carotene during storage has also been reported in recent stability studies done with bottled tomato pulp (*24*). This trend has been attributed to the progressive release of β-carotene from carotene−protein complexes in the sample (*24*).

SIMCA models with lipid fraction spectra from tomato juice rich in δ-carotene (DE, HD) showed major discriminating bands around 1743 and 1220 cm^{-1}. These bands were the most important to differentiate fresh, hot-break, freshly canned, and 1-year-old canned tomato juice. Evidence of isomerization and chemical changes during storage was provided by the change of intensity and shifting of the bands at 964 and 1708 cm^{-1} (**Figure 7**). Reduction of *all-trans*-δ-carotene during canning accounts for 34% (HD variety), and no significant changes were observed during storage.

Tomato varieties with high content of *all-trans*-lycopene and *all-trans*-δ-carotene also contain higher levels of lutein (*15*) and therefore are useful for the evaluation of lutein changes during tomato juice production. A strong reduction of *trans*-lutein was observed after canning (67%, average of HD, ML, HL). Results

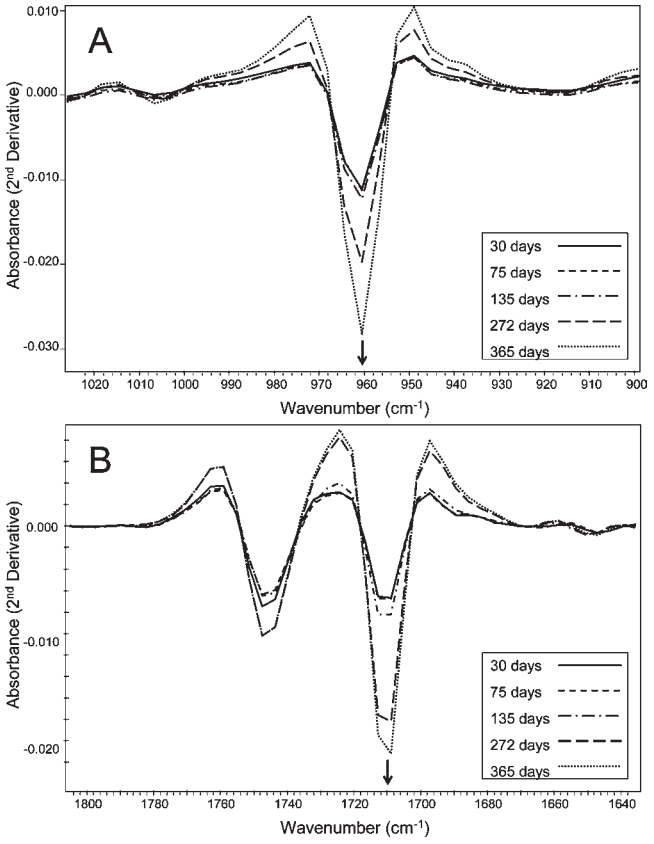

Figure 7. Evolution of bands at (**A**) 960 cm^{-1} and (**B**) 1708 cm^{-1} of the HD variety (high δ-carotene) during storage.

Table 3. PLSR Models Correlating Lipid Fraction Infrared Spectra with Time of Storage

tomato variety	IR region (cm^{-1})	factors	*r* value	SECV[a] (days)
BE	1400−1800	2	0.996	10.03
HB	1400−1800	10	0.994	13.09
HD	1400−1800	13	0.998	8.25
ML	1500−1800	9	0.997	8.90
T3	1500−1800	9	0.997	9.10
DE	800−1800	8	0.994	14.18
HC	1500−1800	11	0.997	9.64
HL	1400−1800	8	0.994	13.17
NO	1500−1800	9	0.996	10.64
T9	1500−1800	5	0.995	12.08

[a] Standard error of cross-validation.

agreed with the observations of Nguyen et al. (*23*), indicating that upon thermal treatment β-carotene and lutein isomerize to a greater extent than lycopene.

Using PLSR, the infrared spectra of lipid fractions collected at five time points were correlated with the number of days in storage (30, 75, 135, 272, and 365 days). Results showed that spectra from juice of all 10 tomato varieties correlated well with time, demonstrating progressive chemical changes in the lipid fraction of tomato juice. Bands with the highest loadings in first latent variables were associated with carbonyl groups (1712 cm^{-1}) and unsaturation (*trans* 960 cm^{-1}, *cis* 3006 cm^{-1}). **Figure 7** shows the progressive increase of absorbance in the ~960 and ~1712 cm^{-1} infrared regions.

The PLSR models were able to predict the time of storage with standard errors of cross-validation of ~11 days (depending on the variety) and coefficients of determination (*r* value) higher than 0.99 (**Table 3**). **Figure 8** shows a graph plotting real number of

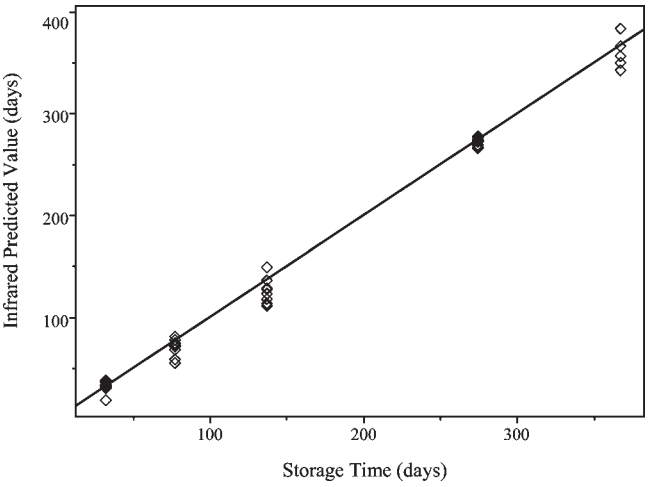

Figure 8. PLSR model correlating infrared spectra from HD variety (high δ-carotene) with storage time. Model based on the 1400−1800 cm^{-1} infrared region. Standard error of cross-validation, 8.25 days; *r* value = 0.998.

days versus predicted time of storage using the PLSR model for the HD variety. This observation may provide a useful tool for quality control purposes and for the identification of tomato products in storage. Spectra collected from tomato juice paste were also successfully correlated with time of storage, but larger standard errors of cross-validation were associated with these models (~20 days).

Results from this study depict a dynamic system with lipid isomerization and oxidation reactions taking place during production and storage of tomato juice. Data from 10 different tomato varieties suggest that individual carotenoids and their isomeric forms behave differently during thermal treatment and storage. In general, hot-break improved the extraction efficiency of carotenoids from tomato juice and caused some degree of isomerization, whereas canning reduced the content of *all-trans* carotenoids. *tetra-cis*-Lycopene, *trans*-lutein, and *trans*-β-carotene were strongly affected by heat treatment. During storage, there was evidence of progressive lipid oxidation and further isomerization. Excellent correlations between changes in infrared spectra and storage time were found for juice from all 10 tomato varieties. Infrared regions associated with carbonyl groups and *cis* and *trans* unsaturations consistently stood out as main factors explaining chemical changes in tomato juice lipids. Infrared spectroscopy provided a rapid and powerful technique for the analysis of bioactive lipids by providing evidence of specific chemical changes during the production and storage of tomato juice. By complementing the information obtained by infrared spectroscopy combined with chemometrics and HPLC, it was possible to characterize particular biochemical changes to assess the impact of thermal processing and storage on tomato juice stability.

LITERATURE CITED

(1) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55*, 207–216.

(2) Zechmeister, L.; LeRosen, A. L.; Went, F. W.; Pauling, L. Prolycopene, a naturally occuring stereoisomer of lycopene. *Proc. Natl. Acad. Sci. U.S.A.* **1941**, *27*, 468–474.

(3) 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.* **1992**, *122*, 2161–2166.

(4) Unlu, N. Z.; Bohn, T.; Francis, D. M.; Nagaraja, H. N.; Clinton, S. K.; Schwartz, S. J. Lycopene from heat-induced *cis*-isomer-rich tomato sauce is more bioavailable than from all-trans-rich tomato sauce in human subjects. *Br. J. Nutr.* **2007**, *98*, 140–146.

(5) Lambelet, P.; Richelle, M.; Bortlik, K.; Franceschi, F.; Giori, A. M. Improving the stability of lycopene Z-isomers in isomerised tomato extracts. *Food Chem*. **2009**, *112*,
(6) van het Hof, K. H.; de Boer, B. C. J.; Tijburg, L. B. M.; Lucius, B. R. H. M.; Zijp, I.; West, C. E.; Hautvast, J. G. A. J.; Weststrate, J. A. Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. *J. Nutr*. **2000**, *130*, 1189–1196.
(7) Lin, C. H.; Chen, B. H. Stability of carotenoids in tomato juice during processing. *Eur. Food Res. Technol*. **2005**, *221*, 274–280.
(8) Xianquan, S.; Shi, J.; Kakuda, Y.; Yueming, J. Stability of lycopene during food processing and storage. *J. Med. Food* **2005**, *8*, 413–422.
(9) Seybold, C.; Fröhlich, K.; Bitsch, R.; Otto, K.; Böhm, V. Changes in contents of carotenoids and vitamin e during tomato processing. *J. Agric. Food Chem*. **2004**, *52*, 7005–7010.
(10) Nguyen, M. L.; Schwartz, S. J. Lycopene stability during food processing. *Proc. Soc. Exp. Biol. Med*. **1998**, *218*, 101–105.
(11) Shi, J.; Le Maguer, M. Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit. Rev. Biotechnol*. **2000**, *20*, 293–334.
(12) Gärtner, C.; Stahl, W.; Sies, H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am. J. Clin. Nutr*. **1997**, *66*, 116–122.
(13) Halim, Y.; Schwartz, S. J.; Francis, D.; Baldauf, N. A.; Rodriguez-Saona, L. Direct determination of lycopene content in tomatoes (*Lycopersicon esculentum*) by attenuated total reflectance infrared spectroscopy and multivariate analysis. *J. AOAC Int*. **2006**, *89*, 1257(6).
(14) De Nardo, T.; Shiroma-Kian, C.; Halim, Y.; Francis, D.; Rodriguez-Saona, L. Rapid and simultaneous determination of lycopene and β-carotene contents in tomato juice by infrared spectroscopy. *J. Agric. Food Chem*. **2009**, *57*, 1105–1112.
(15) Rubio-Diaz, D. E.; De Nardo, T.; Santos, A.; de Jesus, S.; Francis, D.; Rodriguez-Saona, L. E. Profiling of nutritionally important carotenoids from genetically-diverse tomatoes by infrared spectroscopy. *Food Chem*. **2010**, *120*, 282–289.
(16) Mossoba, M. M.; Milosevic, V.; Milosevic, M.; Kramer, J. K. G.; Azizian, H. Determination of total trans fats and oils by infrared spectroscopy for regulatory compliance. *Anal. Bioanal. Chem*. **2007**, *389*, 87–92.
(17) Vogt, N. B.; Knutsen, H. SIMCA pattern recognition classification of five infauna taxonomic groups using non-polar compounds analysed by high resolution gas chromatography. *Mar. Ecol.: Prog. Ser*. **1985**, *26*, 145–156.
(18) De Maesschalck, R.; Candofi, A.; Masart, D. L.; Heuerding, S. Decision criteria for soft independent modeling of class analogy applied to near infrared data. *Chemom. Intell. Lab*. **1999**, *47*, 65–77.
(19) Kvalheim, O. M.; Karstang, T. V. SIMCA − classification by means of disjoint cross validated principal components models. In *Multivariate pattern Recognition in Chemometrics, Illustrated by Case Studies*; Brereton, R. G., Ed.; Elsevier: Amsterdam, The Netherlands, 1992; pp 237.
(20) Lessin, W. J.; Catigani, G. L.; Schwartz, S. J. Quantification of *cis-trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. *J. Agric. Food Chem*. **1997**, *45*, 3728–3732.
(21) Unlu, N. Z.; Bohn, T.; Francis, D.; Clinton, S. K.; Schwartz, S. J. Carotenoid absorption in humans consuming tomato sauces obtained from Tangerine or high-β-carotene varieties of tomatoes. *J. Agric. Food Chem*. **2007**, *55*, 1597–1603.
(22) Ishida, B. K.; Roberts, J. S.; Chapman, M. H.; Burri, B. J. Processing Tangerine tomatoes: effect on lycopene-isomer concentrations and profile. *J. Food Sci*. **2007**, *72*, C307–C312.
(23) Nguyen, M.; Francis, D.; Schwartz, S. Thermal isomerisation susceptibility of carotenoids in different tomato varieties. *J. Sci. Food Agric*. **2001**, *81*, 910–917.
(24) Ordonez-Santos, L. E.; Vazquez-Oderiz, L.; Arbones-Macineira, E.; Romero-Rodriguez, M. A. The influence of storage time on micronutrients in bottled tomato pulp. *Food Chem*. **2009**, *112*, 146–149.

Received for review April 5, 2010. Revised manuscript received June 16, 2010. Accepted June 23, 2010. We acknowledge the USDA National Research Initiative, Improving Food Quality and Value, for their financial support of this research.