

## Isomerization of Carotenoids During Processing of Tangerine Tomatoes

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### **Abstract:**

Epidemiological evidence, as well as *in vitro* and *in vivo* studies suggests that tomato-rich diets may be protective against different cancers, especially prostate cancer. Lycopene is the predominant carotenoid in tomatoes and Tangerine varieties are naturally high in *tetra-cis* lycopene, imparting an orange color. Human nutrition studies have demonstrated that *cis* isomers of lycopene are more bioavailable than the all-*trans* form. This has prompted interest in Tangerine tomatoes, as they are high in *cis*-lycopene when raw. The carotenoid profile of Tangerine tomatoes is quite different than red tomatoes. Little has been done to determine the effects of different degrees of thermal treatment and differing fat levels on the carotenoid profile of these tomatoes. The purpose of this study is to develop a Tangerine tomato juice, optimized for bioavailability and acceptability for use in a clinical trial. The second aim is to use Tangerine tomato sauce as a model system to investigate the effects of different degrees of thermal treatment and fat levels on carotenoid profiles and isomerization. An experimental hybrid of Tangerine tomatoes was hot break processed into juice and canned separately. Juice was then re-processed with varying levels of fat (0, 1, 2 and 3% w/w) for varying amounts of time (0, 30, 60 and 120min) at 88°C. Sauce was freeze dried to concentrate and re-processed with varying levels of fat (0, 1, 5, 15 and 30% w/w) for varying amounts of time (0, 30, 60, 120, 180 min) at 100°C. Samples in replicate were extracted for carotenoids. Phytoene, phytofluene, zeta-carotene, neurosporene, *tetra-cis* lycopene, all-*trans* lycopene and other *cis* lycopene were

quantified using HPLC-PDA. Total carotenoids decreased with longer heating times. Phytoene and phytofluene were relatively heat stable. *Tetra-cis* lycopene decreased significantly with processing time but not with fat level. All-*trans* lycopene and other-*cis* lycopene increased significantly with processing. These results show the capability to modulate carotenoid bioavailability by food processing.

### **Introduction:**

Epidemiological evidence suggests that diets rich in tomatoes and tomato products may be protective against risk for certain cancers, particularly prostate cancer (1). Many *in vitro*, *in vivo* and human clinical studies have suggested similar findings. The carotenoid lycopene has gotten the most attention as the compound in tomatoes responsible for this noted decrease in cancer risk. In raw, red tomatoes, lycopene is found predominantly in the all-*trans* form, imparting its characteristic red color. However, Tangerine tomatoes lack the enzyme carotenoid isomerase, which converts *poly-cis* lycopene into all-*trans*-lycopene (2). As a result, Tangerine tomatoes accumulate *cis*-lycopene, specifically *tetra-cis* lycopene (7Z, 9Z, 7'Z, 9'Z-lycopene, also called prolycopene, Figure 1) at the expense of the all-*trans* form. *Tetra-cis*-lycopene absorbs light approximately 30nm below all-*trans*-lycopene, resulting in tomatoes with an orange color. Tangerine tomatoes also contain considerable levels of phytoene, phytofluene,  $\zeta$ -carotene, neurosporene and other-*cis*-lycopene isomers. Additionally, it is hypothesized that other carotenoids upstream in the biosynthesis of tomato products, like phytoene and phytofluene may play a role in disease prevention (3).

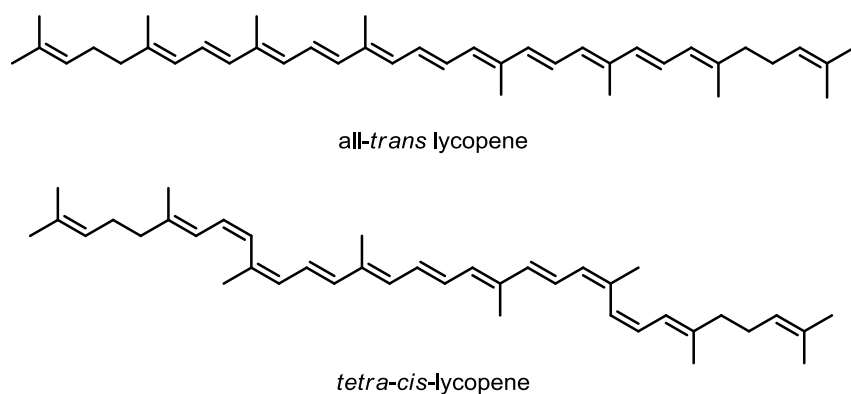


Figure 1. Structures of all-*trans*-lycopene and *tetra-cis*-lycopene.

Lycopene contains 11 conjugated (and 2 unconjugated) double bonds and can theoretically form 1056 geometrical (*cis/trans*) isomers. However, steric hindrance favors the formation of those isomers that exist in the lowest energy state, and the true number of geometrical isomers is likely significantly less (4). In raw, red tomatoes, approximately 95% of the lycopene present is in the all-*trans* form (5). In contrast, *cis*-isomers account for 58-73% of total lycopene in serum, and a surprisingly high 79-88% of total lycopene in benign or malignant prostate tissue (6). It is generally accepted that all-*trans*-lycopene is converted to *cis* isomers *in vivo*, and/or that *cis* isomers are more bioavailable and thus preferentially absorbed compared to all-*trans* (7). The *cis* isomers tend to be more polar, less likely to crystallize and more oil/hydrocarbon soluble compared to the all-*trans* forms (8) while being preferentially micellarized (9) and taken up by intestinal cells (10). This suggests biological relevance of *cis*-lycopene isomers.

The effects of thermal processing and fat concentration on carotenoid profiles in red tomatoes have been extensively studied. Lycopene from tomato products seems to be relatively stable to heat processing, especially in the absence of fat (11,12), although lycopene is more bioavailable from processed tomato products (13). Little research has been conducted to investigate the effects of different levels of thermal treatment and fat on a Tangerine tomato

product. It has, however, been shown that lycopene from a *cis*-isomer rich tomato sauce is more bioavailable than from an all-*trans* rich sauce in human subjects (14).

The aim of this study is to investigate the effects that thermal processing and fat levels (both separately and combined) have on isomerization and content of lycopene and lycopene precursors in a Tangerine tomato juice and sauce.

### **Methods/Materials:**

Tomato juice/sauce processing:

Tangerine tomato hybrid FG04-164 was grown and harvested at the The Ohio State University North Central Agricultural Research Station in Fremont, OH. Tomatoes were juiced at The Ohio State University Food Industries Center Pilot Plant in Columbus, OH and stored in #10 cans. Juice (pH 4.3, 5.1°Brix) was then further processed after come up to 88°C for 0, 30, 60 or 120 minutes with 0, 1, 2 or 3% fat in a 4x4 factorial design. Tangerine tomato sauce was produced by concentrating juice via lyophilization to 12.7°Brix. Sauce was then further processed to 100°C for 0, 30, 60, 120 or 180 minutes with 0, 1, 5, 15 or 30% fat in a 5x5 factorial design.

Pure lycopene was isolated and crystallized from tomato paste and was >95% spectrally pure. Acetone, hexane, methanol and methyl tert-butyl ether (MTBE) were purchased from Fisher Scientific (Pittsburgh, PA). Juice and sauce samples were extracted for carotenoids in duplicate using a method modified from Ferruzzi et al (15). Briefly, approximately 1.5g of tomato sample was placed in a 11mL test tube with 5mL MeOH. Samples were then probe sonicated for 8 seconds and centrifuged for 5 minutes. Supernatant was decanted and saved, and 5mL of 1:1 hexane:acetone was added, probe sonicated for 8seconds and centrifuged for 5

minutes. The hexane:acetone extraction was repeated two more times, or until the pellet was white. The pooled hexane extracts were dried under nitrogen gas and stored at -20°C until further analysis.

Tomato extracts were analyzed using high performance liquid chromatography with a photodiode array detector (HPLC-PDA). A YMC C30 “carotenoid column” (YMC Inc., Wilmington, NC) (4.6x250mm, 3µm pore size) was used to separate carotenoids. Extracts were brought up in 300µL of 1:1 MTBE:MeOH and 10µL injected. A gradient was employed using two solvents, A: 88% MeOH, 5% MTBE, 5% H<sub>2</sub>O, 2% of a 2% solution of aqueous ammonium acetate and B: 78% MTBE, 20% MeOH and 2% of a 2% solution of aqueous ammonium acetate. The flow rate was 1.3mL/min with t=0 min, 45% solvent B, t=20 min, 50% B, t=30 min, 100% B, t=33 min, 100% B, t=33.01 min, 45% B, t=38 min, 45% B. Column was at 30°C and sample at 25°C, monitored from 210-700nm. Phytoene, phytofluene, zeta-carotene, neurosporene, *tetra-cis*-lycopene, *all-trans*-lycopene and other-*cis*-lycopene were quantified at 286, 348, 400, 440, 440, 471 and 471nm respectively. *All-trans*-lycopene was quantified using authentic standard while other carotenoids were quantified using a ratio of molar extinction coefficient relative to *all-trans*-lycopene. Other-*cis*-lycopene were quantified using the molar extinction coefficient of *all-trans*. Multivariate analysis was then performed (SPSS, v. 19.0) using heat and fat as independent factors versus carotenoid content using Tukey’s post-hoc test (p<0.05).

### **Results and Discussion:**

Production of Tangerine tomato sauce concentrated carotenoids linearly in comparison to soluble solids (measured using °Brix). Tangerine tomato sauce is concentrated ~2.25x juice. Carotenoid content of the Tangerine juice and sauce, as well as comparison to a typical red

tomato is listed in Table 1. *Tetra-cis*-lycopene and all-*trans*-lycopene makes up about 60% and 34% respectively of the total lycopene in Tangerine tomato juice and sauce. Phytoene and zeta-carotene (upstream of lycopene in carotenoid biosynthesis in the tomato plant), which are present in much lower concentrations in red tomatoes, are primary carotenoids in the Tangerine tomato. Tangerine tomatoes only contain about 30% of the total lycopene found in red tomatoes, but overall have almost 300% of the total carotenoid found in red tomatoes, because of the high concentration of lycopene precursors.

<b>Carotenoid</b>	<b>Tangerine tomato juice average content (mg/100g wet weight)</b>	<b>Tangerine tomato sauce average content (mg/100g wet weight)</b>	<b>Typical red tomato juice average content (mg/100g wet weight)</b>
<b>Phytoene</b>	7.61	18.35	0.17
<b>Phytofluene</b>	2.55	6.13	0.23
<b>Zeta-carotene</b>	5.77	13.63	ND
<b>Neurosporene</b>	1.16	2.87	ND
<b><i>Tetra-cis</i>-lycopene</b>	1.34	2.77	ND
<b>Other-<i>cis</i>-LYC</b>	0.13	0.36	0.11
<b>All-<i>trans</i>-lycopene</b>	0.72	1.72	6.81
<b>Total lycopene</b>	2.19	4.85	6.92
<b>Beta-carotene</b>	ND	ND	0.17
<b>Lutein</b>	ND	ND	ND*
<b>Total carotenoid</b>	20.62	45.83	7.49

Table 1. Carotenoid content of Tangerine tomato juice, Tangerine tomato sauce and typical red tomato juice. \*Some red tomatoes contain appreciable lutein.

A representative chromatogram of Tangerine tomato sauce is shown in Figure 2. Four isomers of phytoene, two of phytofluene, five of zeta-carotene, three of neurosporene and eight other-*cis*-lycopene isomers were identified and quantified. Chromatograms for Tangerine tomato juice have similar separations with lower carotenoid concentrations.

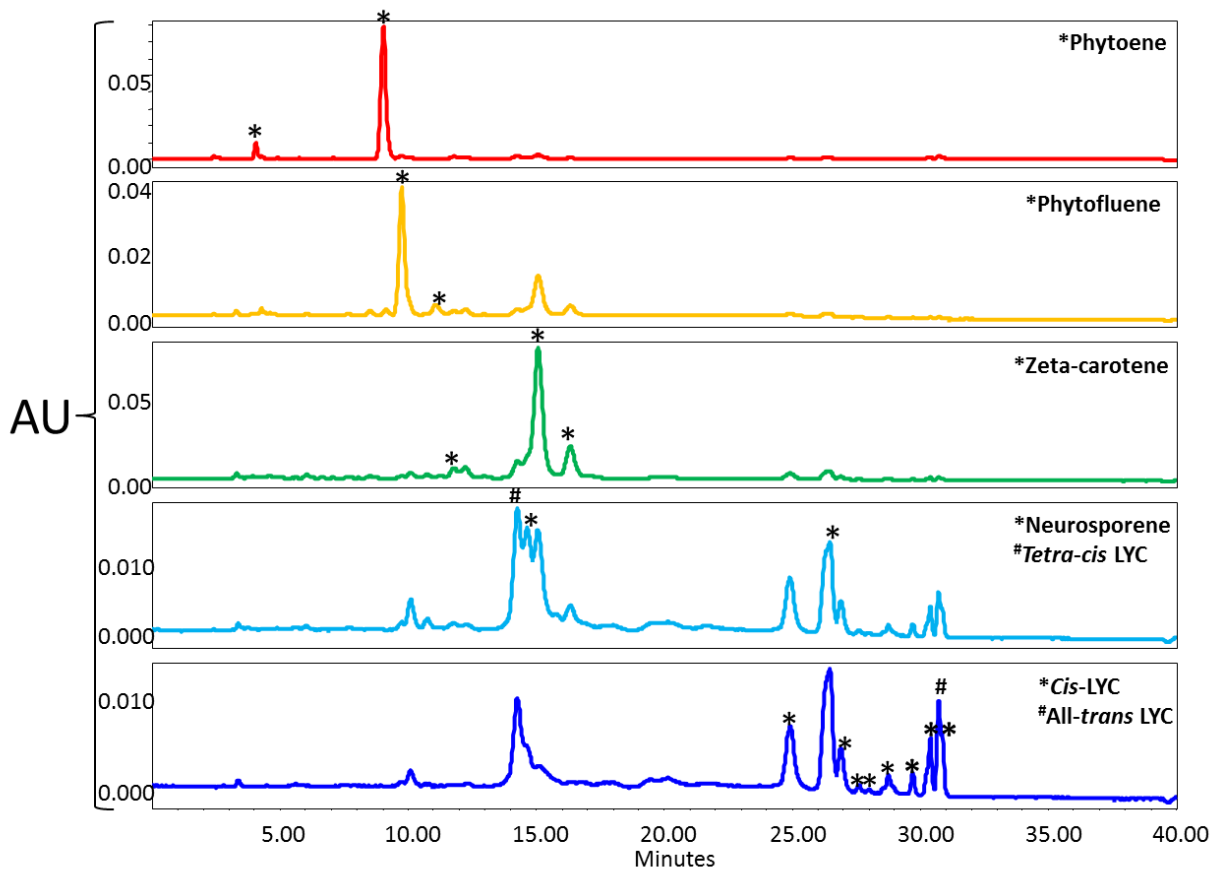


Figure 2. A representative chromatogram of Tangerine tomato sauce. Chromatograms for phytoene (red), phytofluene (orange), zeta-carotene (green), neurosporene (light blue), *tetra-cis*-lycopene (light blue), other-*cis*-lycopene (royal blue) and all-*trans*-lycopene (royal blue) are extracted at 286, 348, 400, 440, 440, 471 and 471nm respectively.

*Tetra-cis*-lycopene significantly decreased in Tangerine tomato sauce with each additional step of heat treatment (Figure 3). By 180 minutes of heating, *tetra-cis*-lycopene has decreased ~80% compared to sauce heated for 0 minutes. With increased heating time, all-*trans*-lycopene and other-*cis*-lycopene significantly increases while total lycopene decreases. This suggests that *tetra-cis*-lycopene is isomerizing to other-*cis* forms, as well as to all-*trans*. The magnitude of this conversion is unknown since total lycopene goes down. No significant differences were found in carotenoid content between fat levels processed for the same amount of time.

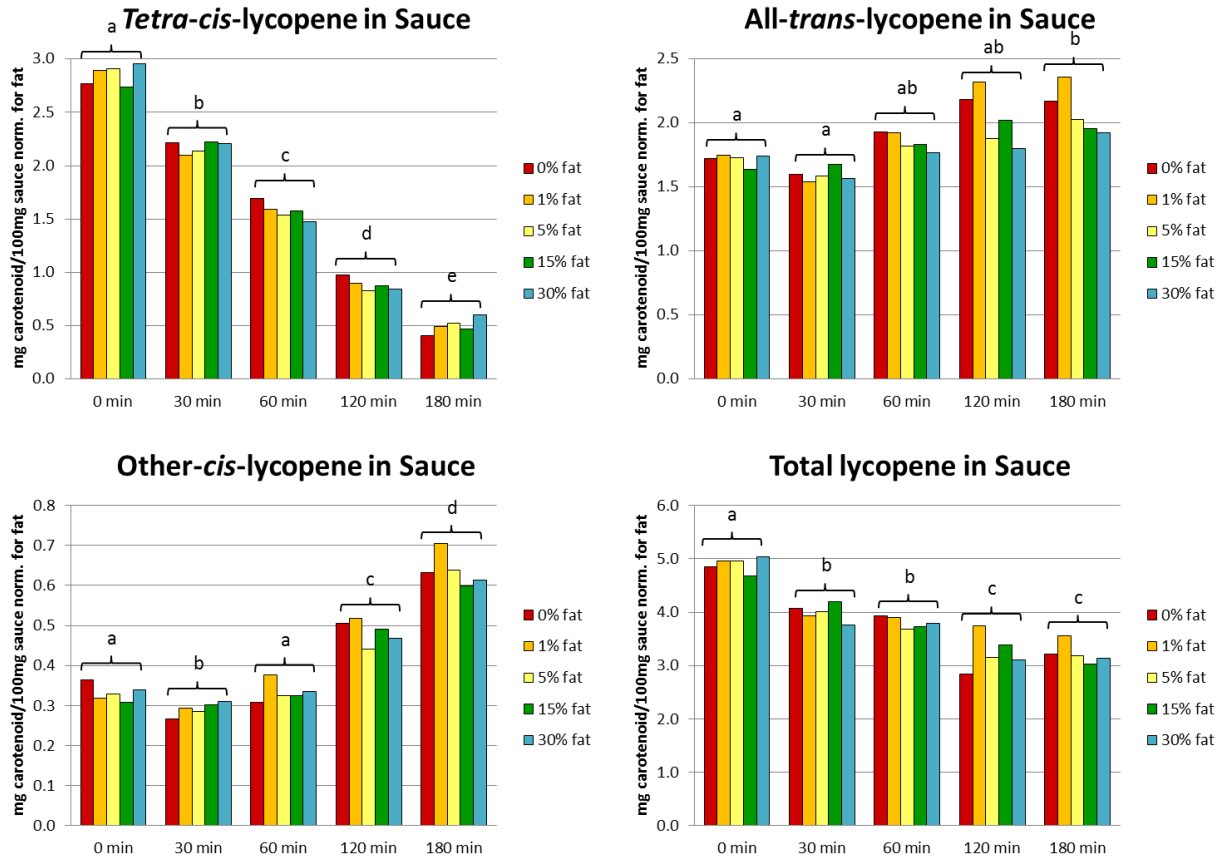


Figure 3. Lycopene content of Tangerine tomato sauce processed at 100°C for 0, 30, 60, 120 or 180 minutes with 0, 1, 5, 15 or 30% fat. Different letters denote statistically significant differences between heat treatments using Tukey’s post-hoc test ( $p < 0.05$ ). No significant differences exist between fat levels.

Phytoene and phytofluene did not significantly differ from initial after 180 minutes of boiling (Figure 4). Zeta-carotene and neurosporene also significantly decreased after 180 minutes of heating, but the magnitude of this decrease is much less compared to the decreased noted with *tetra-cis*-lycopene (Figure 4).



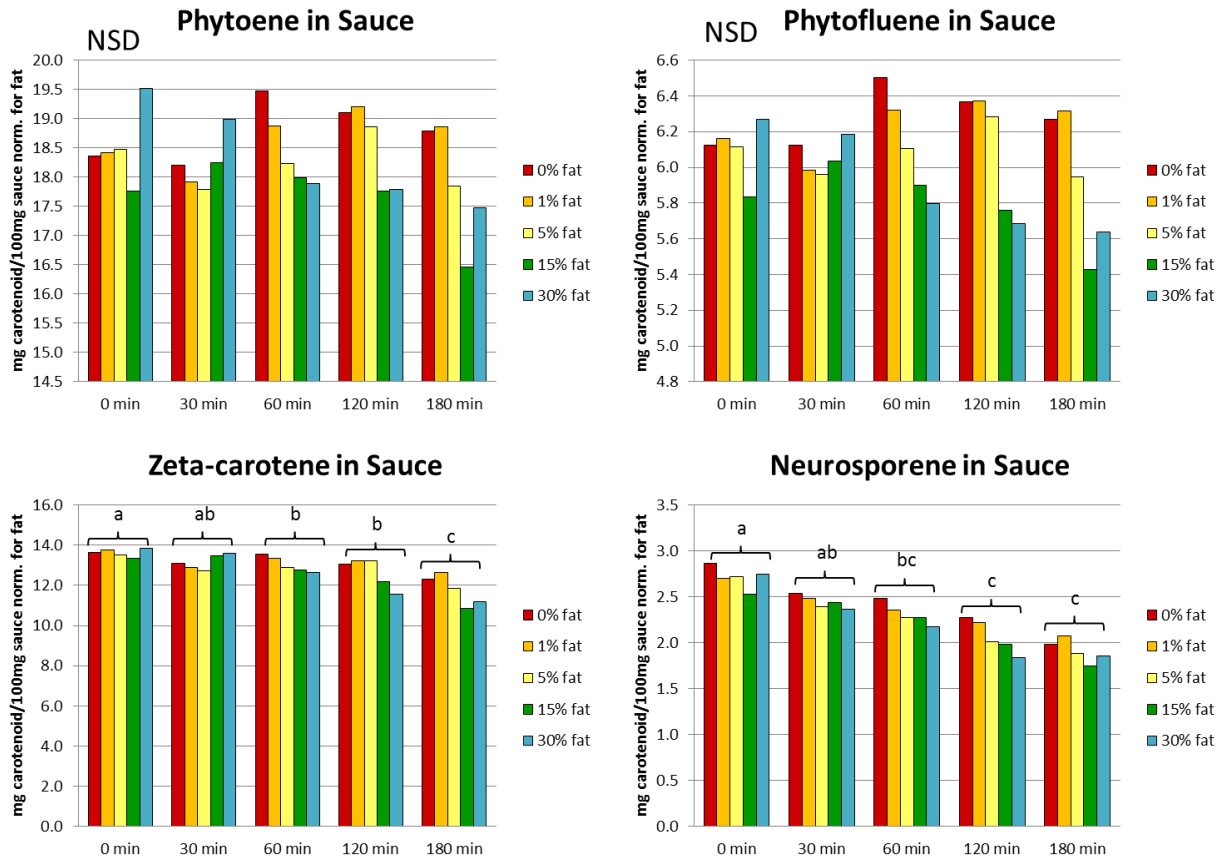


Figure 4. Lycopene precursor content of tomato sauce processed at 100°C for 0, 30, 60, 120 or 180 minutes with 0, 1, 5, 15 or 30% fat. Different letters denote statistically significant differences between heat treatments using Tukey's post-hoc test ( $p < 0.05$ ). No significant differences exist between fat levels.

## Conclusions:

*Tetra-cis*-lycopene in Tangerine tomatoes seems to be relatively liable to heat. If the goal is to administer *tetra-cis*-lycopene for utilization by the body, extreme heat processing should be avoided. *All-trans*-lycopene and other *cis*-lycopene content increases, while total lycopene, zeta-carotene and neurosporene content decreases with longer processing times. Overall, carotenoids tended to be more affected by thermal processing compared to fat. This research additionally suggests that *tetra-cis*-lycopene is not a thermodynamically stable lycopene isomer and tends to isomerize to more stable forms.

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