Processing Effects on Lycopene Content and Antioxidant Activity of Tomatoes

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Consumption of tomato products has been associated with decreased risk of some cancer types, and the tomato antioxidant, lycopene, is thought to play an important role in the observed health effects. In this study, four carotenoids, trans-lycopene, phytofluene, phytoene, and ζ-carotene, were quantified in tomato products. Samples of raw tomatoes, tomato juice after hot break scalder, and final paste were obtained from two different processing plants over two years. Comparison of carotenoid levels throughout processing indicated that lycopene losses during processing of tomatoes into final paste (25–30 °Brix) ranged from 9 to 28%. The initial Brix level of the raw tomatoes appeared to influence the amount of lycopene loss that occurred, possibly due to the differences in processing time required to achieve the final desired Brix level of the paste. In general, no consistent changes in the other carotenoids were observed as a function of processing. The antioxidant activity of fresh tomatoes, tomato paste, and three fractions obtained from these products (i.e., aqueous, methanol, and hexane fractions) was also determined. In both a free radical quenching assay and a singlet oxygen quenching assay, significant antioxidant activity was found in both the hexane fraction (containing lycopene) and the methanol fraction, which contained the phenolic antioxidants caffeic and chlorogenic acid. The results suggest that in addition to lycopene, polyphenols in tomatoes may also be important in conferring protective antioxidative effects.

Keywords: Carotenoids; phytofluene; phytoene; ζ-carotene; tomato paste

INTRODUCTION

Numerous epidemiology studies have shown an inverse association between lycopene intake or serum lycopene values and cancers of the prostate, pancreas, and possibly stomach (reviewed in refs 1 and 2). The major dietary sources of lycopene are fresh tomatoes and tomato products such as juice, paste, puree, and sauce (3). Other quantifiable carotenoids in these products include phytoene, phytofluene, ζ-carotene, neoxanthin, α-carotene, and β-carotene, but their concentrations are significantly lower than that of lycopene (3).

Giovannucci and co-workers have observed that consumption of processed tomato products, but not tomato juice, was associated epidemiologically with a decreased risk of prostate cancer (4, 5). Possible reasons for this observation are unclear. Stahl and Sies (6) have suggested that uptake of lycopene is greater from heat-processed juice than from unprocessed tomato juice, although a recent study by Rao and Agarwal (7) showed that lycopene from both tomato juice and tomato sauce was readily absorbed. Wang et al. (8) have observed that heat-processed tomato juice had a much higher antioxidant activity than fresh tomatoes; the reason for the increase in antioxidant activity as a function of processing was not evaluated.

In general, studies on lycopene content and antioxidant activity of tomatoes have not systematically followed changes throughout processing. For example, in many cases it is not known if observed changes are due to improved extraction during the processing or to the actual effects of the heat treatment (i.e., production or destruction of other antioxidants, etc.). In other cases, the varieties of the fresh tomatoes and the processed products were different or the processing conditions did not fully simulate commercial processing conditions. Therefore, the overall goal of this project was to evaluate the effect of processing on the lycopene content and antioxidant activity of tomatoes. Sampling and processing were conducted in commercial processing facilities over two growing seasons.

MATERIALS AND METHODS

Sampling and Processing Conditions. Tomato samples were obtained from two different processing plants during 1998 and 1999. In 1998, samples were taken from plant A on September 15 and from plant B on September 23. During the 1999 season, sampling was conducted on September 1 and 7 from plants A and B, respectively. Tomatoes in these samples represented a mixture of varieties harvested in the surrounding areas of northern California. The predominant variety was BOS 3155.
Three sampling points were monitored: (a) raw tomatoes from flume (~1500 g sample); (b) juice after hot break scalding (with seeds and skins) (~2000 g sample); and (c) final paste (~28 °Brix; 5000 g sample). Three separate samples were obtained from point a at 0.5 h intervals. Samplings from points b and c were subsequently delayed by an appropriate length of time (~0.25 and 3.0 h, respectively) with respect to point a to allow for transit through the processing line so that a sample from the same sampling “time” of tomatoes would be obtained for the sampling points. Following sampling, all products were stored on ice and transported to the laboratory. Fresh tomatoes were homogenized and extracted within 24 h. The fresh tomato extracts were stored at −80 °C until HPLC analysis, whereas all processed tomato products were stored at −20 °C until extracted or subjected to antioxidant analysis.

### Soluble Solids

Homogenized fresh tomatoes and hot break juice were filtered through Whatman No. 1 filter paper (90 mm; Whatman Inc., Florham Park, NJ) and concentrated to 10 mL on a rotary evaporator as reported previously (9). The soluble solids content was determined using a Bellingham and Stanley Ltd. (Kent, U.K.) model RMF81 automatic refractometer. To determine soluble solids of tomato paste, the paste was first centrifuged for 10 min at 120000 rpm (Beckman Optima TLX Ultra centrifuge; Beckman Coulter, Inc., Fullerton, CA). A Leica Mark II Abbe refractometer (Leica Microsystems Inc., Buffalo, NY) was used to measure the soluble solids of the resulting supernatant.

### Analysis of Lycopene

**Extraction and HPLC analysis of lycopene and related carotenoids** was based on the method of Tonucci et al. (9). All analyses were done in duplicate.

Tomatoes (~150 g), hot break juice (~150 g), or tomato paste (~50 g) was combined with Celite (10 wt %; Fisher Scientific, Fair Lawn, NJ), magnesium carbonate (10 wt %; Sigma, St. Louis, MO), and 10 mL of a 0.2 mg/mL α-carotene (Fluka Chemical Corp., Milwaukee, WI) internal standard solution (prepared in methylene chloride) in a beaker. The resulting mixture was then homogenized and centrifuged, as previously described, the hexane supernatant was stored at -80 °C until HPLC analysis. The aqueous layer was then frozen and stored until analysis.

#### Methodology

- **Extract preparation**: A 20 g sample of tomato fruit was ground with a PT3100 homogenizer (Brinkmann Instruments, Inc., Westbury, NY) and 100 mL of a mixture of acetonitrile/water (75:25 v/v) containing 0.025% BHT was added. The mixture was sonicated for 5 min with a sonicating probe. Oxidation was initiated by adding 100 g of a 2 mg/mL cupric acetate solution (prepared in methylene chloride) to the solution. The solution was then centrifuged at 3000 rpm for 15 min (1 h for paste) at 10 °C. Vials were topped with nitrogen gas prior to centrifugation to limit oxidation.

- **Detection**: The aqueous supernatant was used directly in the antioxidant assay. The remaining pulp and 100 mL of methanol were combined in the blender and diluted to 10 mL under nitrogen atmosphere. The mixture was then centrifuged at 8000 rpm for 10 min at 10 °C. Vials were topped with nitrogen gas prior to centrifugation to limit oxidation.

#### Antioxidant Activity

Three fractions (aqueous, methanol, and hexane fractions) were prepared from fresh tomatoes and tomato paste and used in the antioxidant assays. To prepare the fractions, tomatoes (100 g) were first blended for 2 min under a nitrogen atmosphere in a Waring blender to yield a homogenate. Tomato paste (50 g) or the fresh homogenate was then centrifuged at 3000 rpm for 15 min (1 h for paste) at 10 °C. Vials were topped with nitrogen gas prior to centrifugation to limit oxidation. The aqueous supernatant was used directly in the antioxidant assay. The remaining pulp and 100 mL of methanol were combined in the blender and diluted to 10 mL under nitrogen atmosphere.

### Lycopene Concentration

Lycopene concentration was determined using authentic lycopene standards. All other carotenoid concentrations were reported relative to the weight equivalent of the internal standard (IS).

#### Antioxidant Activity

Free radical quenching activity was measured in a phosphatidylcholine liposome solution similar to that described by Huang and Franken (16) and Huang et al. (11). Phosphatidylcholine (Sigma Chemical Co.) was dissolved in water in constant stirring for ~45 min to give a concentration of 8 mg/mL. The mixture was then sonicated for 5 min with a sonication probe (Sonic Dismembrator 60, Fisher Scientific) at half power to yield a liposome solution. An aliquot (100 µL) of a previously prepared tomato fraction was placed in a 20 mL glass screw-top vial, and 10 mL of the liposome solution was added. The vials were then sonicated for 5 min to disperse the tomato fraction into the solution. Oxidation was initiated by adding 10 µL of a 2 mg/mL cupric acetate solution (prepared in methanol). The samples were held in a water bath at 37 °C, and the reaction rate was monitored by following the formation of conjugated dienes at 234 nm (Milton Roy, Spectronic 60, Rochester, NY). Prior to spectral measurement 100 µL of sample was diluted with 5 mL of methanol. All samples were replicated a minimum of four times. The antioxidant activity of a 15 mM solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich) was also determined simultaneously as a positive control.

Singlet oxygen quenching activity was monitored in a linoleic acid emulsion using p-dimethylaminophenylendoperoxide (12, 13). Endoperoxides are readily synthesized from their parent compounds by photo-oxidation. In this case, 1,4-
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Effects of Processing on Carotenoid Concentrations. To compare processing effects, carotenoid levels of the fresh and hot break juices were corrected to the Brix levels (i.e., soluble solids) of the final paste (Table 2). No consistent changes in lycopene levels were observed as the fresh tomatoes were processed into hot break juice. However, statistically significant decreases in lycopene levels of ~9–28% occurred as the tomatoes were processed into paste (Table 2). The greatest loss (~28%) occurred at plant A in 1999 and may be related to the slightly lower Brix levels for the fresh tomatoes and hot break juice (4.9 °Brix) requiring a longer processing time to achieve the final Brix value of the paste. The smallest losses (<8.6%) occurred at plant A during the 1998 and were associated with the smallest fold change in Brix level during processing (4.6-fold increase in Brix from fresh/hot break juice to paste compared to >5-fold increase for the other samples). Losses at plant B were consistent between both years, ranging from 11 to 17% and averaging 14%. No consistent changes in the other carotenoids were observed and, in general, levels of these carotenoids were not affected by processing (Tables 1 and 2).

Kinetic studies in model systems (pure lycopene in safflower oil) have shown that lycopene is highly susceptible to oxidative and thermal degradation with a reaction rate approximately double that of other carotenoids studied (β-carotene and lutein) (18). However, Abushita et al. (19) observed no change in lycopene concentration as fresh tomatoes were processed into paste in a commercial processing facility. Nguyen and Schwartz (20) also suggested that in tomato products, lycopene was relatively resistant to degradation, including thermally induced cis–cis isomerization reactions.

Table 1. Mean Carotenoid Levels ± SD in Tomato Extracts (Milligrams per 100 g of Juice or Paste) Determined by HPLC

<table>
<thead>
<tr>
<th>sample</th>
<th>°Brix</th>
<th>recovery of IS (%)</th>
<th>trans-lycopene (cis + trans)</th>
<th>phytfluene (cis + trans)</th>
<th>phytoene (cis + trans)</th>
<th>ζ-carotene (cis + trans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plant A paste</td>
<td>25</td>
<td>94.17 ± 0.98</td>
<td>82.35 ± 3.54</td>
<td>4.14 ± 0.12</td>
<td>3.26 ± 0.13</td>
<td>1.02 ± 0.02</td>
</tr>
<tr>
<td>plant B paste</td>
<td>30</td>
<td>94.17 ± 2.64</td>
<td>82.90 ± 3.13</td>
<td>5.39 ± 0.16</td>
<td>4.48 ± 0.16</td>
<td>1.71 ± 0.18</td>
</tr>
<tr>
<td>plant A hot break</td>
<td>5.4</td>
<td>92.23 ± 4.63</td>
<td>19.46 ± 0.86</td>
<td>0.92 ± 0.06</td>
<td>0.66 ± 0.09</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>plant B hot break</td>
<td>5.5</td>
<td>88.83 ± 4.36</td>
<td>18.19 ± 1.83</td>
<td>1.18 ± 0.11</td>
<td>0.84 ± 0.15</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td>plant A fresh</td>
<td>5.4</td>
<td>75.83 ± 6.55</td>
<td>17.34 ± 5.07</td>
<td>1.34 ± 0.36</td>
<td>0.86 ± 0.42</td>
<td>0.27 ± 0.06</td>
</tr>
<tr>
<td>plant B fresh</td>
<td>5.5</td>
<td>91.17 ± 7.03</td>
<td>17.32 ± 1.53</td>
<td>1.26 ± 0.13</td>
<td>1.16 ± 0.06</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plant A paste</td>
<td>25</td>
<td>87.50 ± 4.64</td>
<td>86.85 ± 10.62</td>
<td>5.02 ± 0.23</td>
<td>3.64 ± 0.28</td>
<td>1.13 ± 0.1</td>
</tr>
<tr>
<td>plant B paste</td>
<td>30</td>
<td>86.00 ± 3.58</td>
<td>83.80 ± 3.88</td>
<td>5.06 ± 0.11</td>
<td>3.50 ± 0.17</td>
<td>1.28 ± 0.15</td>
</tr>
<tr>
<td>plant A hot break</td>
<td>4.9</td>
<td>82.33 ± 4.59</td>
<td>22.72 ± 1.12</td>
<td>1.32 ± 0.09</td>
<td>0.94 ± 0.12</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>plant B hot break</td>
<td>5.4</td>
<td>84.50 ± 4.28</td>
<td>16.87 ± 0.38</td>
<td>0.95 ± 0.07</td>
<td>0.68 ± 0.05</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>plant A fresh</td>
<td>4.9</td>
<td>85.00 ± 4.65</td>
<td>23.65 ± 2.22</td>
<td>0.88 ± 0.17</td>
<td>0.81 ± 0.14</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>plant B fresh</td>
<td>6.0</td>
<td>86.17 ± 3.97</td>
<td>20.14 ± 1.85</td>
<td>0.89 ± 0.19</td>
<td>0.77 ± 0.17</td>
<td>0.17 ± 0.03</td>
</tr>
</tbody>
</table>

* Lycopene and phytfluene were quantified with authentic standards; phytoene and ζ-carotene were quantified by weight equivalent of β-apo-8′-carotene (internal standard).

dimethyl-naphthalene (Sigma) was dissolved in hexane and oxidized to the endoperoxide in a photo chamber with a tungsten filament. Methylene blue (Sigma) was used as the "sensitizer", and compressed air (Puritan Bennet, Lenexa, KS) was bubbled through the chamber as a source of oxygen. The endoperoxide was purified on a silica column and crystallized under a stream of nitrogen gas (Puritan Bennet). All of this was performed at 4 °C to prevent thermolysis. The endoperoxide (1 mM) was added to a 60 mM solution of linoleic acid in hexane/ethanol (1:1 v/v), and 10 mL of this solution was placed in a 20 mL vial. Aliquots (100 µL) of the tomato fractions were added, and oxidation was initiated by placing the vial in a 37 °C water bath. Formation of conjugated dienes was monitored at 234 nm. All assays were replicated a minimum of four times.

Results for all antioxidant assays were expressed as percent inhibition of conjugated diene formation as compared to a control solution that did not contain any antioxidants (14). Percent inhibition was determined 35 h following initiation of oxidation for the free radical antioxidant assays and 5 h postinitiation for the singlet oxygen assays.

Ascorbic Acid Analysis. Tomato samples were sent frozen, on dry ice, to The National Food Laboratory, Inc., Dublin, CA, for HPLC analysis of ascorbic acid (15).

Statistical Analyses. Means and standard deviations for replicate analyses were calculated for all samples. Sample means were compared using Student’s t-test (16) where appropriate.

RESULTS AND DISCUSSION

Analysis of Carotenoids. Four carotenoids in fresh and processed tomatoes were quantified: trans-lycopene, ζ-carotene (cis + trans), phytfluene (cis + trans), and phytoene (cis + trans). Overall recovery of the internal standard (β-apo-8′-carotene) was excellent, ranging from 82 to 95% for hot break juice and paste (Table 1). Internal standard recovery for the fresh tomatoes was slightly lower (76–91%) and more variable for replicate analyses, particularly during the 1998 season. The reason for this lower recovery and higher variability is unknown but may be due to unfamiliarity with the analysis procedure for the initial fresh tomato analyses during the 1998 season and the less homogeneous nature of the fresh tomato matrix (compared to hot break juice and paste). The precision (% CV) for replicate analyses was generally <6% for all samples (except fresh tomatoes during 1998).

Concentrations of the four carotenoids in fresh tomatoes, hot break juice, and final paste at the two processing plants during 1998 and 1999 are given in Table 1. As expected, concentrations of all carotenoids were higher in paste than in fresh tomatoes and hot break juice. In a review of carotenoid content of foods, Mangels et al. (17) calculated average lycopene values of 31000 and 85000 µg/100 g for fresh tomatoes and paste, respectively. Measured lycopene values in fresh tomatoes in this study were slightly lower than the average value reported by Mangels et al.; however, concentrations were consistent between processing plants and between the two years. Lycopene concentrations in the paste were in agreement with average values reported by Mangels et al. and were consistent among all samples.
In our study, significant losses in lycopene were observed, and greater losses appeared to be associated with conditions requiring more extensive processing. However, overall losses were <30% and were small compared to those observed for pure lycopene in model systems under similar conditions.

Reasons for the apparent stability of lycopene in tomato products are unclear. Abushita et al. (19) observed that ascorbic acid, tocopherols, and β-carotene levels decreased as a function of thermal processing. Similarly, we observed a decrease in ascorbic acid content from 10 to 12 mg/100 g in the juice to 2 mg/100 g in the paste. Whether these antioxidants or other components that are present in tomatoes play a role in preventing the degradation of lycopene is unclear. Further studies are needed to fully understand the interrelated effects of heat treatments on the stability of the carotenoids and other antioxidants.

**Antioxidant Activity.** The conjugated double-bond system of lycopene confers strong antioxidant activity including the ability to quench singlet oxygen and peroxyl radicals. The singlet oxygen quenching activity of lycopene has been shown to be greater than that for other carotenoids, including β-carotene (2; reviewed in ref 21). In vivo, lycopene consumption has been associated with decreased levels of serum lipid peroxidation and low-density lipid (LDL) peroxidation (7, 22).

We evaluated both the free radical and singlet oxygen quenching activities of the tomato products. Overall reproducibility of the free radical quenching assay was excellent, with a coefficient of variation for replicate analyses of a control sample being <10% over six different days of analysis (n = 24). However, when juice or paste was used directly in the assays, large variabilities were observed, particularly for the paste. This was thought to be due to the presence of particulate matter in the samples. To eliminate the variability associated with particulates in the samples, the tomatoes were centrifuged to yield an aqueous supernatant and then extracted successively with methanol and hexane. The antioxidant activity of the three fractions was then evaluated.

Antioxidant activity was observed in each of the three fractions, and tomato paste had a greater antioxidant activity in all fractions than fresh tomatoes (Table 3). In comparison, 15 mM Trolox showed a free radical quenching activity of 52% compared to the control; all fractions except the methanol fraction from paste exhibited activities lower than that of 15 mM Trolox in this assay.

In fresh tomatoes, the lycopene-containing hexane fraction had the greatest activity. The activity of the paste was greater than that of the fresh juice; however, when the activity of the fresh juice was corrected to a similar solids basis as the paste (30 °Brix) the observed activity was lower than expected, indicating loss in activity as a result of processing. This is consistent with decreases in lycopene concentration discussed previously.

In paste, the methanol fraction contained the greatest activity. When the activity of the fresh juice was corrected to a similar solids basis as the paste (30 °Brix), the activity of the paste was greater than expected as a result of the processing treatments. Similar increases in antioxidant activity were observed by Wang et al. (8) in heat-processed tomato juice and grape juice compared with fresh products. Reasons for the increase in activity in these studies are unknown, but it may be at least partially explained by the production of new antioxidants during processing. For example, a recent study by Stewart et al. (23) indicated that the free, nonconjugated forms of two polyphenols, quercetin and kaempferol, increased during thermal processing of tomatoes. Whether the free forms of polyphenols have greater antioxidant activity than the conjugated forms is not clear. Lavello et al. (24) also observed a slightly higher concentration of total phenols in commercial tomato paste compared with fresh tomatoes. An HPLC analysis of the methanol phase used in our study identified two polyphenols as major constituents, caffeic and chlorogenic acids, consistent with literature reports (reviewed in ref 25). However, the effect of processing on changes in these constituents was not evaluated. The results of this and other studies point to the critical need for future studies that will fully evaluate changes in

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**Table 2. Mean Carotenoid Levels ± SD in Tomato Extracts (Milligrams per 100 g as Paste) Corrected to °Brix Level of Final Paste**

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh tomatoes</td>
<td>hot break juice</td>
</tr>
<tr>
<td>trans-lycopene</td>
<td>80.27 ± 23.49**</td>
<td>90.11 ± 3.98**</td>
</tr>
<tr>
<td>(2.6%)</td>
<td>(8.6%)</td>
<td>(8.6%)</td>
</tr>
<tr>
<td>phytofluene (cis + trans)</td>
<td>6.20 ± 1.67</td>
<td>4.27 ± 0.29</td>
</tr>
<tr>
<td>phytoene (cis + trans)</td>
<td>3.97 ± 1.9</td>
<td>3.04 ± 0.40</td>
</tr>
<tr>
<td>ζ-carotene (cis + trans)</td>
<td>1.24 ± 0.28</td>
<td>0.84 ± 0.02</td>
</tr>
</tbody>
</table>

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**Table 3. Percent Inhibition of Lipid Peroxidation As Measured by Conjugated Diene Formation for Different Fractions Obtained from Tomatoes**

<table>
<thead>
<tr>
<th>% inhibition compared to control (n = 4)</th>
<th>aqueous</th>
<th>methanol</th>
<th>hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>free radical antioxidant activity</td>
<td>10.9</td>
<td>5.2</td>
<td>18.2</td>
</tr>
<tr>
<td>fresh</td>
<td>25.2</td>
<td>64.8</td>
<td>28.4</td>
</tr>
<tr>
<td>paste</td>
<td>not measured</td>
<td>9.3</td>
<td>4.7</td>
</tr>
</tbody>
</table>

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The **Table 2. Mean Carotenoid Levels ± SD in Tomato Extracts (Milligrams per 100 g as Paste) Corrected to °Brix Level of Final Paste** shows the mean carotenoid levels ± standard deviation (SD) in tomato extracts (milligrams per 100 g as paste) corrected to °Brix level of final paste. The table includes fresh tomatoes, hot break juice, and paste samples, with data for the years 1998 and 1999. The **Table 3. Percent Inhibition of Lipid Peroxidation As Measured by Conjugated Diene Formation for Different Fractions Obtained from Tomatoes** shows the percentage inhibition compared to control for different fractions obtained from tomatoes, including aqueous, methanol, and hexane.
levels of all polyphenol classes as well as changes in the antioxidant activity of these compounds as a function of processing treatments in tomatoes.

Summary. Thermal processing of tomatoes into pastes can result in decreases in lycopene concentration of 9–28%. Longer processing times, required to achieve the desired final solids levels, may be associated with increased losses. In general, however, lycopene in tomatoes is relatively resistant to degradation compared to pure lycopene in model systems. Other constituents, including tocopherols, ascorbic acid, and phenolic antioxidants, may help to stabilize lycopene during processing; however, further studies are needed to evaluate these effects. Changes in the antioxidant activity of tomato products are complex and depend on the specific compounds being studied. Initial results suggest that losses in antioxidant activity associated with decreases in lycopene concentration during processing may be accompanied by increases in antioxidant activity of other components, particularly polyphenolics. Therefore, further studies characterizing changes in polyphenol content and antioxidant activity during thermal processing will be critical to fully understand the role that fresh and processed foods in the diet may play in preventing human disease.

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LITERATURE CITED


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