

## Ultratraces of carotenes in tomato purées: HPLC-TLS study

S. Luterotti<sup>a)</sup>

*Faculty of Pharmacy and Biochemistry, University of Zagreb, HR-10000 Zagreb, Ante Kovačića 1, Croatia*

K. Marković

*Faculty of Food Technology and Biotechnology, University of Zagreb, HR-10000 Zagreb, Pierottijeva 6, Croatia*

M. Franko

*Laboratory of Environmental Research, Nova Gorica Polytechnic, P.O. Box 301, SI-5001 Nova Gorica, Slovenia*

D. Bicanic

*Laser Photoacoustic Laboratory, Biophysics Division, Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands*

N. Vahčić

*Faculty of Food Technology and Biotechnology, University of Zagreb, HR-10000 Zagreb, Pierottijeva 6, Croatia*

O. Doka

*Department of Physics, University of West Hungary, Deak Ter, Mosonmagyaróvár, Hungary*

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The present study was designed to provide information about (i) the profile of carotene pigments and (ii) trace quantities of lycopene and  $\beta$ -carotene left in tomato purées. The ultrasensitive method comprising HPLC and thermal lens spectrometric (TLS) detection enabled us to detect as low as 0.3 and 1.1 ng ml<sup>-1</sup> lycopene and  $\beta$ -carotene in purée extracts, respectively. Total concentration of  $\beta$ -carotene and lycopene (varying from 3 to 170 ng g<sup>-1</sup>) in the examined tomato purées may serve as an indicator of the carotene-specific antioxidative capacity of these products. Although conventional spectrophotometry can be used to rapidly assess the quality of products derived from tomatoes, a highly sensitive and selective method such as HPLC-TLS is needed for reliable analyses of samples such as, for example, those subjected to inappropriate storage and/or handling. © 2003 American Institute of Physics. [DOI: 10.1063/1.1512773]

### I. INTRODUCTION

Production of hydroxy radicals and peroxy compounds is a result of abnormal oxidative processes involved in the initiation and development of both cardiovascular diseases and the cancers. Health benefits of tomato and tomato-derived products have been suggested to relate with the antioxidant ability of carotenoids, the most abundant phytonutrients in tomato: lycopene is the most prominent carotene followed by phytoene, phytofluene,  $\beta$ -,  $\zeta$ -,  $\gamma$ -, and  $\alpha$ -carotene, as well as several minor carotenoids.<sup>1-3</sup> Recent epidemiological studies show that levels of lycopene and risk of coronary heart disease are inversely related.<sup>4</sup> Experimental studies have also implicated the protective role of lycopene during carcinogenesis.<sup>5</sup> Lycopene can act as a potent antioxidant in reacting with highly reactive oxygen species produced during normal aerobic cellular metabolism.<sup>6</sup> Recently, the authors postulated that lycopene could play an important role in recovery and integrity of biological membranes of the liver after radiation injury.<sup>7</sup> Singlet oxygen is quenched by lycopene at a rate almost twice as high as that of  $\beta$ -carotene.<sup>8,9</sup>

The quantity of lycopene in raw tomatoes has been reported to depend upon the ripeness of the fruit at the time of harvesting.<sup>10</sup> During processing, the thermally induced rupture of cell walls and the release of lycopene contributes to the increased lycopene content in processed tomato products.<sup>11</sup>

Lycopene in fresh tomato fruits occurs essentially in the all-*trans* form.<sup>12</sup> The main causes of tomato lycopene degradation during processing are isomerization and oxidation. Heat induces isomerization of *trans* to *cis* isomers, the concentration of *cis* isomers increasing with temperature and processing time.<sup>12</sup> However, according to Nguyen and Schwartz,<sup>13</sup> it follows that unlike  $\beta$ -carotene, lycopene remained relatively resistant to a heat-induced geometrical isomerization during typical food processing of tomatoes and related products. However, even in the absence of light and in the presence of antioxidants lycopene readily isomerized in organic solvents.<sup>13</sup> Although more information on the bioavailability of lycopene is still needed, it seems that its absorption from processed tomato products exceeds that from unprocessed fresh tomatoes.<sup>12</sup> This may partly be due to the fact that the bioavailability of *cis* isomers in food is higher than that of *trans* isomers; therefore evaluating the extent of

<sup>a)</sup>Electronic mail: sluter@pharma.hr

lycopene isomerization during the processing is a necessary task.

High biological activity of lycopene and  $\beta$ -carotene prompted us to monitor their concentration in tomato purées stored under different temperatures and for varying time. It is for the first time that the HPLC hyphenated with thermal lens spectrometric (TLS) detection has been demonstrated as a useful tool to analyze minute quantities of lycopene and  $\beta$ -carotene in tomato products. In the past, this ultrasensitive technique proved suitable for assaying  $\beta$ -carotene in various samples.<sup>14–17</sup>

## II. EXPERIMENT

### A. Samples and standards

Tomato purées prepared from several tomato cultivars were stored under different temperature conditions for varying lengths of time. Afterwards, purées were extracted according to Hakala and Heinonen<sup>18</sup> using petroleum ether and solid-phase extraction procedures. Dry extracts were then dissolved in THF/MeOH (10/90) and their absorbance measured by the spectrophotometry prior to their direct analysis by the HPLC-TLS or after appropriate dilution (2, 5, or 10 times) with the mobile phase.

The lycopene standard was kindly donated by the Department of Human Nutrition (Wageningen University and Research Center, The Netherlands), whereas Betatene® 20% Soy and Lyc-O-Mato™ 6% were the gifts of Cognis Deutschland GmbH, Nutrition and Health (Düsseldorf, Germany) and LycoRed Natural Products Industries Ltd. (Beer-Sheva, Israel), respectively.

$\beta$ -carotene and lycopene were identified and quantified (in peak area mode) versus standards. Standards and sample solutions were protected from light and stored at  $-20^{\circ}\text{C}$  prior to use.

### B. Equipment

The HPLC-TLS setup constructed in the laboratory<sup>17</sup> included an intensity-modulated Ar-ion laser used as the excitation source (476.5 nm, 60 mW) and a helium–neon laser (632.8 nm, 20 mW) acting as a probe beam source. The modulation frequency of the pump beam (40 Hz) was achieved by means of a mechanical chopper. The induced probe beam intensity fluctuations were detected by a photodiode connected to the lock-in amplifier (time constant 1 s). The chromatographic conditions were as follows: column Vydac 218TP54 C<sub>18</sub> (4.6 × 250 mm, 5  $\mu\text{m}$  particle size, 30 nm pore diameter, Separations Group, USA), isocratic LC pump 250 (Perkin Elmer, USA), the mobile phase 5% THF in methanol, eluent flow rate 1.0 ml min<sup>-1</sup>, manual injection switching valve with a 10  $\mu\text{l}$  loop (Rheodyne, USA), solvent delivery system and the 1 × 10 mm analytical flow cell (Milton Roy SM 400, UK).

UV-visible spectra of the extracts were recorded using a Hitachi U-2010 Spectrophotometer Micro Scan 4GP/ADI (Japan).

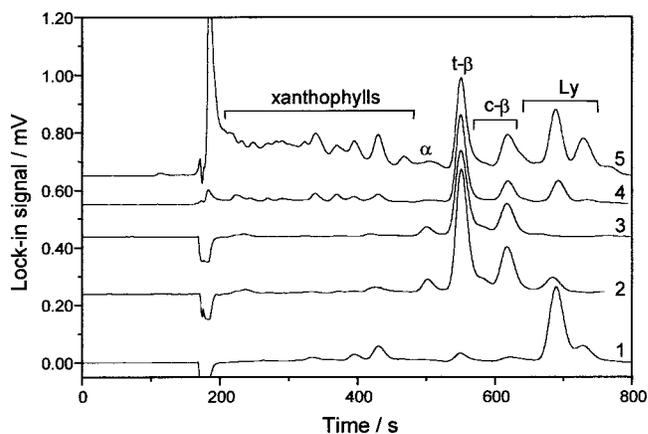


FIG. 1. Chromatograms of standards and two different tomato purée extracts. 1: Lyc-O-Mato™ 6%, 2: Betatene® 20% Soy + lycopene, 3: Betatene® 20% Soy, 4: tomato purée extract (10 times diluted), and 5: tomato purée extract (undiluted). Peaks:  $\alpha$ :  $\alpha$ -carotene,  $c$ - $\beta$ :  $cis$ - $\beta$ -carotene, Ly: lycopene,  $t$ - $\beta$ :  $trans$ - $\beta$ -carotene.

## III. RESULTS AND DISCUSSION

Figure 1 shows chromatograms obtained from standards and tomato purée extracts. Unlike early eluting xanthophylls and carotenes (up to 500 s),  $trans$ - $\beta$ -carotene was found to elute at approximately 550 s followed by  $cis$  isomers (up to 620 s).  $trans$ -lycopene occasionally appeared at approximately 640 s and was followed by  $cis$  isomers eluting after 660 s. Such a carotenoid pattern was recognized in all samples regardless of the tomato cultivar, storage time, or temperature (see Fig. 1). At 476 nm the interference of phytoene and phytofluene was practically eliminated.

The analytical system, very similar to that used in this study, has already been fully validated when assaying  $trans$ - and  $cis$ - $\beta$ -carotene in vegetable oils at ultratrace level.<sup>16</sup> In the current research as little as 0.3 and 1.1 ng ml<sup>-1</sup> lycopene and  $\beta$ -carotene has been measured in the samples. The sensitivity is so high that some extracts needed to be diluted prior to HPLC-TLS analysis. Accordingly, respective concentrations of lycopene and  $\beta$ -carotene in tomato purées were estimated to be 0.4–110 and 3–100 ng g<sup>-1</sup>. These findings suggest a severe loss of both carotenes in the examined tomato purées and a dramatic decrease of their bioantioxidant potency (according to Ref. 3 expected concentrations of lycopene and  $\beta$ -carotene in tomato purées are 166.7 and 4.1  $\mu\text{g g}^{-1}$ ). Total concentration of  $\beta$ -carotene and lycopene served as an indicator of carotene-type antioxidative capacity of our samples and it varied greatly in the examined tomato purées ranging from 3 to 170 ng g<sup>-1</sup>.

The 470 nm absorbance (due to all the carotenoids present in the extracts) measured by the conventional spectrophotometry correlated well with the concentration of  $\beta$ -carotene and lycopene found in tomato purées by HPLC-TLS. It correlated linearly with the concentration of  $\beta$ -carotene, lycopene, and an overall concentration of lycopene and  $\beta$ -carotene; the corresponding  $R^2$  values being 0.77, 0.85, and 0.92 (19–21 data points).

Although the conventional spectrophotometry can be used to rapidly assess the antioxidant quality of tomato products, the need for a more accurate analysis (especially in the

case of samples subjected to inappropriate storage and/or handling), requires the availability of a highly sensitive method such as HPLC-TLS.

In conclusion, a study of extracts from tomato purées benefits substantially from the potential of the HPLC-TLS method to detect carotene pigments at the ultratrace level as well as by the fact that only minute quantities of samples are needed for the analysis.

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