

Communication

## Effect of Cooking Processes on the Contents of Two Bioactive Carotenoids in *Solanum lycopersicum* Tomatoes and *Physalis ixocarpa* and *Physalis philadelphica* Tomatillos

María P. Elizalde-González\* and Socorro G. Hernández-Ogarcía

Centro de Química, Instituto de Ciencias, Universidad Autónoma de Puebla, Apdo. Postal J-55, 72571 Puebla, Pue. Mexico

\* Author to whom correspondence should be addressed; E-mail: melizald@siu.buap.mx

Received: 21 March 2007; in revised form: 31 July 2007 / Accepted: 31 July 2007 / Published: 13 August 2007

---

**Abstract:** Calculation of the HPLC chromatographic retention times of different carotenoids supported our improved chromatographic separation of  $\beta$ -carotene and lutein in four tomatoes and two tomatillo varieties in fresh form and after three different cooking procedures: pot boiling, cooking in a pressure cooker and microwaving. A good separation was achieved experimentally using an Ultrasphere ODS column and gradient elution with an acetonitrile-tetrahydrofuran-water mobile phase. It was shown that diverse tomato species contained different amounts of  $\beta$ -carotene (6-400  $\mu\text{g}/100\text{ mg}$ ) and lutein (2-30  $\mu\text{g}/100\text{ mg}$ ). The concentration in fresh samples was higher than in cooked tomatoes. The  $\beta$ -carotene content in fresh tomatillo varied between 2 and 20  $\mu\text{g}/100\text{ mg}$ . Microwaving caused partial destruction of the  $\beta$ -carotene and lutein in tomatillos.

**Keywords:**  $\beta$ -Carotene; lutein; tomatoes; tomatillos; boiling process.

---

### Introduction

$\beta$ -Carotene acts as a pro-vitamin A, anti-cancer compound and prevents several degenerative diseases [1]. Its retention in carrots during processing and storage has been studied, but not in tomatoes after cooking procedures. It has been known that the carotene and xanthophylls contents in tomato

leaves are almost constant and relatively little affected by environmental factors [2a-b].  $\beta$ -Carotene in carrots was degraded less by low-pressure superheated steam drying than by hot air drying [3]. The unsaturated conjugated chain of carotenoids is very sensitive to air, oxidizing and reducing agents. The relative stability of lutein is the basis of its use as yellow natural dyestuff for food, but the loss of lutein during the storage is known and in many instances it starts at the raw material handling stage. For example, high temperature (50 °C) promoted its degradation [4]. After soaking and drying treatments, the stability of various carotenoids in flowers of *Hemerocallis disticha* was studied and showed loss of carotenoids after air-drying [5].

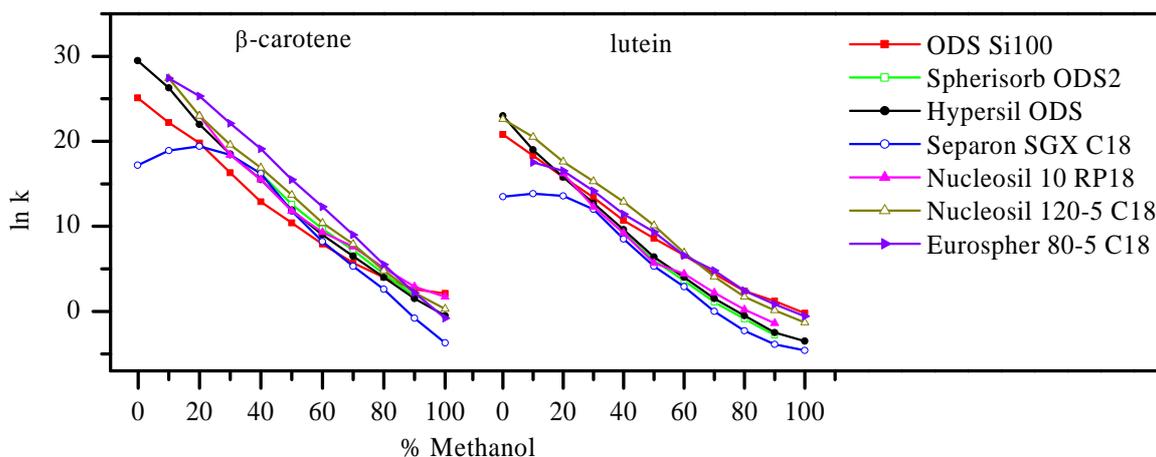
High-pressure treatment is a technique for preserving food quality through the inactivation of endogenous food enzymes. However, enzyme denaturation is caused by rearrangement and/or destruction of noncovalent bonds such as hydrogen bonds and ionic bonds of the tertiary protein structure. During tomato processing, an increase of carotenoids content on a wet weight basis has been observed [6]. HPLC has been used successfully for the analysis of foodstuffs. The determination of carotenoids has been predominantly performed by reversed-phase HPLC on C18 columns [7a-b] and with acetonitrile (MeCN) as eluent. The optimal methodology would appear to be well established, however, it is well known that different C18 phases do not always provide similar performance, i.e. significant differences in peak retention times, selectivity, and even peak shape can be observed. It was therefore of interest to calculate the chromatographic retention of different carotenoids in support of the improvement of the chromatographic separation of the compounds under study. This study attempts to show by means of HPLC how pressure and temperature can influence the content of  $\beta$ -carotene as representative of the carotenes and lutein as the principal xanthophyll found in tomatoes and tomatillo.

## Results and Discussion

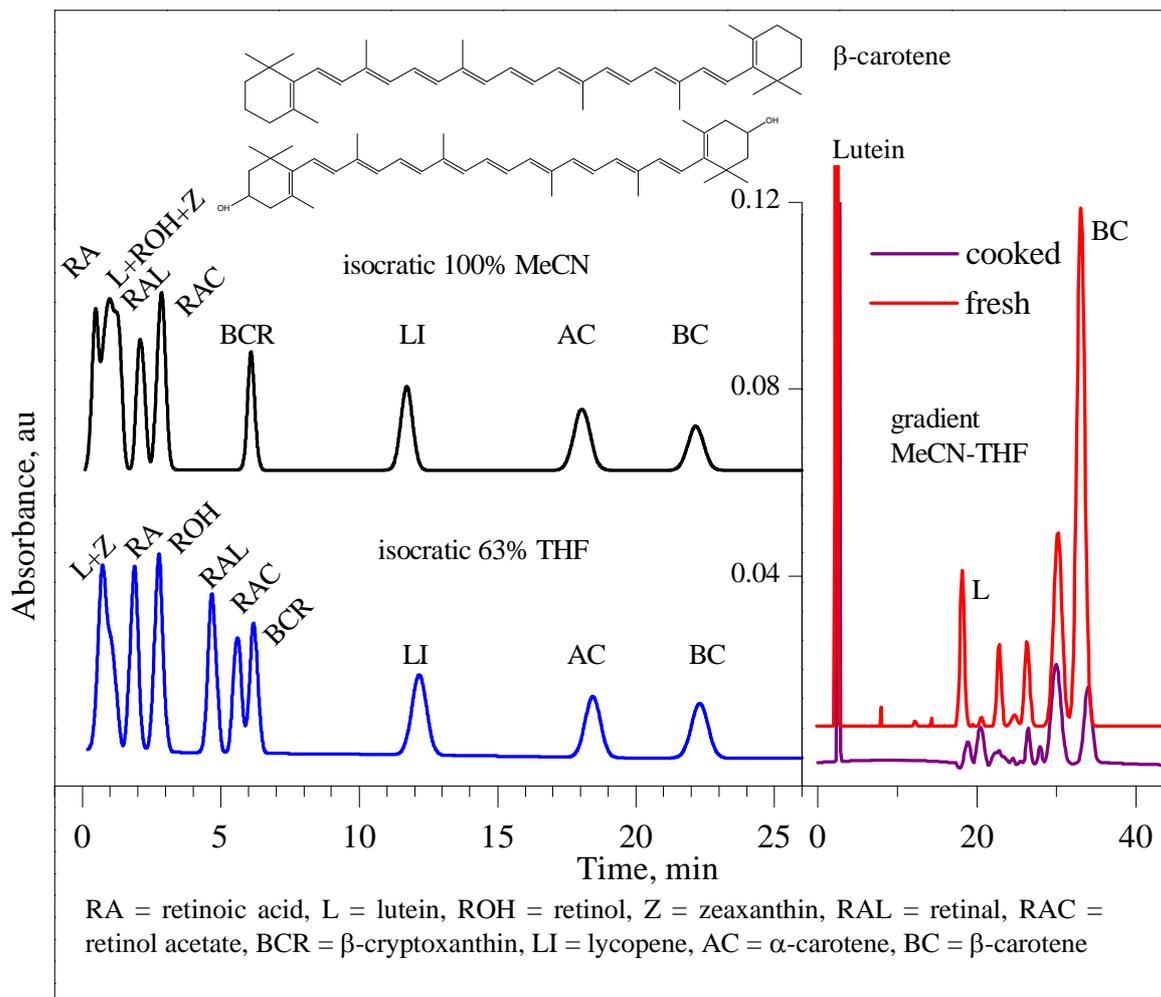
An effort to model theoretically the retention of the carotenoids under study -  $\beta$ -carotene and lutein - on seven different columns and with methanol (MeOH) as eluent is shown in Figure 1. A very similar retention, expressed as the logarithm of the capacity factor over the entire composition range, was found. This result disqualified MeOH, and further retention on the Hypersil ODS column with water-MeCN and water-tetrahydrofuran (THF) mobile phases was modeled.

The optimized theoretical chromatograms on Hypersil ODS using 100 % MeCN and 63 % THF-water are depicted in Figure 2 (left panel). It is worth mentioning, that complete resolution of the mixture components could not be achieved with MeCN. In view that more than two carotenoids could be present in the extracts of the studied fruits, we focused on a superior separation between  $\beta$ -carotene and lutein, minimizing peak overlapping with other compounds and implementing at the same time the use of THF and MeCN.

**Figure 1.** Dependence of the capacity factor  $k$  on commercial C18 columns in water-methanol.



**Figure 2.** Calculated HPLC chromatograms of a model carotenoids mixture on Hypersil ODS and experimental chromatograms of a cherry tomato extract on Ultrasphere ODS.



From our stock columns, Ultrasphere ODS (12 % C) was chosen for the experimental work with real samples due to its carbon load, which is similar to that of Hypersil ODS (10 % C). Based on the theoretical results, the experimental determination of  $\beta$ -carotene and lutein in vegetable samples was

performed using an Ultrasphere ODS column and a MeCN-THF-water mobile phase with gradient elution. Different composition gradients were tested and attention was restricted here to resolution and analysis time of real samples, as shown in Figure 2 (right panel). This is distinct from method validation, which is central for analytical and chromatographic communications. Nevertheless, the theoretical approach facilitated a prompt extrapolation to the mobile phase components and gradient composition.

Since production of tomato foodstuffs and sauces prepared from tomatillo comprises diverse cooking procedures like boiling, roasting or steaming, in this work the content of  $\beta$ -carotene and lutein was determined for different tomato species in fresh fruits and after cooking in a pressure cooker. Results presented in Table 2 evidence variations in the carotenoid content of the fresh vegetables depending on the species. *Lycopersicum cerasiforme* was the lutein richest tomato, while *Lycopersicum periforme Dun* exhibited the highest  $\beta$ -carotene concentration. A mean  $\beta$ -carotene content of 440  $\mu\text{g}/100\text{ mg}$  has been reported [6], which varied from fruit to fruit and could reach values as high as 600 [8] and 780  $\mu\text{g}/100\text{ mg}$  [6]. Due to the lack of information in reports [6] and [8] about the species analyzed, it was not immediately clear if different species are more rich in this component than others. After preparation in a pressure cooker, the  $\beta$ -carotene content decreased in a dissimilar manner in the different species: 25% (in *Lycopersicum periforme Dun*) and 90% (in *Lycopersicum cerasiforme*). Similarly, it has been reported that mangos of different cultivars showed diminished carotene concentration after dehydration [9]. On the other hand, for green beans [10], the effect of cooking could not be revealed due to the wide ranges reported for the fresh and processed vegetables. It is then not surprising, that for example in different Catsup brands [11] the total carotenoids content varied significantly, since tomatoes varieties and manufacture processes differed. Lutein concentration in *Lycopersicum cerasiforme* tomatoes diminished almost 50% after pressure cooking.

**Table 2.** Mean carotenoids concentrations in different tomatoes and tomatillos.

Tomato/tomatillo	<sup>1</sup> State	<sup>2</sup> $\beta$ -carotene, $\mu\text{g}/100\text{ mg}$	<sup>2</sup> lutein, $\mu\text{g}/100\text{ mg}$
Guajillo tomato ( <i>Lycopersicum periforme Dun</i> )	fresh	403 $\pm$ 12	12 $\pm$ 2
	cooked	292 $\pm$ 12	10 $\pm$ 2
Cherry tomato ( <i>Lycopersicum cerasiforme</i> )	fresh	254 $\pm$ 30	32 $\pm$ 3
	cooked	26 $\pm$ 10	13 $\pm$ 1
Saladet tomato ( <i>Lycopersicum esculentum</i> )	fresh	6 $\pm$ 1	9 $\pm$ 1
	cooked	1 $\pm$ 1	1 $\pm$ 1
Beef tomato ( <i>Lycopersicum esculentum Mill</i> )	fresh	6 $\pm$ 1	2 $\pm$ 1
	cooked	bdl	2 $\pm$ 1
Tomatillo ( <i>Physalis philadelphica</i> )	fresh	21 $\pm$ 3	12 $\pm$ 1
	boiled	21 $\pm$ 1	10 $\pm$ 1
	cooked	1 $\pm$ 1	bdl
	microwaved	10 $\pm$ 1	8 $\pm$ 1
Husk tomato ( <i>Physalis ixocarpa</i> )	fresh	2 $\pm$ 1	1 $\pm$ 1
	boiled	2 $\pm$ 1	bdl
	cooked	bdl	1 $\pm$ 1
	microwaved	bdl	bdl

<sup>1</sup> Cooked in pressure cooker, boiled in a covered pot

<sup>2</sup> bdl = below detection limits

Tomatillo is known for its protein (11%) and low fat content (18%) [12]. Nutritional analysis have not examined yet the extraction and quantification of carotenoids, despite its high (58%) carbohydrate content and the quinone reductase activity found in hepatoma cells for one extract of *Physalis philadelphica* [13]. In this work, a low concentration of  $\beta$ -carotene and lutein was demonstrated in two species of tomatillo and the relative amounts varied with the cooking procedures, as reported in Table 2.

## Conclusions

The concentrations of  $\beta$ -carotene and lutein were higher in the fresh form of four tomato species, compared with those obtained in the vegetables subjected to pressure cooking. The relative content of the bioactive compounds in the fresh form was: *Lycopersicum periforme* Dun > *Lycopersicum cerasiforme* > *Lycopersicum esculentum* ~ *Lycopersicum esculentum* Mill for  $\beta$ -carotene and *Lycopersicum cerasiforme* > *Lycopersicum periforme* Dun > *Lycopersicum esculentum* ~ *Lycopersicum esculentum* Mill for lutein. The  $\beta$ -carotene and lutein contents of two species of fresh and cooked tomatillos was also determined.

## Experimental

### General

Lutein and  $\beta$ -carotene standards were purchased from Aldrich and Fluka, respectively. Stock solutions were prepared in THF. All reagents used in the extraction of the carotenoids from the tomatoes samples were purchased from Merck. Methanol, acetonitrile and tetrahydrofuran HPLC grade (Burdick & Jackson) and deionised water (Milli-Q reagent grade water) were used in the chromatographic analysis.

### Equipment

Samples were analyzed using a Beckman Gold automated liquid chromatograph (Beckman Coulter, Inc., Fullerton, CA, USA) comprising a Beckman 168 diode array detector. RP-separations were carried out at 30 °C using a 250 x 4.6 mm column packed with Ultrasphere ODS (5  $\mu$ m particles, 80 Å pore diameter) at flow rate 1 mL·min<sup>-1</sup> with a 60 minute linear gradient, starting with 25% water-75% mixture MeCN-THF (85%:15%, v/v). After each run the column was re-equilibrated to initial conditions during 20 minutes. Eluates were monitored at 355 nm and 40  $\mu$ L were injected in triplicate. Peak identification was performed by retention time matching and the spectra provided by the DAD-detector. Quantification was achieved by calibration curves. Retention of the carotenoids was calculated with the ChromDream software (Knauer, Germany). Baseline correction of the gradient chromatograms was performed using the Peak Fitting Modul from the Origin 5.0 software.

### Sample preparation

Fresh tomatoes and tomatillos were purchased in a local market in Zacatlán, Puebla (Mexico) located at 97°58'E and 19°57'N. Commercial maturity of the fruits corresponded with the USDA sixth stage "red" [14] according to manual grading. Three lots (200 g fresh weight) were prepared on the day of purchase for each cooking and extraction procedure. Batches were obtained by washing and cooking in deionized water (150 mL) during five minutes. The time was recorded when the water reached its boiling point (94 °C) for the boiling trials, when the valve got closed in the pressure cooker experiments (pressure 15 psi), and after pressing the start button of the microwave oven (650 W). The final temperature in the vegetable matter was 90 °C. After cooling to room atmosphere, extraction was carried out in triplicate for each lot according to a modified procedure of [15]: raw or processed material (25 g) were ground in a mortar with CaCO<sub>3</sub> (0.1 g), Na<sub>2</sub>SO<sub>4</sub> (2 g) and sand (0.2 g); then extracted with of a petroleum ether-acetone mixture (1:1, v/v, 120 mL) using a Büchner funnel. The extract was evaporated to dryness in a water bath. The residue was dissolved in THF (2 mL) and chromatographic vials were filled with this solution using syringe filters.

### Acknowledgements

SGHO thanks the SNI, CONACYT (Mexico) for an assistant scholarship.

### References

1. Aust, O.; Sies, H.; Stahl, W. Analysis of lipophilic antioxidants in human serum and tissues: tocopherols and antioxidants. *J. Chromatogr. A* **2001**, *936*, 83-93.
2. (a) Went, F.W.; LeRosen, A.L.; Zechmeister, L. Effect of external factors on tomato pigments as studied by chromatographic methods. *Plant Physiol.* **1942**, *17*, 91-100; (b) Dharmapuri, S.; Rosati, C.; Pallara, P.; Aquilani, R.; Bouvier, F.; Camara, B.; Giuliano, G. Metabolic engineering of xanthophyll content in tomato fruits. *FEBS Lett.* **2002**, *22*, 30-4.
3. Suvarnakuta, P.; Devahastin, S.; Mujumdar, A.S. Drying kinetics and  $\beta$ -carotene degradation in carrot undergoing different drying processes. *J. Food Sci.* **2005**, *70*, 520-526.
4. Shi, X.-M.; Chen, F. Stability of lutein under various storage conditions. *Nahrung* **1997**, *41*, 38-41.
5. Tai, C.-Y.; Chen, B.H. Analysis and stability of carotenoids in the flowers of Daylily (*Hemerocallis disticha*) as affected by various treatments. *J. Agric. Food Chem.* **2000**, *48*, 5962-5968.
6. Baranska, M.; Schütze, W.; Schulz, H. Determination of lycopene and  $\beta$ -carotene content in tomato fruits and related products. Comparison of FT-Raman, ATR-IR, and NIR spectroscopy. *Anal. Chem.* **2006**, *78*, 8456-8461.
7. (a) Barua, A.B. Improved normal-phase and reversed-phase gradient high-performance liquid chromatography procedures for the analysis of retinoids and carotenoids in human serum, plant and animal tissues. *J. Chromatogr. A* **2001**, *936*, 71-82; (b) Liu, H.L.; Kao, T.H.; Chen, B.H. Determination of carotenoids in the Chinese medical herb Jiao-Gu-Lan (*Gynostemma Pentaphyllum* MAKINO) by liquid chromatography. *Chromatographia* **2004**, *60*, 411-417.

8. Vuong, L.T.; Dueker, S.R.; Murphy, S.P. Plasma  $\beta$ -carotene and retinol concentrations of children increase after a 30-d supplement with the fruit *Momordica cochinchinensis* (gac). *Am J. Clin. Nutr.* **2002**, *75*, 872-9.
9. Pott, I.; Marx, M.; Neidhart, S.; Mühlbauer, W.; Carle, R. Quantitative determination of  $\beta$ -carotene stereoisomers in fresh, dried, and solar-dried mangoes (*Mangifera indica* L.). *J. Agric. Food Chem.* **2003**, *51*, 4527-4531.
10. De La Cruz-García, C.; González-Castro, M.J.; Oruña-Concha, M.J.; López-Hernández, J.; Simal-Lozano, J.A.; Simal-Gándara, J. The effects of various culinary treatments on the pigment content of green beans (*Phaseolus vulgaris*, L.). *Food Res. Int.* **1997**, *30*, 787-791.
11. Ishida, B.K.; Chapman, M.H. A comparison of carotenoid content and total antioxidant activity in catsup from several commercial sources in the United States. *J. Agric. Food Chem.* **2004**, *52*, 8017-8020.
12. Bock, M.A.; Sanchez-Pilcher, J.; McKee, L.J.; Ortiz, M. Selected nutritional and quality analyses of tomatillos (*Physalis ixocarpa*). *Plant Foods Hum. Nutr.*, **1995**, *48*, 127-133.
13. Kennelly, E.J.; Gerhäuser, C.; Song, L.L.; Graham, J.G.; Beecher, Ch.W.W.; Pezzuto, J.M.; Kinghorn, A.D. Induction of quinine reductase by withanolides isolated from *Physalis philadelphica* (Tomatillos). *J. Agric. Food Chem.* **1997**, *45*, 3771-3777.
14. Choi, K.; Lee, G.; Han, Y.J.; Bunn, J.M. Tomato maturity evaluation using color image analysis. *Trans. Am. Soc. Agric. Eng.* **1995**, *38*, 171-176.
15. Gonzalez, E.; Montenegro, M.A.; Nazareno, M.A.; López de Mishima B.A. Carotenoid composition and vitamin A value of an Argentinian squash (*Cucurbita moschata*). *Arch. Latinoam. Nutr.* **2001**, *51*, 395-399.

*Sample Availability:* Contact the authors.