Carotenoids, Health Benefits and Bioavailability

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Phytochemicals in Fruits and Vegetables for Health
October 2, 2013
Lycopene Biosynthesis

Phytoene
Phytofluene
ζ-Carotene
Neurosporene
Lycopene

Biosynthesis of common β and ε cyclic carotenes


Tomatoes Varieties with Unique Carotenoid Profile

RED OR1 OR2 YEL GRE
### Common Carotenoids

<table>
<thead>
<tr>
<th>Xanthophylls</th>
<th>Hydrocarbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>(\alpha)-Carotene</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>(\beta)-Carotene</td>
</tr>
<tr>
<td>(\beta)-Cryptoxanthin</td>
<td>Lycopene</td>
</tr>
</tbody>
</table>

### Biological Functions of Carotenoids

- Provitamin A Activity
- Non-provitamin A Activity:
  - Singlet Oxygen Quenching Activity
  - Antioxidant Activity (Trap Free Radicals)
  - Enhancement of Immune Response
  - Potential Chemopreventive Properties

### Conversion to Vitamin A

\[
\beta\text{-carotene} \xrightarrow{O_2} 15,15'-\text{oxygenase} \xrightarrow{H^+} \text{reductase} \rightarrow \text{retinol} \rightarrow \text{retinol}
\]
Vitamin A - WHO Facts and Figures

• An estimated 250 million preschool children are vitamin A deficient
  – It is likely that in vitamin A deficient areas, a substantial proportion of pregnant women are vitamin A deficient

• An estimated 250,000 to 500,000 vitamin A-deficient children become blind every year,
  – Half of these die within 12 months of losing their sight

Carotenoids and Health Benefits

• Epidemiological
• Cell culture
• Animal (experimental)
• Human (clinical)
Dietary carotenoids, vitamin-A, vitamin-C, and vitamin-E, and advanced Age-related Macular Degeneration

Seddon et al.

“Conclusion.-Increasing the consumption of foods rich in certain carotenoids, in particular dark green, leafy vegetables, may decrease the risk of developing advanced or exudative AMD, the most visually disabling form of macular degeneration among older people.”

Lutein & Zeaxanthin in the Macula

- Macula is the Region Directly Behind the Lens, Receiving the Most Light.
- Lutein & Zeaxanthin are Deposited Specifically.
- The Role Is To Prevent Photo-Oxidation.
Vegetables, fruit, and cancer prevention: A review
Steinmetz KA, Potter JD
Journal of the American Dietetic Association 96: (10) 1027-1039, 1996

“The types of vegetables or fruit that most often appear to be protective against cancer are raw vegetables, followed by allium vegetables, carrots, green vegetables, cruciferous vegetables, and tomatoes.”

Absorption and Bioavailability

Bioavailability is the fraction of an ingested nutrient that is available for utilization in normal physiological functions, metabolism and/or storage.

In the context of this discussion, bioavailability of carotenoids is defined as the amount of these micronutrients that are absorbed by the intestinal enterocytes and transported in the bloodstream.
Food Processing and Preparation

- Physical and thermal treatment of foods causes degradation of plant cell structural constituents.
- Thermal processing brings about disruption of the carotenoid protein complexes.
- Inactivation of oxidizing enzymes results in less degradation and greater stability of carotenoids.
- These changes result in enhanced uptake and efficiency of carotenoid absorption.

Altered “Bioavailability” By Food Processing

<table>
<thead>
<tr>
<th>Percentage Change</th>
<th>Raw</th>
<th>Processed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Carotene</td>
<td>120</td>
<td>90</td>
</tr>
<tr>
<td>Total Beta Carotene</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>


Intake of carotenoids and retinol in relation to risk of prostate cancer

Giovannucci et al.
*Journal of the National Cancer Institute*, 87(23): 1767-76, 1995

“Combined intake of tomatoes, tomato sauce, and pizza (which accounted for 82% of lycopene intake) was inversely associated with risk of prostate cancer.”
“This comprehensive prospective analysis suggests women with higher circulating levels of α-carotene, β-carotene, lutein+zeaxanthin, lycopene, and total carotenoids may be at reduced risk of breast cancer.”

CARET and ATBC
randomized, double blind, placebo controlled
- Supplemented with high dose of beta-carotene (20mg and 30mg)
- Followed for 5-8 years
- Risk of lung cancer increased in both studies in smokers
Carotenoids as Antioxidants

Endogenous and Exogenous Reactive Oxygen Species (ROS)

- Antioxidants

Lipids, Proteins, DNA Damage

- Chronic Disease

Lycopene as a Singlet Oxygen Quencher

- Lycopene is the most efficient singlet oxygen quencher and phenoxyl radical scavenger among the naturally occurring carotenoids.


Other Hypothesized Mechanism of Action

- Stimulate enzymes that detoxify toxins/carcinogens
- Enhance DNA repair mechanisms
- Increase apoptosis of cancer cells
- Decrease cell proliferation
- Possess anti-angiogenesis activity
- Restore gap-junction communication
PDA Chromatogram of Tomato Carotenoids

Selected Geometrical Isomers of Lycopene

- all-trans lycopene
- 5-cis lycopene
- 9-cis lycopene
- 13-cis lycopene
- 7,9,9',7'-cis lycopene
Lycopene is more bioavailable from tomato paste than from fresh tomatoes

Christine Gärtner, Wilhem Stahl and Helmut Sies

ABSTRACT Lycopene bioavailability, from a single dose of fresh tomatoes or tomato paste (23 mg lycopene) ingested together with 15 g corn oil was compared by analyzing carotenoid concentrations in the chylomicron fraction. The lycopene isomer pattern was the same in both fresh tomatoes and tomato paste. The triacylglycerol response in chylomicrons was not significantly different after both treatments. Ingestion of tomato paste was found to yield 2.5-fold higher total and all-trans-lycopene peak concentrations (P<0.05 and P<0.005, respectively) and 3.8-fold higher area under the curve (AUC) responses (P<0.001) than ingestion of fresh tomatoes. The same was calculated for lycopene cis-isomers, but only the AUC response for the cis-isomers was significantly higher than ingestion of tomato paste (P<0.005). No difference was observed in the α- and β-carotene response. Thus, in humans, the bioavailability of lycopene is greater from tomato paste than from fresh tomatoes.
**Electron Microscopy**

Electron micrograph of lycopene crystalloids in mature red tomato chromoplast.

A. Lycopene crystalloids.
B. Plastoglobulin-type sacs in which \( \beta \)-carotene reportedly accumulates.

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**Crystalloid Carotenoids in Tomato and Carrot**

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**Tomato Product Consumption – Correlation with Plasma Lycopene**

<table>
<thead>
<tr>
<th>Product</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato Sauce</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>&lt; 0.056</td>
</tr>
</tbody>
</table>

Lower Prostate Cancer Risk in Men with Elevated Plasma Lycopene Levels: Results of a Prospective Analysis (Gann et al., *Cancer Research*, 1999, 59: 1225-1230)
Commercial Products Study – Experimental Design

- **Vegetable Juice**
- **Tomato Sauce**
- **Tomato Soup**

<table>
<thead>
<tr>
<th>Wash-out</th>
<th>Meals</th>
<th>Analysis</th>
</tr>
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<tbody>
<tr>
<td>(14 days)</td>
<td>(28 days)</td>
<td></td>
</tr>
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</table>

**Plasma Lycopene Response**

**Total Lycopene Levels in Buccal Cells**

*Allen et al. J. Nutr. 2003*
Clinical Study Design – Prostate Cancer Subjects

N = 32
8/Group

Washout

Randomize

V8 Vegetable Juice

Tomato Soup

Tomato Sauce

Soy Protein

PROSTATECTOMY

7 days 3 weeks

↑ = blood draw  ↑ = 24-h urine

Change in Plasma Lycopene Concentrations Following 21 Days of Dietary Intervention with Tomato-Based Products or Soy

$P < 0.05; \ N = 8/\text{group}$

Prostate Lycopene Isomers Following 3 Weeks of Dietary Intervention with Tomato Products or Soy Protein

$P < 0.01; \ N = 8/\text{group}; \text{Other prostate carotenoids were not sig. across groups}$
HPLC-MS/MS Analysis

Prostate Isoflavones
Prostate beta-carotene and lycopene

Two Stage *In Vitro* Digestion


Caco-2 Human Intestinal Cells

- Human colon carcinoma cells
- Differentiate spontaneously at confluence under normal cell culture condition
- Enterocyte: polarized monolayer, microvilli, tight junctions
Massive doses of a carotene-rich vegetable to the basic diet over a long period did not greatly increase serum carotene and vitamin A levels and that most carotene was excreted.
Addition of fats to the diet may contribute to the relief of vitamin A deficiency in this region.

Carotenoids

Roels, Trout and Dujacquier. J. Nutr. 1958

Carotenoids Absorption & Metabolism


Post-prandial Response

440 mV Applied Potential 20.0 nA Full Scale
Carotenoid Response at Three Different Lipid Levels

Fat Free 0 g
Reduced Fat 6 g
Full Fat 28 g


Avocado as Source of Dietary Lipid

40g Lettuce  100g Carrots  80g Spinach
+  
75g Avocado / 150g Avocado / 24g Avocado Oil

Baseline Corrected Mean AUC Values for Each Treatment

Values are based upon mean AUC ± SEM
Post-prandial Response of Lycopene and Beta-Carotene - Salsa With and Without Avocado

Tomato Varieties

Postprandial Absorption of Tangerine vs High Lycopene (Pasta Sauce)
High β-Carotene Tomatoes

- Monitor absorption of carotenoids from a novel high β-carotene tomato sauce when co-consumed with and without lipid (avocado as source of fat).
- Quantify bioconversion of provitamin A β-carotene as influenced by the presence of lipid in the diet.

High β-Carotene Tomato Sauce

![Graph showing absorption and bioconversion]

LCMS Analysis of β-Carotene and Retinyl Esters

![Graph showing LCMS analysis]

*Modified method also analyzes for α-tocopherol, phyloquinone, lutene, β-cryptoxanthin, α-carotene, lycopene

Delivery of β-Carotene and Retinyl Esters (Vitamin A)

- Sauce + Avocado
- Sauce only

Baseline-corrected carotenoid level in Blood Plasma (nmol/L ± SEM)

Hours after meal consumption (h)

Kopec et al. (2013)

Unilever’s Wish-Bone Salad Dressing

Metabolism of Carotenoids
Bioactive Metabolites – Retinoids

β-carotene → Lycopene → Lycopenealdehyde → Lycopenoic Acid
RAR Activation → Transcription

Metabolites - Lycopensals

- all-trans lycopene
- apo-6'-lycopenal
- apo-8'-lycopenal
- apo-10'-lycopenal
- apo-12'-lycopenal
- apo-14'-lycopenal
- apo-15'-lycopenal

Kopec et al. (2010)

Lycopensals – LC-MS

Kopec et al. (2010)
Apo-Lycopenals in Human Plasma

Standards
- Lycopene
- apo-6'-lycopenal
- apo-8'-lycopenal
- apo-10'-lycopenal
- apo-12'-lycopenal
- apo-14'-lycopenal


Human Plasma

13C-Lycopene Production using Tomato Cell Cultures

Tomato flower

Tomato callus culture (13C-Glucose)


Tomato cell suspension

13C Lycopene Plasma Response After Dosing

subject 1

subject 2
Metabolomics (MS & $^{13}$C NMR)

Mass Spectrum of Lycopene Isotopomers in Human Plasma after Single Dose of $^{13}$C Lycopene Showing Native and $^{13}$C Enriched Forms

$^{13}$C NMR Spectra of Urine

Baseline

Post-Dose

β-apo-Carotenals in Human Plasma

apo-carotenal standards (isomeric of beta-carotene)
Accumulating evidence continues to associate health benefits with carotenoid rich fruit and vegetable consumption.

“Bioavailability” of carotenoids from specific food products is influenced by a number of factors including food processing treatments, matrix and product formulation (particularly lipid content) and should be determined to more accurately assess delivery of these phytochemicals from the diet.

Unique varieties of vegetables and fruit (high lycopene, tangerine, high beta carotene tomatoes) can be considered to study the bioavailability and bioconversion of carotenoids from the diet.

Apo-carotenoid metabolic oxidation products may exhibit biological activity by binding to retinoid receptors and modulate gene expression.

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