Changes in Carotenoid Content and its Composition during Maturation of *Fructus lycii* Fruits

(Received November 13, 2004)  
(Accepted January 28, 2005)

Meilan Piao a), Yoshiyuki Murata b), Beiwei Zhu  
Yasuaki Shimoishi b), Mikiro Tada b)

\[ a) \text{Graduate School of Natural Science and Technology, Okayama University} \\
\text{b) Faculty of Agriculture, Okayama University} \\
c) \text{College of Bio \\& Food Technology, Dalian Institute of Light Industry} \]

\[ \text{Abstract} \]

Carotenoids in *Fructus lycii* fruits at 7 different ripening stages were analyzed using a high-performance liquid chromatography-mass spectrometry (LC-MS) method. As fruits of *F. lycii* matured, the total amount of carotenoid increased and the amount of chlorophyll decreased in the fruits. The LC-MS analysis after saponification of carotenoids showed that 65% of total carotenoids in the full matured fruit was zeaxanthin and that 13% was lutein. The other detectable carotenoids were identified as -cryptoxanthin and -carotene.

Key words: carotenoid, *Fructus lycii*, lutein, zeaxanthin, maturation

\[ \text{I. Introduction} \]

Carotenoids is characterized by a 40-carbon atoms polyene chain, which is sometimes terminated by rings. Carotenoids where some of the double bonds are oxidized are known as xanthophylls and the un-oxidized carotenoids are known as carotenoids. Both of them provide the intensive yellow, orange or red color of a great number of plant-based foodstuffs.

*Fructus lycii* is one of Chinese medicinal plants, whose fruit is a Chinese medicine to prescribe for the treatment of infertility. The fruits of *F. lycii* are effective in preventing skin wrinkle 1 as well as in preserving good vision based on centuries of traditional experience in China 2, 3. Carotenoids are usually synthesized during fruit maturation and the accumulation of carotenoid pigments greatly differs among plant species 4. However, there is no report for the change of chlorophyll and carotenoid at same mature stage in the fruits of *F. lycii*.

This study focused on the change in the amounts of carotenoids and chlorophyll in the fruit of *F. lycii*, during ripening and also analyzed the composition of carotenoids.

\[ \text{II. Materials and methods} \]

1. Samples

Fruits of *F. lycii* were freshly harvested at Nisiiwai, Japan on October 2003. The fruits at different ripening stages were harvested at the same day. The fruits were classified into 7 groups by color: stage 1, green; stage 2, green-yellow; stage 3, yellow; stage 4, yellow-orange; stage 5, orange; stage 6, red and stage 7, full-ripe 4, 5.

To determine water contents of the fruits of *F. lycii*, 5 g of the fruits at each stage was dried in an oven at 50°C for overnight and then was weighted.

2. Extraction of carotenoids from *F. lycii*

The fresh fruits were homogenized using a commercial mixer. Acetone was added to the homogenate and the homogenate was filtrated with No. 2 filter. Then hexane and water were added to the filtrate and then shaken. The upper (organic) layer was collected and was washed with water. The lower (aqueous) layer was repeatedly extracted with hexane until the color of the layer disappeared. All of the hexane solutions were combined and then
were evaporated to dryness under nitrogen. After evaporating, the residue was saponified with 10% methanolic potassium hydroxide and incubated in the dark at room temperature for 24 h. The reaction mixture was then heated at 90°C for 10 min. Hexane and water were added to the mixture and then collected the organic layer. The organic layer was washed with water. Meanwhile, the aqueous layer was repeatedly extracted with hexane until the color of the layer disappeared. All hexane solutions were combined and then were dehydrated with anhydrous sodium sulphate. After the solution was filtered to remove sodium sulphate with cotton, the filtrate was evaporated to dryness under nitrogen. The residue containing carotenoids was dissolved in a mixture of acetonitrile/ethanol (3:2). The carotenoid solution was immediately analyzed by HPLC (model LC-6A; Shimadzu Co., Kyoto, Japan). The stationary phase was YMC carotenoid C-30 5 μm, 4.6 mm × 250 mm, the mobile phase was acetonitrile/ethanol (3:2 V/V), and the flow-rate was 1.0 ml/min. When analyzing absorption spectrum, HPLC system was equipped with a photodiode array detector (SPD-M10AVP, Shimadzu Co.). As investigating molecular ion peak, the entire effluent volume was directed to LC-TOF MS (JMS-T100LC AccuTOF; JEOL Ltd., Tokyo, Japan) and mass spectra were monitored in the mass range m/z 100-1000 on a mass spectrometer equipped with an APCI positive interface.

3. Identification and quantification of chlorophylls and carotenoids

Each carotenoid was identified by comparing a visible absorption spectrum, a mass spectrum and a retention time from HPLC analysis with those of an authentic sample. Total chlorophyll amounts were determined according to Mackinney method 7 and total carotenoid amounts were calculated from absorbance at 450 nm, where ε450 value of zeaxanthin in hexane is 2350 8.

III. Results

The water content of fresh fruits of F. lyii was found to be from 73.6±0.8% at stage 3 to 80.6±0.6% at stage 7. There were no statistically significant differences in the different ripening stages.

The chlorophyll and carotenoid amounts of the F. lyii fruits are shown in Fig. 1. The amount of chlorophyll was 14.1 mg/100 g-FW at stage 1 and decreased to 0.8 mg/100 g-FW at stage 4 during the development of ripening (P<0.01) (Fig. 1). No chlorophyll was detected at stage 6 and 7 (Fig. 1). After most part of the chlorophyll content reduced, the carotenoid content began to increase. The amount of carotenoids rapidly increased with ripening of the fruits. The change was significant from stage 4 to stage 7 (P<0.05) (Fig. 1).

The HPLC and LC-MS analysis of carotenoids in the F. lyii fruits was performed. Fig. 2 shows representative chromatograms from HPLC analysis of samples at different stages of carotenoids (A) and identified structural formulae (B). Each peak was determined by comparison with the retention time, absorption spectrum and mass spectrum of the pure authentic samples. The retention time of P3 and P4 are consistent with those of lutein and zeaxanthin, respectively. Furthermore, P3 exhibited a molecular ion at m/z 569 and showed absorption spectrum with absorption maximum at 447 nm. P4 showed a molecular ion peak at m/z 569 and absorption spectrum with absorption maximum at 454 nm. By comparison with pure authentic samples, P3 was identified as lutein and P4 was determined as zeaxanthin 9. P6 exhibited a molecular ion at m/z 553 and showed absorption spectrum with absorption maximum at 454 nm. The retention time and absorption spectrum with absorption maximum of the pure authentic β-cryptoxanthin indicated that P6 was β-cryptoxanthin. P7 exhibited a molecular ion at m/z 537 and showed absorption spectrum with absorption maximum at 453 nm. By comparison with the retention time and absorption spectrum with absorption maximum of the authentic β-carotene, P7 was found to be β-carotene.

The amounts of zeaxanthin, lutein, β-carotene, β-cryptoxanthin, and other carotenoids in the F. lyii fruits at seven different ripening stages were summarized in Table 1. The amount of zeaxanthin was largest at stage 7 (26.04±8.89 mg/100 g-FW). The amount of lutein decreased from stage 1 to stage 4 and then increased from stage 4 to stage 7. The amount of lutein was lowest in stage 4 (0.57±0.04 mg/100 g-FW), and then increased to 5.21±1.72 mg/100 g-FW in stage 7 (P<0.01). The amounts of β-cryptoxanthin and β-carotene slightly increased with ripening (Table 1).

IV. Discussion

In this study, there was an inverse correlation between the content of chlorophylls and the content of carotenoids at different
maturity of the fruits of *F. lycii*. The chlorophyll/carotenoid molar ratio (pigment balance) was strongly changed in the fruits of *F. lycii*, that is, carotenoids increased and chlorophylls were degraded. The fruits at stage 7 was characterized by about 8-fold increase of total carotenoids with comparing to the fruits at stage 1, that is, the pigment balance drastically decreased during maturation (Fig. 1). The changes in color of the fruits during matura-
tion were caused by the change in composition of carotenoid pig-
ments. The yellow-orange fruits of *F. lycii* (stage 4) were character-
ized by few content of chlorophyll and low content of caro-
tenoids. The orange-red fruits of *F. lycii* (stage 6) were character-
ized by the complete disappearance of chlorophyll and by the 
rapid synthesis of carotenoids, in particular, zeaxanthin. More 
than 75% of total carotenoids were composed of lutein and zeax-
anthin in stage 7, whereas sum of \(\beta\)-cryptoxanthin and \(\beta\)-caro-
tene was less than 10% of total carotenoid at each stage (Table 1). These results are consistent with the previous reports that ze-
axanthin is a major carotenoid in fruits and that zeaxanthin is the 
characteristic carotenoid in *F. lycii*\(^{10}\). In this study, lutein was 
detected at all ripening stages, which does not agree with the 
result reported by Lam and But\(^{13}\). This difference might are from 
efficiency to extraction of carotenoids from the fruits of *F. lycii*.

Total carotenoid content described in some previous reports 
ranged from 4.0 to 8.8 mg carotenoid per 100 g-DW of *F. lycii*\(^{3,11,12}\). Lam and But reported that the total carotenoid con-
tent in the fruits of *F. lycii* purchased from the stores was 13.4 
mg/100 g-DW\(^{13}\). Li *et al.* reported there was 295 mg/100 g-DW 
in the fruits of *F. lycii* harvested in China\(^{14}\). Our studies showed

---

**Table 1.** The amount of individual carotenoids in the fruits of *F. lycii* at seven ripening stages.

<table>
<thead>
<tr>
<th>STAGE</th>
<th>total carotenoid (mg/100 g-FW)</th>
<th>lutein (mg/100 g-FW)</th>
<th>zeaxanthin (mg/100 g-FW)</th>
<th>(\beta)-cryptoxanthin (mg/100 g-FW)</th>
<th>(\beta)-carotene (mg/100 g-FW)</th>
<th>others (mg/100 g-FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAGE 1</td>
<td>5.56±0.23</td>
<td>2.50±0.10</td>
<td>0.22±0.01</td>
<td>0.13±0.01</td>
<td>1.07±0.03</td>
<td>1.62±0.86</td>
</tr>
<tr>
<td>STAGE 2</td>
<td>4.24±0.18</td>
<td>2.89±0.12</td>
<td>0.10±0.02</td>
<td>0.18±0.01</td>
<td>0.22±0.01</td>
<td>0.86±0.09</td>
</tr>
<tr>
<td>STAGE 3</td>
<td>4.07±0.67</td>
<td>1.84±0.30</td>
<td>0.33±0.05</td>
<td>0.10±0.02</td>
<td>0.42±0.07</td>
<td>1.17±0.30</td>
</tr>
<tr>
<td>STAGE 4</td>
<td>4.60±0.06</td>
<td>0.57±0.04</td>
<td>2.26±0.33</td>
<td>0.70±0.12</td>
<td>0.11±0.02</td>
<td>0.95±0.17</td>
</tr>
<tr>
<td>STAGE 5</td>
<td>15.64±0.65</td>
<td>2.04±0.33</td>
<td>8.48±2.66</td>
<td>2.08±1.26</td>
<td>0.30±0.21</td>
<td>2.75±0.85</td>
</tr>
<tr>
<td>STAGE 6</td>
<td>24.90±3.22</td>
<td>3.46±0.31</td>
<td>15.96±6.02</td>
<td>2.09±0.18</td>
<td>0.48±0.05</td>
<td>4.58±5.86</td>
</tr>
<tr>
<td>STAGE 7</td>
<td>40.21±4.01</td>
<td>5.21±1.72</td>
<td>26.04±8.89</td>
<td>2.97±3.04</td>
<td>0.75±0.64</td>
<td>5.24±5.34</td>
</tr>
</tbody>
</table>
the full matured fruits of *F. lycii* gathered in Japan contained 228.47 ± 22.78 mg/100 g-DW of carotenoids in stage 7, where the carotenoid content per dry weight was calculated from the carotenoid content per fresh weight, 40.21 ± 4.01 mg/100 g-FW, and the water content, 83.4%. When the carotenoid contents of *F. lycii* cultivated in China was analyzed by following the same procedure, the contents were 160 to 173 mg/100 g-DW. The large variations cannot be explained because of poor description for the method of analysis in their reports. However, one of the reasons could be that our extract efficiency is higher than theirs.

Zhou et al. reported that *F. lycii* berry contained the large amount of zeaxanthin but the trace amount of lutein. Lutein in *F. lycii* fruits was not detected by Li et al. In our studies, the HPLC and LC-MS analysis of the carotenoids showed that lutein, zeaxanthin, \( \beta \)-cryptoxanthin, \( \beta \)-carotene (P3, P4, P6 and P7) and three unknown carotenoids (P1, P2 and P5) existed in the fruits of *F. lycii*. Some preliminary evidences suggest that P5 was stereoisomer of zeaxanthin (data not shown).

Over 40 types of carotenoids are present in a typical American diet and only zeaxanthin and lutein are present in human retina. Recent studies showed that high consumption of lutein and zeaxanthin reduced the risk for age-related macular degeneration. Our results support that the fruit of *F. lycii* containing the remarkably high amounts of zeaxanthin is one of good natural sources for health benefit to human and *F. lycii* as well as corn are good source to provide zeaxanthin and are less risky than egg yolk because of low cholesterol. To identify unknown carotenoids in *F. lycii* and to assess the functionality of these carotenoids would enhance the availability of *F. lycii*.

**V. Acknowledgments**

We thank Dr. T. Miyake, Industrial Technology Center of Okayama Prefecture for LC-MS analysis of carotenoids. We thank Dr. S. Arita for discussion.

**VI. References**


ノート

クコ実実中の成熟過程におけるカロテノイドの量及び組成変化
(2004年11月13日受理)
(2005年1月28日受理)

朴美蘭a)、村田芳行b)、朱薇薇c)、下石靖昭c)、多田幹郎b)

a) 岡山大学大学院自然科学研究科
b) 岡山大学農学部
c) 中国大連軽工業学院生物・食品工程学院

キーワード：カロテノイド、クコ、ルテイン、ゼアキサンチン、成熟

概要
新鮮クコの7つの異なる成熟段階において、LC-MSを用いてカロテノイド含量と組成分析を行った結果、成熟につれ、カロテノイド含量は増加したが、クロロフィル含量は減少した。アルカリ水解後、完熟クコの全カロテノイドのうちゼアキサンチンが65%、ルテインが13%を占めた。他にβ-クリプトキサンチンとβ-カロテンも同定した。