# Variation in Carotenoid Content across Different Bitter Melon Cultivars

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**Abstract:** Bitter melon (*Momordica charantia* L.) belonging to the family Cucurbitaceae is traditionally used as a medicinal herb because of its anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial, anti-diabetic, and anti-tumor properties. Carotenoids are a diverse group of pigments that accumulate in the plastids of leaves, flowers, and fruits and play important roles in many physiological processes in plants. In humans, some carotenoids are essential nutrients, whereas others have protective effects against several diseases. In this study, nine different bitter melon cultivars collected from Japan and the Philippines were analyzed to distinguish the levels of three different carotenoids, i.e., lutein, α-carotene, and β-carotene by using high-performance liquid chromatography. The carotenoid content varied significantly among the cultivars. In general, the carotenoid content in the cultivars from the Philippines was higher than that in the ones from Japan, with the highest content found in Galaxy from the Philippines and the lowest content found in the cultivar Kyushu from Japan. The levels of lutein in the Galaxy cultivar were 2.9 and 2.8 times higher than those in Kyushu and Trident 357 cultivars, respectively. The cultivar Galaxy contained 3.7, 3.1, and 3.0-fold higher α-carotene and 3.9, 2.9 and 2.8 times higher β-carotene than those in Kyushu, Sta.Rita Strain. L., and Peacock, respectively. The results of our analysis suggest that the bitter melon cultivar Galaxy could potentially be used as a source of carotenoid.
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## 1. Introduction

Bitter melon (*Momordica charantia* L.) belonging to the family Cucurbitaceae is a medicinal plant indigenous to temperate and tropical regions of Asia and grown in other parts of the world. The fruit of *M. charantia* has a bitter taste and is oblong, resembling a cucumber. It is emerald green when young and turns orange-yellow upon ripening (Basch *et al*., 2003; Krawinkel and Keding, 2006). At present, bitter melon is used for the treatment of diabetes and colic and as a carminative in many countries (Cefalu *et al*., 2008; Leung *et al.*, 2009; Nahas and Moher, 2009). The fruit and seeds of bitter melon have traditionally been used as medicinal herbs because of their anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial, anti-diabetic, and anti-tumor properties (Zafar and Neerja, 1991; Ng *et al.*, 1992; Scartezzini and Speroni, 2000; Grover and Yadav, 2004; Beloin *et al.*, 2005).

Carotenoids are a diverse group of more than 600 naturally occurring red, orange, and yellow pigments (Sergio and Robert, 1999) that accumulate in the plastids of leaves, flowers, and fruits. These pigments

play important roles in many plant physiological processes. For example, some act as light absorbers in photosynthetic membranes and prevent damage to photo-oxidative processes (Penuelas and Munne-Bosch, 2005). Carotenoids are responsible for the characteristics colors of flowers and fruits and help attract pollinators and seed-dispersal agents (Howitt and Pogson, 2006). The carotenoid composition may varies with variety, culture, cultivation conditions, the state of maturity, the post-harvest and storage handling, the climate and the geographical localization, the type of sample and the part of plant (Mercadante and Amaya, 1990, Yamini *et al.* 2001). In humans, some carotenoids are essential nutrients, whereas others have protective effects against several diseases. For example, provitamin A carotenoids such as α- carotene and β-carotene- precursors of vitamin A- are necessary for the prevention of xerophthalmia,

blindness, and premature death (IMFNB, 2000; Mayne, 1996).

Because of the importance of carotenoids and their variation across plants due to different factors, especially due to variety/cultivars and geographical localization, reliable assessment of carotenoids in bitter melon, which is used as a vegetable, is necessary for the accurate quantification of dietary intake. To our knowledge, no study has used high performance liquid chromatography (HPLC) to determine carotenoid contents in bitter melon. Hence, the present study was designed to collect bitter melon cultivars from different sources and assess their carotenoid levels.

## 2. Material and Methods

**Plant material:** Seeds of nine bitter melon cultivars (4 from Japan and 5 from the Philippines) were germinated, and the seedlings were transferred to a greenhouse maintained at 25°C, and 60% RH. The greenhouse was maintained under normal day light conditions and located in an experimental farm at Chungnam National University (Daejeon, Korea). The fruits (at green edible stage) of the cultivars were harvested and used to compare their carotenoid contents.

**Carotenoid extraction and HPLC analysis:** Ascorbic acid and β-apo-8′-carotenal were purchased from Sigma Chemical Co. (St. Louis, MO). Lutein and β-carotene were obtained from CaroteNature (Lupsingen, Switzerland). The extraction method used for carotenoid analysis was slightly modified from that described in a previous report (Howe et al., 2006). Briefly, carotenoids were extracted from 0.1 g bitter melon samples using 3 mL of ethanol containing 0.1% ascorbic acid (w/v). This mixture was vortexed for 20 s and incubated in a water bath at 85 °C for 5 min. Next, 120 µL of potassium hydroxide (80% w/v) was added to saponify any potentially interfering oils. The samples were vortexed and incubated at 85 °C for 10 min, and then placed on ice. Subsequently, 1.5 mL cold deionized water and 0.05 mL β-apo-8'-carotenal (12.5 $\mu g \cdot mL^{-1}$) as an internal standard were added. The carotenoids were extracted twice by using 1.5 mL hexane, followed by centrifugation at 1200 ×*g* each time to separate the layers. The extracts were freeze-dried under a stream of nitrogen gas and resuspended in 50:50 (v/v) dichloromethane/methanol. For quantification purposes, calibration curves were drawn by plotting four different concentrations of carotenoid standards according to the peak area ratios with -apo-8'-carotenal. The linear equations and regression coefficients for lutein, α-carotene, and -carotene were $y = 0.1928\ x - 0.027$, $r = 0.9999$, $y = 0.1126\ x - 0.0212$, $r = 0.9985$, and $y = 0.1126\ x - 0.0212$, $r = 0.9985$, respectively.

For HPLC analysis, the carotenoids were separated on an Agilent 1100 HPLC system equipped with a $C_{30}$ YMC column (250 × 4.6 mm, 3 µm; Waters Corporation, Milford, MA, USA) and detected using a photodiode array (PDA) detector at 450 nm. Solvent A and B consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate and 100% methyl *tert*-butyl ether (MTBE), respectively. The flow rate was maintained at 1 $mL \cdot min^{-1}$ and the samples were eluted using the following gradient program: 17% solvent B at 0 min; 30%, 23 min; 41%, 29 min; 70%, 35 min; 70%, 40 min; 17%, 44 min; and 17%, 55 min. The identification and peak assignment of carotenoids were primarily performed by comparing their retention time and UV-visible spectrum data with those of standards by using previously reported guidelines (Fraser et al., 2000; Howe et al., 2006).

**Statistical analysis:** All data were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect trial effects by using SAS Software release 9.2 (SAS, 2010), and means were compared using Duncan's multiple range t. Significance was set at $P = 0.05$.

## 3. Results

**Carotenoid content in different cultivars of bitter melon:** In all, three carotenoids, i.e., lutein, α-carotene, and β-carotene, were determined in the 9 cultivars. Carotenoids content varied among the bitter melon cultivars and was significantly greater in the cultivars obtained from the Philippines than in those obtained from Japan. Galaxy from Philippines contained the highest amount of carotenoids, whereas Kyushu from Japan contained the lowest amount of carotenoids. There was no significant difference in lutein content between Galaxy and Sta Monica cultivars from the Philippines and Nikko from Japan. The levels of lutein in the Galaxy cultivar were 2.9 and 2.8 times higher than those of lutein in Kyushu and Trident 357 cultivars, respectively. The variation in α-carotene and β-carotene levels between Galaxy and Kyushu was considerably higher than that in lutein content. Galaxy contained 3.7, 3.1, and 3.0-fold higher α-carotene and 3.9, 2.9 and 2.8-fold higher β-carotene levels than those in Kyushu, Sta.Rita Strain. L., and Peacock, respectively.

## 4. Discussions

Carotenoid levels varied widely among the cultivars studied. The Galaxy cultivar from the Philippines showed the highest level of all kinds of carotenoids. Variation in carotenoid content depending on cultivar type and geographical location has been reported in some vegetables. For example, Park et al (2012) reported variation in the carotenoid

content between the skins and flesh of two cultivars of kohlrabi. Our findings are consistent with those of Habicht *et al*. (2011) who reported variations in the levels of saponin, linoleic acid, and linolenic acid among the cultivars of bitter melon. They found that the saponin concentration of white bitter gourd varieties (0.25%) was significantly lower than that of the green varieties (0.67%). Differences in the findings of our study and those of Oishi *et al.* (2007) might result from the differences in the extraction methods and the cultivars used. Oishi *et al* did not provide any information about the variety or plant part used. Nonetheless, their results also suggested that bitter gourd is rich in saponins. Charantin is an important steroidal saponin in bitter gourd, with β -sitosterol and stigmasterol as aglycones (Dans *et al*., 2007; Harinantenaina *et al*., 2006).

Table 1. Carotenoid contents in different cultivars of *Momordica charantia*

| Country | Cultivar | Carotenoid (µg/g dry weight) | | |
|---|---|---|---|---|
| | | lutein | α-carotene | β-carotene |
| Japan | Erabu | 8.09 c | 0.50 d | 0.89 c |
| | Kyushu | 4.77 e | 0.35 f | 0.46 f |
| | Nikko | 13.06 a | 0.49 d | 0.73 d |
| | Peacock | 7.75 c | 0.43 e | 0.64 e |
| | Galaxy | 13.65 a | 1.28 a | 1.78 a |
| | Sta Monica | 13.40 a | 0.94 b | 1.41 b |
| Philippines | Sta. Rita Strain. L | 5.88 d | 0.42 e | 0.66 e |
| | Trident 357 | 4.80 e | 0.46 de | 0.63 e |
| | Verde Buenas | 11.1 b | 0.63 c | 1.35 b |

Mean values (mean of three replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Duncan Multiple Range Test)

Because carotenoids are known to be the precursors of vitamin A, cultivars such as Galaxy that is rich in carotenoids could be used as a food supplement by people earning lower incomes in developing countries.

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