Note

Carotenoid Pigments in GAC Fruit (Momordica cochinchinensis SPRENG)

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The carotenoids in Gac fruit (Momordica Cochinchinensis spreng) were analysed by high-performance liquid chromatography (HPLC), and the concentrations of β-carotene, lycopene, zeaxanthin and β-cryptoxanthin were measured. Lycopene was found to be predominantly present in the Gac seed membrane at a concentration of up to 380 μg/g of seed membrane. The concentration of lycopene in the Gac seed membrane was about ten-fold higher than that in known lycopene-rich fruit and vegetables, indicating that Gac fruit could be a new and potentially valuable source of lycopene.

Key words: carotenoid; momordica; β-carotene; lycopene; Gac

The protective effects of carotenoids against disease are assumed to be through such antioxidative activities as quenching of singlet oxygen and scavenging of peroxyl radicals.1) Carotenoids in nature are responsible for the characteristic colors of various kinds of fruit, vegetables and shellfish. Tomatoes and watermelon with the red color of lycopene, carrots with the orange color of β-carotene, and dark green vegetables are important sources of carotenoid compounds. In Vietnam, a subtropical region, among the wide variety of fruit and vegetables, Momordica cochinchinensis called “Gac” is very popular because of its perfect combination of a natural red color and high β-carotene content.2) Gac is used in Vietnam as a colorant for cooking red glutinous rice, a popular dish in this country, similar to Sekihan (rice boiled together with red beans) in Japan. However, carotenoid data for Gac fruit are limited,2) and a quantitative analysis of the carotenoid pigments in Gac is therefore necessary. In addition, Gac fruit are cheap and easy to grow. Quantitative measurement of the carotenoid compounds in Gac will provide information to support the use of Gac as a useful and economical carotenoid food source.

The inside of Gac contains two parts: (1) fruit meat (yellow part) and (2) red membrane surrounding the seeds (seed membrane), as shown in Fig. 1. In order to obtain data on the whole Gac fruit, we measured the carotenoid pigments in both the fruit meat and seed membrane. Five samples of Gac fruit were purchased from different markets in Ho Chi Minh City and separately analysed. The fruit meat and seed membrane from each sample were separately removed and frozen at −20°C until needed.

The carotenoids were analysed after being extracted and saponified. Briefly, 56 g of fruit meat or 10 g of seed membrane was homogenized and then extracted with a four-fold volume of acetone until complete exhaustion of the color. The extract was filtered and transferred to a separating funnel, together with an equal volume of diethyl ether. The obtained upper phase was washed three times with a saturated NaCl solution, dehydrated with Na2SO4 anhydride, and dried under reduced pressure at 25°C. The dried sample of fruit meat was dissolved in 4 ml of dichloromethane, that of the seed membrane was dissolved in 4 ml of diethyl ether, and acetonitrile was added to each sample to make up to 20 ml.

Fig. 1. Gac Fruit.
1, Fruit meat (yellow part); 2, Seed membrane (red part)
These solutions were analysed by HPLC.

The saponification process was carried out for an analysis of the carotenoid esters. To the dried sample of fruit meat or seed membrane were added 50 ml of ethanol and 5 ml of 60% KOH, and the solution saponified in N₂ gas for 24 h in the dark at 5°C. The solution was then adjusted to pH 4 with HCl and acetic acid, before being transferred to a separating funnel together with 50 ml of petroleum ether. This procedure was repeated once, and the combined upper phase was washed four times with water, dehydrated with Na₂SO₄ anhydride, dried under reduced pressure at 25°C, and analysed by HPLC.

The HPLC analysis was performed with an AS-950 autosampler (Jasco), two PU-980 pumps (Jasco), a DG-980-50 degasser (Jasco), a UV-970 UV/VIS detector (Jasco), a CS-300B column casket (Chromato Science), and a Symmetry C18 column (250 × 4.6 mm i.d., 5 µm; Waters) at 40°C. To analyze the extracted fruit meat samples, the mobile phase consisted of a mixed solution of (A) acetonitrile:dichloromethane: methanol (v/v 7:2:1) and (B) dichloromethane. The column was eluted by using consecutive linear gradients 100% of solution A for 30 minutes, 0–50% of solution B for 5 minutes, and 50% of solution B for 5 minutes. The flow rate was 1 ml/min, detection was at 450 nm, and the injection volume was 25 µl. To analyze the saponified fruit meat, the mobile phase was made up of A acetonitrile and mixed solution B of acetonitrile:dichloromethane:methanol (7:2:1 v/v). The column was eluted by using consecutive linear gradients of 0–100% of solution B for 20 minutes and 100% of solution B for 30 minutes. The flow rate was 1 ml/min, detection was at 450 nm, and the injection volume was 25 µl. The HPLC conditions for the analysis of the extracted and saponified seed membrane were similar to those used for the analysis of the extracted fruit meat. The results of the HPLC analysis were completely reproducible.

The carotenoid pigments were identified by comparing their HPLC retention times (Fig. 2) with those of authentic samples as standards. The content of an authentic sample solution for the HPLC analysis was confirmed from the extinction coefficient of E (1%, 1 cm) in the petroleum ether of lycopene (3450, λₘₐₓ 472 nm), β-carotene (2592, λₘₐₓ 453 nm), zeaxanthin

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**Fig. 2.** Carotenoids in Gac Fruit Meat and Seed Membrane. 
(a) and (b) were analysed under different HPLC conditions. (c) and (d) were analysed under the same HPLC conditions.
of lycopene and 7–37 μg/g of fresh fruit meat. In the saponified samples detected lycopene, 0.1–0.7 μg/g, zeaxanthin and 0.2–1.6 μg/g of β-cryptoxanthin (Fig. 2b). One g of saponified fruit meat contained 4–26 μg of β-carotene, 0.1–0.7 μg of lycopene, 1–2 μg of zeaxanthin, and 2–5 μg of β-cryptoxanthin, giving a total of 8–33 μg of carotenoid pigments. The red color was reduced about 40% by saponification.

The lycopene and β-carotene content in the seed membrane were found to be much higher than that in the fruit meat, both in the extracted and saponified samples (Table 1). As an average, 1 g of extracted seed membrane contained 310–460 μg of lycopene and 60–140 μg of β-carotene, compared to 0.2–1.6 μg of lycopene and 7–37 μg of β-carotene in the extracted fruit meat. In the saponified samples, 1 g of the seed membrane contained 300–400 μg of lycopene and 50–110 μg of β-carotene, compared to 0.1–0.7 μg of lycopene and 4–26 μg of β-carotene in the fruit meat. Zeaxanthin and β-cryptoxanthin were also detected in the saponified seed membrane (Fig. 2d), but their contents were very low (Table 1). About 10% of the red color was reduced by the saponification procedure.

Carotenoids are usually found in fruit and vegetables in the free form or as fatty acid esters. Zeaxanthin and β-cryptoxanthin have hydroxyl groups in the 6-membered ring, and these hydroxyl groups can bind with fatty acids to form carotenoid esters. This is the reason why the carotenoid esters, zeaxanthin and β-cryptoxanthin, were found in the saponified samples, but not in the extracted samples. The degree of esterification of carotenoids in some fruit increases during ripening. Breithaupt et al. have analysed the carotenoid esters in 64 types of fruit and vegetables, and found the highest carotenoid ester concentration in red chili (171 μg/g), while most of the investigated fruit and vegetables showed concentrations up to 15 μg/g.

Many plants are known to contain β-carotene, but few plants containing lycopene are known, except for tomato (31 μg/g of lycopene), watermelon (41 μg/g), guava (54 μg/g) and pink grapefruit (33.6 μg/g) (French food data). Among them, guava had the highest concentration of lycopene. However, the concentration of lycopene in the Gac seed membrane was seven-fold higher than that (380 μg/g). The edible seed membrane in Gac fruit made up an average of 30% (30.2 ± 4.9%, n = 5) and the inedible fruit meat averaged 50% (52.1 ± 7.7%, n = 5). Lycopene exhibits the highest singlet oxygen-quenching capability among carotenoids. The antioxidative effect of lycopene in lowering the risk of developing major chronic disorders such as coronary heart disease and cancer has been reported through many studies. In a clinical trial at Hanoi University in Vietnam, an oil extract of Gac was found to be effective in the treatment of liver cancer.

Our study has revealed that Gac contained a higher lycopene quantity than that in other fruits known to contain lycopene. Gac fruit are easily grown in a subtropical climate; therefore, this new finding is expected to contribute a new promising source of lycopene worldwide.

### References


