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Fatty Acid and Carotenoid Composition of Gac (*Momordica cochinchinensis* Spreng) Fruit

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In this study, we analyzed fatty acid and carotenoid composition of fruit tissues, including seed (which are surrounded by a bright red, oily aril) of *Momordica cochinchinensis* Spreng, known as gac in Vietnam. Carotenoid content was analyzed by reversed-phase HPLC, using a C_{30} column and a method separating cis- and trans-isomers of the major carotenoids in this fruit. Mean values obtained in aril tissues were 1342 μg trans-, 204 μg cis-, and 2227 μg total lycopene; 597 μg trans-, 39 μg cis-, and 718 μg total β-carotene; and 107 μg α-carotene/g FW. Mesocarp contained 11 μg trans-, 5 μg cis-β-carotene/g FW, trace amounts of α-carotene, and no lycopene. Gac aril contained 22% fatty acids by weight, composed of 32% oleic, 29% palmitic, and 28% linoleic acids. Seeds contained primarily stearic acid (60.5%), smaller amounts of linoleic (20%), oleic (9%), and palmitic (5−6%) acids, and trace amounts of arachidic, cis-vaccenic, linolenic, and palmitoleic, eicosa-11-enoic acids, and eicosa-13-enoic (in one fruit only) acids.

KEYWORDS: ***Momordica cochinchinensis* Spreng; fatty acids; carotenoids; HPLC; lycopene; β-carotene; aril; mesocarp; seed; oil.**

INTRODUCTION

Momordica cochinchinensis Spreng, a *Cucurbitaceae*, is indigenous throughout Asia and used as food and for medicinal purposes. The fruit, called gac in Vietnam, are only picked there at maturity from August through February when they are red and the seeds are hardened. Aril, the oily, red, fleshy pulp surrounding the seeds, has a palatable, bland to nutty taste and is cooked along with seeds to impart its red color and flavor to a rice dish, *xoi gac*, served at festive occasions (e.g., weddings) in Vietnam (*1*). Seeds are used in Chinese traditional medicine. Early recognition of the value of gac fruit focused on β-carotene concentration (*2*). West and Poortvliet (*3*) measured 188.10 μg of β-carotene and 891.50 μg total carotenoids/g fresh weight (FW) in gac aril. Chemical analyses by Vuong et al. (*1*) showed that gac aril contained 175 μg of β-carotene and 802 μg of lycopene/g FW (*1*). Lycopene concentration in gac aril is in marked contrast to the 40−60 μg lycopene/g FW found in field-grown tomatoes (*4*, *5*), which is the major source of lycopene in the Western diet. Lycopene, of course, is of interest, because of the correlation of reduced risk of certain cancers, such as prostate (*6*−*8*) and lung (*7*, *9*), with the consumption of tomato products, which is attributed to protection by free radical-quenching lycopene (*10*, *11*). In addition, studies on African-American men having prostate cancer show that daily consumption of lycopene from tomato sauce significantly increased lycopene content of plasma and the prostate gland, decreased their prostate-specific antigen levels (a marker for prostate cancer), and showed significant clinical and metabolic improvements (*12*). Antioxidants seem to have protective effects against cardiovascular diseases (*13*−*16*) and a number of common eye diseases, such as cataracts and age-related macular degeneration (*17*−*20*). In addition, because β-carotene is a precursor to Vitamin A, gac fruit is a potentially valuable source of this vitamin and could be extremely useful in fighting Vitamin A deficiency, which is common in third world countries (*1*).

According to a report by Vuong et al. (*1*), gac aril also contains 102 mg oil/g of FW. These authors also found that, of the total fatty acids in gac aril, 69% are unsaturated, and 35% of these are polyunsaturated (*21*). Vuong and King (*21*) reported that the oil in gac aril contains significant amounts of Vitamin E (334 μg/mL), as well as 3020 μg of lycopene and 2710 μg of β-carotene (and isomers)/mL, making gac aril with its oil a valuable potential source of antioxidants.

Gac seed composition is of interest because of its use in traditional Chinese medicine. Recently, a pentacyclic triterpenoid ester was isolated from the seed (*22*).

Since the completion of this study, a report on carotenoid pigments in gac fruit was published (*23*). Our study, in addition to identification of major carotenoids, includes carotenoid profiles, measuring both trans- and cis-isomers of lycopene and β-carotene. We also provide a detailed fatty acid analysis of gac seed and aril, as well as the weight distribution of anatomical components of the fruit.

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MATERIALS AND METHODS

Fatty Acid Analysis. *Materials.* Gac fruit were purchased from two Asian markets (Vinh Phat and Shun Fat) in Sacramento, California. Fruit had been shipped frozen by commercial exporters from Vietnam to California (storage temperature during transport unknown) and were left frozen in a −20 °C freezer until ready for analyses. Gac fruit were divided carefully into its anatomical components: skin, mesocarp, connective tissue, aril, and seed. Most of the seeds used for analyses were taken from purchased, frozen fruit that had been shipped from Vietnam; a few were a gift from the Guangzhi Province in Western China.

Trifluoroacetic anhydride, 3-pyridyl carbinol, 4-(dimethyl amino) pyridine, and cyclohexane were obtained from Sigma-Aldrich (St. Louis, MO). Nonadecanoic acid methyl ester and GLC-68 fatty acid methyl ester (FAME) standard mixture were obtained from Nu-Chek Prep, Inc. (Elysian, MN). Heptadecanoic acid methyl ester and anhydrous acetyl chloride were purchased from Alltech (Deerfield, IL), and butylated hydroxytoluene (BHT) was obtained from Spectrum Chemical MFG Corp. (Gardena, CA). Anhydrous sodium sulfate was purchased from J. T. Baker Inc. (Philipsburg, NJ), and 2-propanol, methanol, hexane, toluene, diethyl ether, and dichloromethane were obtained from Fisher Scientific (Fair Lawn, NJ). Potassium hydroxide, sodium thiosulfate, sodium chloride, and potassium bicarbonate were obtained from Mallinckrodt Laboratory Chemicals (Philipsburg, NJ). Ethanol was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY). The water used was double distilled, and all chemicals and solvents used were of reagent grade.

Method. Gac aril and mesocarp were thoroughly homogenized using a household-type coffee grinder (Mr. Coffee, Cleveland, OH; Model IDS59) and then dried using a vacuum centrifuge (7−8% dry weight). Gac seed was homogenized using a mortar and pestle. Gac sample (0.05 g) was accurately weighed into 10-mL glass tubes. The lipids were extracted using 2 mL of hexane/2-propanol (8:2, v/v) containing 50 μg/mL of BHT. Internal standard (nonadecanoic acid methyl ester) was added, and the extraction took place at 55 °C for 30 min with shaking every 10 min. Extracts were filtered and dried over sodium sulfate, and the solvent was evaporated under nitrogen. Oil weight was determined gravimetrically. Toluene (0.5 mL) was then added, and the lipids were methylated for 1 h at 80 °C using methanolic hydrogen chloride (3%), as described by Christie (*24*). Resulting FAMEs were dissolved in 10 mL of cyclohexane (0.01% BHT) for GC analysis.

Quantitative analysis was carried out by GC-FID using a Hewlett-Packard 6890 GC system with split injection connected to a 7673 automatic liquid sampler (Agilent Technologies, Palo Alto, CA). Separation was achieved on a DB-WAX column (20-m × 0.12-mm i.d., 0.18-μm film thickness) purchased from J & W Scientific, Agilent Technologies. The injector and detector temperatures were 250 and 280 °C, respectively. The column temperature program was 100 °C for 1 min, then increased by 5 °C/min to 250 °C, and held at 250 °C for 1 min. Standard solutions of a mixture of FAMEs at three different concentrations in the range of 5 to 150 μg/mL were used for generating standard calibration curves. A 50-μL sample of methyl heptadecanoate (1 mg/mL) was added as internal standard to 1-mL aliquots of each standard sample. Injections of 1 μL were used, and duplicate determinations were performed.

Identification of peak components was achieved on a Hewlett-Packard 5890 GC system connected to a 5970A mass selective detector (Agilent Technologies). Split injection was applied, and the same type of column and temperature program as described above was used. Comparison to mass spectra of known FAMEs was used to identify each peak. In addition, double-bond locations for the unsaturated fatty acids were determined by interpreting spectra from picolinyl derivatives of free fatty acids (FFAs), employing the methodology described by Christie (*24*).

Carotenoid Analysis. *Materials.* Dichloromethane, 99.9%, HPLC grade and anhydrous tetrahydrofuran (THF), 99.9%, were purchased from Aldrich Chemical Co. (Milwaukie, WI). Methanol (MeOH), HPLC grade, methyl-*tert*-butyl ether (MTBE), and ethyl acetate (EtOAc), HPLC grade, were purchased from Fisher Scientific (Fair Lawn, NJ). Lycopene for standard solutions was extracted and purified from berries of autumn olive (*Elaeagnus umbellata* Thunberg) plants, which were a gift from Beverly A. Clevidence (Beltsville Human Nutrition Research Center, UDSA, ARS, Beltsville, MD). β-Carotene (type IV from carrots), mixed isomer carotene (from carrots), and lutein (from alfalfa) were purchased from Sigma Chemical Company (St Louis, MO).

Methods. Dry weights of gac aril and mesocarp tissues were determined using a Model AVC-80 microwave moisture/solids analyzer (CEM Corporation, Mathews, NC). Samples of tissue were placed between two tared glass-fiber pads and heated at 50% power for 4.5 min. Moisture content (or percent solids) was determined by difference in weight after drying.

Carotenoids were extracted from gac fruit tissues by the modification (*25*) of the method described by Ishida et al. (*26*). Tissues were excised carefully from gac fruit to avoid cross contamination, especially between aril and mesocarp, then homogenized, using an Omni-Mixer (Sorvall/DuPont Medical Products, Newtown, CT). Gac samples were first extracted, using 5 mL of ice-cold MeOH/homogenate, then the suspension was vacuum-filtered through two layers of Whatman No. 1 filter paper on a Büchner funnel and washed with an additional volume of ice-cold MeOH. The filtrate was saved. The remaining dehydrated residue on the filter was carefully resuspended in 5 mL of dichloromethane and extracted by vacuum filtration three times to remove the red/orange color. The filtrate from the MeOH used to dehydrate the tissue homogenate was combined with dichloromethane extracts. Water (5 mL) was then added to the combined extracts and mixed thoroughly, using a vortex mixer. After phase separation, the bottom yellow layer was transferred to a small vial and dried under nitrogen gas. The residue was then resuspended in 2 mL of THF and passed through a 0.45-mm poly(tetrafluoroethylene) filter (Alltech Associates, Inc., Deerfield, IL). Throughout these procedures, care was taken to keep samples ice-cold and protect them from exposure to light.

Extracts of gac fruit tissue were analyzed for carotenoid content by separation followed by quantitation using a reversed-phase HPLC system, consisting of a Waters (Milford, MA) 2690 Separation Module, 996 Photodiode-Array Detector, auto injector, and column temperature regulator. Separations were accomplished using a reversed phase, analytical (250 × 4.6-mm I. D.), 3-μm particle diameter polymeric C_{30} column (YMC Inc. Wilmington, NC). The system was purged daily for 3 min each with MTBE, MeOH, and EtOAC. The C_{30} column was then conditioned with elution solvent at a flow rate of 1 mL/min for 10 min. Carotenoids were separated isocratically using a mobile phase of 40% MTBE 50%, MeOH, and 10% EtOAc (v/v). Injection volumes ranged from 5 to 20 μL. Column temperature was maintained at 28 °C. The photodiode array detector was set between 300 and 700 nm to detect all of the peaks of interest eluted from the column. Standard compounds: xanthophyll (Sigma; 70% pure from alfalfa); lycopene extracted from autumn olive (*Elaeagnus umbellate* Thunberg) (gift from B. A. Clevidence, USDA Beltsville Human Nutrition Research Center), purified and found to be 97% trans isomer, was used as a standard for quantitation; β-carotene (Sigma; synthetic, Type 1, 95% pure), and α-carotene (Sigma; from spinach, substantially free of β-carotene) were used to check retention times on the HPLC. Phytoene, phytofluene, zeaxanthin, and β-cryptoxanthin were detected by examining spectra of compounds under chromatographic peaks and comparing to known, published spectra of carotenoids to identify these compounds, which are found commonly in fruit such as tomato, guava, and citrus.

RESULTS AND DISCUSSION

Weight Distribution of Fruit Components. Table 1 shows fresh and dry weights and percent weight distributions of the anatomical components of a typical gac fruit. Two whole fruits were analyzed in this way. Most of the fruit is composed of mesocarp and seeds with their surrounding oily pulp (aril). Of these tissues, the mesocarp represents almost half of the weight of the entire fruit.

Fatty Acid Analysis. Data on total FAME content of aril from two gac fruit were collected. Total % weight content of FAME in these two fruit was nearly identical (22%, with relative standard deviations of 2.3 and 12.2%), even though the aril from

Table 1. Weight Distribution of Gac (*Momordica cochinchinensis*, Spreng) Fruit, % Total Fresh Weight ($n = 2$)

fruit part	fresh weight (g)	% dry wt	% total fresh wt
whole fruit	772.0		100.0
aril	190.0	21.7	24.6
seed[a]	130.0		16.8
skin	55.0		7.1
mesocarp	373.7	I = 8.0, O = 6.9[b]	48.4
connective tissue	22.6	10.71	2.9

[a] No. of seeds per fruit = 28, average seed weight = 4.67 g. [b] I = inner mesocarp, O = outer mesocarp.

Table 2. FAME Composition of Gac Aril, % Total FAMEs (n = 2)

FAME	fruit no. 1	fruit no. 2	avg %
myristic (14:0)	0.5	0.5	0.5
palmitic (16:0)	32.1	26.4	29.2
palmitoleic (16: 1 Δ^9)	0.2	0.3	0.3
stearic (18:0)	3.2	12.2	7.7
oleic (18:1 $\Delta^{9)}$	33.7	30.8	32.3
cis-vaccenic (18:1 Δ^{11})	0.9	0.7	0.8
linoleic (18:2 $\Delta^{9,12}$)	28.7	27.5	28.1
α-linolenic (18:3 $\Delta^{9,12,15}$)	0.3	0.8	0.5
arachidic (20:0)	0.1	0.5	0.5
eicosa-11-enoic (20:1 Δ^{11})	0.5	0.3	0.4

Table 3. FAME Composition of Gac Seeds, % Total FAMEs ($n = 3$)

FAME	fruit no. 1	fruit no. 2	fruit no. 3	avg
palmitic (16:0)	6.2	5.2	5.3	5.6
palmitoleic (16:1 Δ^9)	0.1	n.d.[a]	n.d.	0.1
stearic (18:0)	71.7	55.2	54.5	60.5
oleic (18:1 Δ^9)	4.8	11.2	11.0	9.0
cis-vaccenic (18:1 Δ^{11})	0.4	n.d.	0.7	0.5
linoleic (18:2 $\Delta^{9,12}$)	11.2	24.8	25.0	20.3
α-linolenic (18:3 $\Delta^{9,12,15}$)	0.5	0.6	0.4	0.5
arachidic (20:0)	1.3	2.0	1.7	1.6
eicosa-11-enoic (20:1 Δ^{11})	0.8	1.0	1.4	1.1
eisoa-13-enoic (20:1 Δ^{13})	3.0	n.d.	n.d.	3.0

[a] n.d. = not detected.

these fruit were dried differently, one by oven and the other by vacuum centrifugation.

Table 2 shows data on FAME composition (as % total FAME) in the aril of each of the two fruit, as well as average values. Gac aril has high concentrations of oleic, palmitic, and linoleic acids. These data are similar to those reported by Vuong et al. (*1*), although our data show a somewhat higher content of palmitic and lower contents of linoleic and α-linoleic acids. The aril also contains a significant, but varying, amount of stearic acid and small amounts of *cis*-vaccenic, myristic, eicosa-11-enoic, arachidic, and palmitoleic acids.

Our data on total FAME content in gac seed ranged from 15.7 to 36.6% of the total weight of the seed (relative standard deviations varied from 2.6 to 6.1). In **Table 3**, data on FAME composition of gac seed are given. The analysis of average percent composition by weight shows that the primary FAME in the seeds is stearic acid, with an average of 60.5% weight and values ranging from 54.5 to 71.7% weight. Linoleic acid contributed an average of 20.3% weight (range, 11.2−25.0), oleic 9.0% (range, 4.8−11.2), and palmitic acid contributed 5.6% (range, 5.2−6.2), while eicosa-113-enoic acid was found at 3.0%, but only in one fruit. Small amounts of arachidic, cis-vaccenic (in two fruit), α-linolenic, and eicosa-11-eneoic acids were also detected.

Table 4. Carotenoid Composition of Gac Fruit (μG/g FW)[a]

carotenoid	gac aril	gac mesocarp[b]
trans lycopene	1902.9	0.0
SD[c]	122.2	
RSD[d]	6.4	
cis-lycopene isomers	117.0	0.0
SD	17.3	
RSD	14.8	
trans β-carotene	641.0	43.7
SD	70.7	6.0
RSD	11.0	13.7
cis β-carotene	128.7	14.6
SD	7.5	1.6
RSD	5.8	11.0
α-carotene	84.3	13.3
SD	9.7	1.0
RSD	11.5	7.5

[a] Mean values of three samples from a single fruit. [b] Samples were at first divided into inner, outer, top, middle, and bottom to detect gradients, if any, along the thickness and axis of the fruit. No gradients were found, but variations from one location to another occurred. [c] SD = standard deviation, %. [d] RSD = relative standard deviation, %.

The fatty acid composition of the aril and seed are interesting, and they reflect the origin of the extracted oil (*27*−*29*). Aril is considered a "fruit-coat" fat, as described in Hilditch and Williams (*27*), analogous to the pulp surrounding the seed in avocado, olive, and palm. The principal fatty acid components of such fats are palmitic, oleic, and linoleic acids. Gac is somewhat unusual in having a higher proportion of linoleic acid, and given the similar percentage of the three fatty acids, may also have a limited distribution of TAG species. The oil of gac aril also has been reported to have significant amounts of Vitamin E and omega-3 fatty acids (*21*), although our data show only 0.3−0.8% linolenic acid (**Table 2**).

Seeds of tropical plants may contain high levels of saturated fatty acids, with palmitic acid predominant through the plant world. However, some tropical fruits produce seeds with high levels of stearic acid (*28*), including mangosteen (*29*). Because the seed is capable of producing a high stearic acid fat, it has been used as a source for a gene encoding an acyl-ACP thioesterase, which has been used to engineer high stearic acid content in canola (*30*). Increasing the stearic acid content of an oil generally raises its melting point. This approach produces a solid, oxidatively stable fat for shortening, margarine, and frying and obviates the production of trans fatty acids that result from partial hydrogenation of liquid oils to obtain a solid fat.

Carotenoid Profile. For carotenoid analyses, we chose three of the ripest gac fruit that we could find. A typical chromatogram of carotenoids extracted from gac aril is shown in **Figure 1**. Concentrations of the major carotenoids in aril of a single fruit are given in **Table 4**, along with relative standard deviation (RSD) values, which were between 5 and 15%. The ranges of carotenoid values obtained from three fruit are given in **Table 5**. The primary carotenoid in aril is lycopene (range, 1546.5−3053.6 μg/g FW). Of this amount, 2.7−13.2% was present as *cis*-lycopene (82.1−204.4 μg/g FW), and 86.8−97.3% was in the trans-isomeric form (1342.1−2971.5 μg/g FW). The carotenoid having the next highest concentration was β-carotene at 636.2−836.3 μg/g FW, predominately as the trans isomer (74.7−93.9%; 509.7−701.2 μg/g FW). The cis isomer of β-carotene comprised 6.1−25.3% (39.1−172.6 μg/g FW) of the total β-carotene. Of the major carotenoids in aril, α-carotene was present at the lowest concentration (67.0−106.8 μg/g FW).

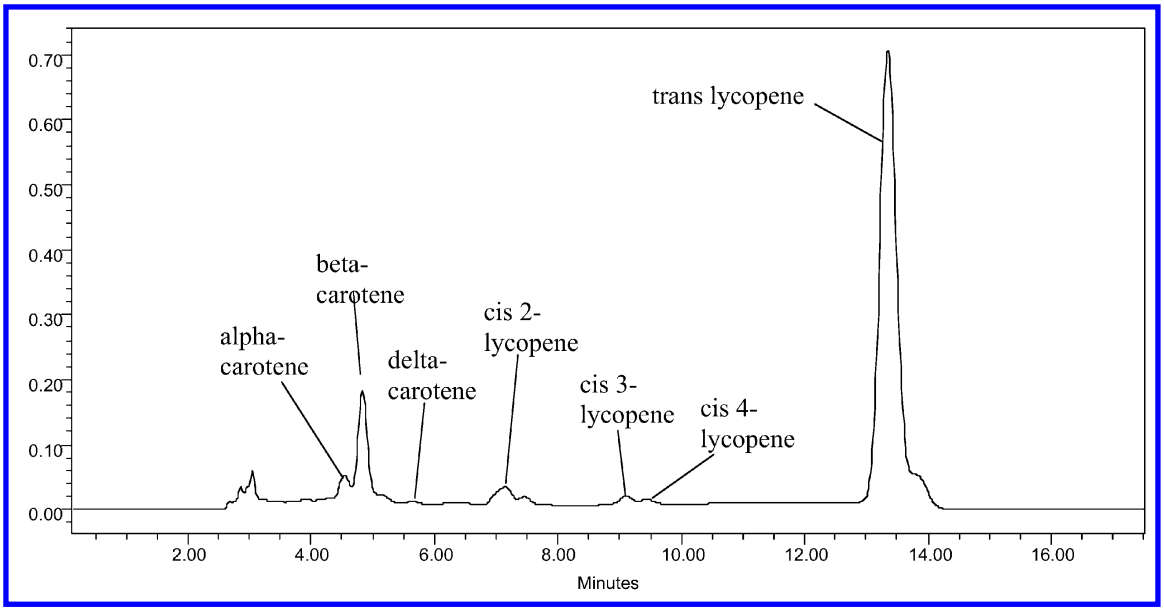

Figure 1. A typical chromatogram of carotenoids obtained after extraction from gac aril and separation by HPLC.

Table 5. Carotenoid Concentrations in Gac Aril Fruit Tissue, Range (μg/gFW)[a]

tissue	lycopene				β-carotene				α-carotene
	trans-	cis-	total	% cis	trans-	cis-	total	% cis	total
aril	1342.1–2971.5	82.1–204.4	1546.5–3053.6	2.7–13.2	509.7–701.2	39.1–172.6	636.2–836.3	6.1–25.3	67.0–106.8
mesocarp	0	0	0	0	11.3–43.7	5.0–14.6	16.3–58.3	25–30.7	6–13.3

[a] Samples from three fruits analyzed in triplicate.

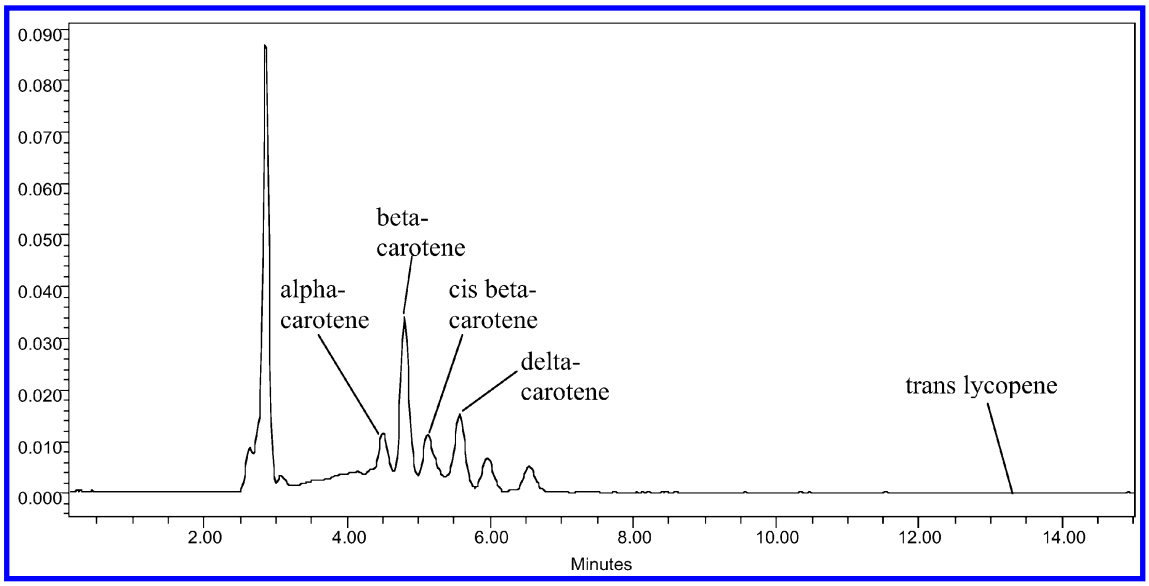

Figure 2. A typical chromatogram of carotenoids obtained after extraction from gac mesocarp and separation by HPLC.

Our data (**Tables 4** and **5**, **Figure 2**) on gac mesocarp show no lycopene, substantial amounts of the trans isomer of β-carotene (range, 11.3−43.7 μg/g FW) and smaller amounts of *cis*-β-carotene. (5.0−14.6 μg/g FW; 25−30.7% of the total), giving a total β-carotene concentration of 16.3−58.3 μg/g FW. Smaller amounts of α−carotene (6−13.3 μg/g FW) were found in gac mesocarp. We also detected phytofluene, phytoene, and trace amounts of zeaxanthin and β-cryptoxanthin, but no lutein in either aril and mesocarp tissues.

In contrast, Aoki et al. (*23*) reported 380 ± 71 μg/g of lycopene in gac mesocarp, compared to our findings of none detectable. The authors also reported 101 ± 38 and 22.1 ± 15.2 μg β-carotene/g FW in extracted samples of aril and mesocarp, respectively, and 16 and 9 μg/g of zeaxanthin and 35 and 2 μg/g of β-cryptoxanthin in saponified samples of mesocarp and aril tissues, respectively. We suggest that the presence of lycopene in gac mesocarp samples was probably a result of contamination of samples with oil from gac aril. In preparing samples for carotenoid analysis, care must be taken to use mesocarp tissues that have not been in direct contact with aril. This is somewhat difficult, because oil from the aril tends to spread over surfaces when the fruit is first cut open.

Carotenoid composition is especially noteworthy, because the aril is such a good source of lycopene and β-carotene, providing

concentrations that exceed most other sources. Our data on lycopene concentration show that the fruit are capable of forming concentrations that are more than 76 times the concentration found in commercial tomato fruit. The concentration of total lycopene in the ripest of the three fruit samples was 3053 μg/g FW, compared to 40−50 μg/g FW in commercially available tomato. Its total β-carotene concentration was 682.3 μg/g FW or 22.3% of the total lycopene concentration in the aril (this ratio of β-carotene/lycopene varied among the three sampled fruit, ranging between 22.3 and 41.1%). β-Carotene concentrations in gac mesocarp were also high, but much lower than those in aril. Our data show higher concentrations of both lycopene and β−carotene extracted from gac fruit tissues than those of others (*1*, *3*, *23*). This might reflect variability of carotenoid concentrations among individual fruits, depending on factors such as degree of ripeness and conditions of culture. In addition, our modified extraction procedure was designed specifically to avoid the loss of *cis*-lycopene isomers, which are of interest because of evidence that shows that the cis-isomers of lycopene and β-carotene are more bioavailable (more readily absorbed) than the trans forms (*11*, *31)*. We also evaluated carotenoid components after HPLC separation of stereoisomers.

The coexistence of both high concentrations of unsaturated fatty acids and carotenoids in gac aril serves to enhance the bioavailability of these carotenoids. Studies show that co-ingestion of lycopene with fat increases the intestinal uptake of both β-carotene and lycopene (*32*, *33*). Thus, it is evident that gac fruit is a valuable source of lycopene and β-carotene, two carotenoids that have been shown to have protective antioxidant effects against the deleterious consequences of various major degenerative diseases.

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