Note

Carotenoid Content and In Vitro Bioaccessibility of Lutein in Some Leafy Vegetables Popular in Sri Lanka

Udumalagala Gamage CHANDRIKA¹,
Basnayake Mudiyansele Lohitha Bandara BASNAYAKE², Indika ATHUKORALA¹,
Pula Wahampurage Nilanka Madurangi COLOMBAGAMA³ and Anil GOONETILLEKE²

¹Department of Biochemistry, Faculty of Medical Sciences, ²Department of Pharmacology, Faculty of Medical Sciences, and ³Department of Food and Science and Technology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

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Summary Lutein is one of the major carotenoids in green leafy vegetables (GLVs), which show a marked antioxidant activity. The study was carried out to identify and quantify the carotenoid content of selected Sri Lankan GLVs. RP-HPLC with photodiode array detection method was followed for the separation, identification and quantification of carotenoids. Among the vegetables analyzed, *Ipomoea batatas* leaves showed the highest β -carotene content ($743.9 \pm 35.0 \mu\text{g/g}$ dry weight (DW)) while *Synгонium angustatum* leaves contained the highest amount of lutein ($1,728.2 \pm 168.3 \mu\text{g/g}$ DW). Percentage in vitro bioaccessibility of lutein from cooked GLVs ranged from 10.1 to 48.0% in stir-fried preparations of *Centella asiatica* and *Cucurbita maxima*, respectively. Hence these GLVs can be exploited as a rich source of β -carotene and lutein to overcome vitamin A deficiency and age-related macular degeneration.

Key Words β -carotene, lutein, HPLC-DAD, green leafy vegetables, in vitro bioaccessibility

Lutein is one of the major carotenoids in green leafy vegetables (GLVs), which show a marked antioxidant activity. It is a predominant carotenoid of human macular pigment and it is known to protect the eye by giving protection against age-related macular degeneration (AMD) by preventing light-initiated oxidative damage to the retina, mediated by its ability to quench singlet oxygen and blue light in the retina (1). Previous studies using humans suggested that the bioavailability of the more hydrophilic lutein from vegetables is 5 times higher than that of β -carotene (2). The content of lutein of even the most widely consumed Sri Lankan GLVs has not been reported. Also no reliable information is available on the relative bioaccessibility of lutein from GLVs. Therefore the content and in vitro accessibility of lutein from Sri Lankan cooked GLVs was studied.

Lutein is the second most prevalent carotenoid in human serum (3) and is abundantly present in dark GLVs such as *Spinacea oleracea* (4). Not only important for eye health, consumption and serum levels of lutein have been shown to be inversely related to the risk of AMD (5, 6) and cataracts (7–9). Recent findings suggest that ingestion of lutein-containing food or supplements results in increased macular pigment optical density (10, 11) and may help to improve visual function in patients suffering from AMD and other ocular diseases (12).

Several studies suggest that lutein and zeaxanthin, a

stereoisomer of lutein, may help maintain the condition of the heart by reducing the risk of atherosclerosis (13, 14). The objective of the present study was (i) to identify and quantify the main carotenoids of thirteen GLVs popular in Sri Lanka and (ii) to determine the in vitro bioaccessibility of lutein in selected heat processed GLVs with high lutein content.

Materials and Methods

Sampling. Thirteen types of fresh green leafy vegetable samples (4 samples from each type) were collected from different points in and around Colombo district, Sri Lanka. They consisted of *Gymnema lactiferum* (Kurigan), *Polyscias scutellaria* (Koppa kola), *Synгонium angustatum* (Wel kohila), *Boerhavia diffusa* (Pitasudu sarana), *Triathema monogyna* (Sarana), *Manihot esculenta* (manioc), *Cucurbita maxima* (Pumpkin leaves), *Ipomoea batatas* (Sweet potato leaves), *Spinacea oleracea* (Nivithi), *Amaranthus caudatus* (Thampala), *Sesbania grandiflora* (Katurumurunga), *Alternanthera sessilis* (Mukunuwanna) and *Centella asiatica* (Gotukola). These plants were taxonomically identified by a botanist (Dr. P. Mahagamasekara) and voucher specimens were deposited in the Botany Laboratory, University of Sri Jayewardenepura for future reference.

RP-HPLC-DAD analysis of carotenoids. Quantification was performed using high performance liquid chromatography with Diode Array Detection (HPLC-DAD), using HPLC series 1200 (Agilent, Germany) equipped with Chem-Station software, a degasser G 13322 A, quaternary gradient pump G1311A, an auto sampler G

E-mail: udumalagala2002@yahoo.com

Fig. 1. RP-HPLC chromatograms of the carotenoids of (a) *Boerhavia diffusa*, (b) *Syngonium angustatum*, (c) *Polyscias scutellaria*, (d) *Gymnema lactiferum*. Peak identification: 1, neoxanthin; 2, violaxanthin; 3, lutein; 4, β -cryptoxanthin; 5, α -cryptoxanthin; 6, β -carotene 5,6,5',6' diepoxide; 7, β -apo-8'-carotenal (internal standard); 8, chlorophyll peak; 9, α -carotene; 10, β -carotene.

1329 A, a column oven G 1316 A and a diode array detector G1315B. The mobile phase consisted of acetonitrile, methanol and ethyl acetate containing 0.05% of triethylamine used at a flow rate of 0.5 mL/min with diode array detection, using a monomeric C₁₈ column (Agilent), 3 μ m, 4.6 $\times$ 250 mm. A gradient was applied from 95 : 05 : 00 to 60 : 20 : 20 in 70 min maintaining this proportion until the end of the run. Purity of the peaks was confirmed using a UV visible photodiode array detector. Quantification of carotenoids was done using external standardization. The internal standard used was β -apo-8'-carotenal.

Isolation of neoxanthin and violaxanthin standards

was performed with open column chromatography (OCC) using *Sesbania grandiflora* leaves. β -Carotene and lutein standards were purchased from Sigma (USA).

Carotenoids for HPLC analysis were isolated as we have done in our previous studies (15). Identification of carotenoids was carried out according to the Rodriguez-Amaya (16).

Preparation of green leaves with scraped coconut. The preparation procedure of GLVs before cooking included washing the sorted leafy vegetables (200 g) after which the leaves were finely cut and mixed with scraped coconut (200 g). Cooking of leafy vegetables involved heating this mixture for 5–10 min in an uncovered pan

Table 1. Carotenoid compositions of some raw green leafy vegetables popular in Sri Lanka.

Green leafy vegetable		β -Carotene ($\mu\text{g/g DW}$)	Lutein ($\mu\text{g/g DW}$)	Neoxanthin ($\mu\text{g/g DW}$)	Violaxanthin ($\mu\text{g/g DW}$)
Botanical name	Local name (Sinhalese)				
<i>Polyscias scutellaria</i>	koppa kola	165.5 $\pm$ 32.1	892.4 $\pm$ 121.9	77.6 $\pm$ 12.5	137.3 $\pm$ 11.9
<i>Gymnema lactiferum</i>	kurignan	162.9 $\pm$ 48.0	928.9 $\pm$ 174.9	90.8 $\pm$ 14.9	162.5 $\pm$ 26.4
<i>Syngonium angustatum</i>	wel kohila	263.4 $\pm$ 42.5	1,728.2 $\pm$ 168.3	109.9 $\pm$ 32.5	280.2 $\pm$ 19.8
<i>Boerhavia diffusa</i>	pitasudu sarana	386.3 $\pm$ 43.6	1,472.6 $\pm$ 205.9	144.5 $\pm$ 22.3	273.3 $\pm$ 17.2
<i>Manihot esculenta</i>	manioc	517.5 $\pm$ 75.6	1,655 $\pm$ 23.4	246.9 $\pm$ 14.5	439.2 $\pm$ 25.6
<i>Triathema monogyna</i>	sarana	560.3 $\pm$ 30.8	760 $\pm$ 20.7	231.6 $\pm$ 12.5	579.8 $\pm$ 20.0
<i>Spinaces oleracea</i>	nivithi	300.9 $\pm$ 18.5	640 $\pm$ 18	96.7 $\pm$ 7.9	241 $\pm$ 13.9
<i>Amaranthus caudatus</i>	thampala	400.4 $\pm$ 25.8	490 $\pm$ 19.5	146.8 $\pm$ 10.6	333.2 $\pm$ 22.7
<i>Cucurbita maxima</i>	pumpkin leaves	655.0 $\pm$ 40.5	1,534.4 $\pm$ 48.2	327.8 $\pm$ 13.7	818.5 $\pm$ 28.0
<i>Ipomoea batatas</i>	sweet potato leaves	743.9 $\pm$ 35.0	1,685.8 $\pm$ 101.7	256.3 $\pm$ 19.4	640.4 $\pm$ 30.5
<i>Sesbania grandiflora</i>	katurumurunga	402.4 $\pm$ 31.1	1,084.8 $\pm$ 21.3	321.8 $\pm$ 13.2	335.9 $\pm$ 20.0
<i>Alternanthera sessilis</i>	mukunuwanna	280.4 $\pm$ 25.4	295.5 $\pm$ 18.2	112.8 $\pm$ 12.6	233.5 $\pm$ 15.3
<i>Centella asiatica</i>	gotukola	255.5 $\pm$ 22.1	980.2 $\pm$ 53.2	102.9 $\pm$ 11.3	255.6 $\pm$ 9.4

$n=4$. DW, dry weight.

with occasional stirring, and salt (1 g) was added at the same time. This preparation is locally called “malluma.”

Preparation of green leaves with coconut oil. Same samples mentioned above were used. Cooking of vegetables (400 g) involved frying for 5–10 min in an uncovered frying pan in boiling coconut oil (100 mL) with occasional stirring. Salt (1 g) was added. This is locally called “stir-fried” preparation of green leaves.

Determination of bioaccessibility of lutein in vitro. Prior to digestion, cooked leafy vegetables were homogenized into pieces similar in size to chewed particles and subjected to an in vitro digestion procedure according to Chandrika et al. (15) that simulated the human gastrointestinal digestion. Briefly, about 5 g of the sample was weighed and put into a screw-capped flask containing 5 mL of distilled water and 1% (w/v) ascorbic acid. The gastric phase (pH 2) was simulated by adding 5 mL of pepsin solution to the sample and pH adjusted with 2 M HCl. The solution was incubated in an orbital shaking water bath at 37°C for 1 h. In the subsequent intestinal phase the pH was increased from 2 to 7.5 and a mixture of pancreatin and bile extract was added. The sample was incubated in a shaking water bath for 30 min. After centrifugation the carotenoids released from the food matrix and appearing in the supernatant were extracted with organic solvents. The in vitro bioaccessible lutein was quantified with the RP-HPLC-DAD method described above.

Statistical analysis. Statistical analysis was carried out in Microsoft Excel. All the results were presented as mean $\pm$ SD, p -values of less than 0.05 were considered to be significant.

Results and Discussion

Major pigments in GLVs used in the present study consist of xanthophylls, chlorophylls and carotenes. A total of eight different major peaks were noted in the 13 vegetables. A range of carotenoids was observed in the

GLVs but for each vegetable, only four main carotenoids were observed as usual. Though the HPLC chromatogram of all GLVs found to be similar with four main carotenoids, namely neoxanthin, violaxanthin, lutein and β -carotene, the major difference appeared to be their concentrations as shown in Fig. 1 for four types of vegetables.

Lutein and β -carotene were common carotenoids present in all leafy vegetables at measurable levels. These carotenoids are of importance in human health (17). The leafy vegetable species studied were rich in β -carotene and lutein as shown in Table 1. β -Carotene has a high pro-vitamin A activity whereas lutein is essential in reducing risk of cancer and macular degeneration. *I. batatas* was the richest source of β -carotene (743.9 $\pm$ 35.0 $\mu\text{g/g}$ dry weight (DW)) while *S. angustatum* showed the highest amount of lutein (1,728.2 $\pm$ 168.3 $\mu\text{g/g}$ DW). The highest concentrations of both neoxanthin and violaxanthin were found in *C. maxima* (327.8 $\pm$ 13.7 $\mu\text{g/g}$ DW and 818.5 $\pm$ 28.0 $\mu\text{g/g}$ DW, respectively).

Lutein showed a wide variation in the concentration in cooked GLVs (Table 2), ranging from 288.5 $\pm$ 35.6 to 1,235.3 $\pm$ 70.4 $\mu\text{g/g}$ DW. It was also evident that cooking caused a reduction in lutein levels (18) (Tables 1 and 2) in stir-fried preparations ($p<0.001$).

The percentage in vitro bioaccessibility of lutein in cooked GLVs varied from 10.1 to 72.9%. It was significantly more than that of β -carotene (12 to 43%) according to previous studies (15). This may be due to the fact that lutein is more hydrophilic than β -carotene.

Conclusion

This study shows that among the selected GLVs, less commonly consumed leaves, namely *Polyscias scutellaria*, *Gymnema lactiferum*, *Syngonium angustatum*, *Boerhavia diffusa*, *Cucurbita maxima*, *Ipomoea batatas* and *Sesbania grandiflora* contain higher lutein content when

Table 2. Influence of heat processing on the bioaccessibility of lutein from test vegetables.

Cooked leafy vegetable	Traditional preparations	Lutein in traditionally cooked vegetable ($\mu\text{g/g DW}$) ^a		
		Total ($\mu\text{g/g DW}$) Mean $\pm$ SD ^b	In vitro accessible ($\mu\text{g/g DW}$) Mean $\pm$ SD ^b	In vitro (%)
<i>Cucurbita maxima</i>	Malluma	835.3 $\pm$ 35.8	300.7 $\pm$ 31.4	35.9
	Stir fried	956.8 $\pm$ 60.2	459.8 $\pm$ 34.6	48.0
<i>Ipomoea batatas</i>	Malluma	903.6 $\pm$ 40.2	300.5 $\pm$ 18.5	33.2
	Stir fried	980.0 $\pm$ 50.5	450.6 $\pm$ 40.5	45.9
<i>Sesbania grandiflora</i>	Malluma	338.5 $\pm$ 30.6	56.2 $\pm$ 10.6	16.6
	Stir fried	833.5 $\pm$ 46.4	359.0 $\pm$ 25.4	43.0
<i>Centella asiatica</i>	Malluma	288.5 $\pm$ 35.6	55.5 $\pm$ 9.8	19
	Stir fried	305.5 $\pm$ 36.5	31.3 $\pm$ 6.3	10.1
<i>Manihot esculenta</i>	Malluma	975.6 $\pm$ 40.9	580.5 $\pm$ 9.3	59.5
	Stir fried	1,097.5 $\pm$ 62.4	800.8 $\pm$ 15.6	72.9
<i>Polyscias scutellaria</i>	Malluma	302.3 $\pm$ 14.5	125.5 $\pm$ 8.4	41.5
	Stir fried	505.6 $\pm$ 20.3	200.6 $\pm$ 10.4	39.6
<i>Gymnema lactiferum</i>	Malluma	400.5 $\pm$ 16.3	137.8 $\pm$ 8.4	34.4
	Stir fried	505.5 $\pm$ 22.6	258.9 $\pm$ 14.6	51.2
<i>Syngonium angustatum</i>	Malluma	965.5 $\pm$ 38.9	376.9 $\pm$ 23.5	39.0
	Stir fried	1,035.7 $\pm$ 48.9	450.4 $\pm$ 20.8	43.5
<i>Boerhavia diffusa</i>	Malluma	855.3 $\pm$ 56.7	238.4 $\pm$ 12.2	27.8
	Stir fried	1,235.3 $\pm$ 70.4	594.3 $\pm$ 23.5	48
<i>Triathema monogyna</i>	Malluma	392.6 $\pm$ 20.4	56.4 $\pm$ 4.5	14.4
	Stir fried	485.8 $\pm$ 37.8	98.2 $\pm$ 7.5	20.2
<i>Spinaces oleracea</i>	Malluma	243.8 $\pm$ 15.4	106.2 $\pm$ 10.4	43.7
	Stir fried	320.2 $\pm$ 13.2	154.2 $\pm$ 25.7	48.1
<i>Amaranthus caudatus</i>	Malluma	138.4 $\pm$ 18.0	85.6 $\pm$ 34.5	61.8
	Stir fried	280.7 $\pm$ 12.6	143.5 $\pm$ 50.3	51.1
<i>Alternanthera sessilis</i>	Malluma	100.7 $\pm$ 11.8	50.2 $\pm$ 13.4	50.2
	Stir fried	125.8 $\pm$ 13.6	60.3 $\pm$ 14.5	48.2

^a Dry matter one gram (g) includes vegetable and coconut oil or scraped coconut solid (fat, protein, etc.).

^b Mean $\pm$ SD of all samples ($n=5$ for total content, $n=5$ for in vitro content).

compared to that of commonly consumed GLVs. Percentage in vitro bioaccessibility of lutein from cooked GLVs is also considerably high in traditional preparations. Hence these GLVs could be exploited as a rich source of β -carotene and lutein to overcome vitamin A deficiency and AMD. These data on the composition of carotenoids could be used in intervention programs to improve the quality of life of the population in Sri Lanka as well as other developing countries.

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