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Comparison of three chosen vegetables with others from South East Asia for their lutein and zeaxanthin content

Yeting Liu, Conrad O. Perera *,[1], Valiyaveettil Suresh

Department of Chemistry, Food Science and Technology Programme, National University of Singapore, S3 Level 6, Science Drive 4, Singapore 117543, Singapore

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Abstract

Three local leafy vegetables chekup manis (*Sauropus androgynus*), West Indian pea tree leaves (*Sesbania grandiflora* (L.) Pers.), and drumstick tree leaves (*Moringa oleifera*), are consumed by local South East Asian populations and are believed to have beneficial effects on improved vision and prevention of eye diseases. High performance liquid chromatography equipped with photodiode array detection was used to investigate their lutein and zeaxanthin contents, which were compared with those from other commonly found vegetables in the region. It was found that these three leafy vegetables contained significantly higher amounts of lutein namely, 19.5, 28.3, and 24.8 mg/100 g edible fresh leaves, respectively, compared to other vegetables in the region. It was also found that cooking in boiling water increase the extractable lutein content in chekup manis by almost 20%, within 4 min.

Keywords: Lutein; Zeaxanthin; Chekup manis; West Indian pea; Drumstick; South East Asia

1. Introduction

Lutein and zeaxanthin (L & Z) are the dominant carotenoids in the human retina (Sommerburg et al., 1999). They represent about 36% and 18% of the total carotenoid content of the retina, respectively (Landrum & Bone, 2001). They are also the only carotenoids detected in the human lens (Yeum, Taylor, Tang, & Russell, 1995). L & Z are selectively deposited from the blood into the macular (Chan, Leung, Lam, & Tso, 1998), which is rich in cone receptors that permit us to have our maximal visual acuity (Krinsky, Landrum, & Bone, 2003). L & Z play very important roles in the human vision perception, although their biological function in the eye is not fully understood. Two functions have been proposed, namely their role as antioxidants (Dagnelie, Zorge, & McDonald, 2000; Semba & Dagnelie, 2003) and the absorption of damaging near-to-UV blue light, the most energetic portion of visible light (Krinsky et al., 2003).

Today, high intake or high serum levels of L & Z have been associated with lower risk of developing cardiovascular disease (Granado, Olmedilla, & Blanco, 2003), several types of cancer (Granado et al., 2003), cataracts (Olmedilla, Granado, Blanco, & Vaquero, 2003) and age-related macular degeneration (AMD) (Krinsky et al., 2003) in a number of epidemiological studies.

L & Z, like the other carotenoids, cannot be synthesized by humans and must be obtained through the diet (Semba & Dagnelie, 2003). Lutein-rich dietary sources are dark green leafy vegetables, such as spinach, broccoli, kale, and lettuce (Hart & Scott, 1995). Although the high intake of lutein has shown to have potential health benefits, it is still not considered as an essential nutrient (Harper, 1999). However, lutein has been considered as a 'conditionally' essential nutrient (Semba & Dagnelie, 2003).

Three dark green leafy vegetables, chekup manis (*Sauropus androgynus*), West Indian pea tree leaves (*Sesbania*

* Corresponding author. Tel.: +65 6516 1655; fax: +65 6775 7895.
E-mail address: pereraco@hotmail.com (C.O. Perera).
[1] Present address: P.O. Box 462, Palmerston North 5301, New Zealand. Tel.:+64 6 3554622; fax: +64 67757895.

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grandiflora), and drumstick tree leaves (*Moringa oleifera*) are consumed by South East Asian populations and are believed to have beneficial effects on improved vision and prevention of eye diseases. So their lutein and zeaxanthin contents were investigated. Chekup manis is a leafy vegetable commonly used in Malay cuisine, and is known to have a pleasant taste, similar to fresh garden peas, and a slightly nutty flavour. They are normally eaten raw in salads or steamed and added to stir-fry rice and egg dishes, soups, or casseroles (Fletcher, 1998). Drumstick tree leaves are eaten as greens in salads and in vegetable curries in Malaysia and the Indian sub-continent. They have been applied as poultice to sores, rubbed on the temples for headaches, and said to have purgative properties (Duke, 1983a). West Indian pea tree leaves are commonly used in Indian cuisine. They are lightly boiled or steamed and served as a vegetable or mixed with curries or salad. It is also considered as a folk medicine for bruises (Duke, 1983b).

Some L & Z rich leafy vegetables, such as spinach and kailan (Chinese kale), were also studied for the purpose of comparison. Other vegetables probably unique to Asian diets are amaranth (Chinese spinach) and Ceylon spinach (Malabar spinach). Knowing their lutein content is very important to understand the dietary lutein intake of the Asian populations. Hence the objective of this research was to study the lutein content in three green leafy vegetables known for their ability to improve eye health and to compare their levels with those from other popular vegetables consumed in the region.

2. Materials and methods

2.1. Materials

Fresh samples of chekup manis (*Sauropus androgynus*), West Indian pea tree leaves (*Sesbania grandiflora*), drumstick tree leaves (*Moringa oleifera*), red and green-leaf amaranth (*Amaranthus tricolor*), green-leaf amaranth (*Amaranthus tricolor*), local kailan (*Brassica oleracea* var. *alboglabra*), Chinese kailan (*Brassica oleracea* var. *alboglabra*), mini kailan (*Brassica oleracea*var. *alboglabra*), spinach (*Spinacea oleracea* L.), red-stemmed Ceylon spinach (*Basella rubra*), and green-stemmed Ceylon spinach (*Basella alba*) were purchased from the local wet-market early in the morning to ensure their freshness. They were brought to the laboratory and were prepared immediately for analysis.

2.2. Sample preparation

The fresh leaves bought from the local market were picked off from the branches and washed with tap water. The water remaining on the surface was removed by blotting with tissue paper and freeze-dried for 48 h until the samples were completely dry. During the freeze-drying process, the samples were protected from light by covering the sample container with an aluminum foil. The dried samples were ground into powder at room temperature under dim light. The powdered samples were vacuum packed in plastic–aluminum bags and kept below −20 °C until they were used for extraction.

2.3. Lutein and zeaxanthin extraction

A sample of 0.1 g freeze-dried powder was extracted in 25 ml acetone solution at room temperature in the dark and with continuous shaking. Butylated-hydroxy-toluene (0.3 g) was added to prevent the oxidation of carotenoids. After 4 h of extraction, the sample was centrifuged (4500*g*, 4 °C, 15 min) and the supernatant was collected. The residue was re-extracted with 25 ml acetone as before for another 4 h. The acetone extract was collected after centrifugation in the same way as described above. The residue was extracted a third time as above but with 22 h continuously shaking. The acetone extract was collected as before. All the three acetone extracts were combined and dried under vacuum by using the vacuum rotary evaporator at 40 °C in dim light. In addition, the rotary extractor was also covered with aluminum foil to prevent light falling on the sample. The dried extract was re-dissolved in 5 ml methanol and used for HPLC analysis.

2.4. High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) equipped with photodiode array detector (PAD) is widely used for separation and quantification of carotenoids (Almela, Fernandez-Lopez, & Lopez-Roca, 1992). ODS-1 column (a non-capped, lightly carbon loaded C_{18} column) has been tested for separation of carotenoids and has been recommended by Gilmore and Yamamoto (1991).

The HPLC method used by Gilmore and Yamamoto (1991) was adapted and improved as given below. The eluents used were HPLC-grade acetonitrile and methanol, and Tris–HCl buffer (0.1 M; pH 8). They were filtered through a 0.45 μm cellulose acetate syringe filter (Schleicher & Schuell Ltd., Dassel, Germany) and sonicated for half an hour to remove dissolved air bubbles before use. In the whole separation process, the column temperature was maintained at 25 °C and the sample in auto-sampler was maintained around 4 °C. PAD was used to record the chromatogram simultaneously at wavelengths 450 nm, 431 nm, 461 nm, and 290 nm and to determine the peak spectra for identification of carotenoids and for the checking of peak purity. The run time for each sample was only 35 min. The column was equilibrated with 20% methanol, 73% acetonitrile, 7% Tris–HCl buffer for half an hour before analyses and it was regenerated by washing with acetonitrile after analysis. The elution conditions are shown in Table 1.

Compounds were identified according to their chromatographic behavior on HPLC and UV–vis absorption spectra. Both the retention times and characteristics of the absorption spectra of each peak were compared with those available for standards. PAD measurements of spectral properties for the individual peaks (from 200 to

Table 1
Gradient table of HPLC separation

Time (min)	Flow rate (ml/min)	Buffer composition (%)		
		Methanol	Acetonitrile	Tris–HCl
0	2	20	73	7
3	2	20	73	7
3.1	1	20	73	7
21	1	20	73	7
40	2	20	73	7
41	2	0	100	0
71	2	0	100	0
72	0.1	0	100	0

600 nm) were determined at the up-slope, apex, and down-slope. The matching of the three spectra indicated the degree of peak purity. The chromatograms were evaluated quantitatively by relating the peak areas of the individual carotenoids at 450 nm.

In this experiment, a Waters Alliance HPLC system, equipped with Waters 2996 photodiode array detector (Waters, Milford, MA, USA) was used for the analyses. The separating column used was Waters ODS-1 column, 250 × 4.6 mm, 5 μm particle size (Dublin, Ireland).

2.5. Study of lutein changes during cooking

A sample of 10 g fresh chekup manis leaves was immersed in 150 ml of boiling water in a Schott conical flask and was boiled with continuous stirring on a hotplate. They were removed at regular intervals of 4 min, 8 min, 12 min, 16 min, and 20 min. After that, they were prepared and analyzed using the same method described for fresh leaves. The water used for cooking was extracted using 20 ml hexane using a separation funnel. This process was repeated twice. The combined hexane extract was dried and reconstituted in 5 ml methanol using the same method described in lutein extraction section. The methanol extract was used for HPLC analysis.

2.6. Moisture determination

The moisture contents of different leafy vegetables were measured in triplicate as the loss in weight using vacuum-oven method (AOAC, 2002, Method No. 925.09).

2.7. Standards

The reference standards of lutein (purity 93%) and lycopene (purity 98%) were purchased from Sigma–Aldrich Chemicals (St. Louis, USA). Chlorophyll *a* (purity 95%), chlorophyll *b* (purity 95%), and β-carotene (purity 97%) were purchased from Fluka (Buchs, Switzerland). Zeaxanthin (purity 95%) and β-cryptoxanthin (95%) were obtained from ChromaDex (Santa Ana, California). Stock solutions of 200 ppm of each of the standards was prepared by dissolving 1 mg standard in HPLC grade methanol to make 5 ml solution using 5 ml volumetric flask. The standard solutions with various concentrations were then prepared by diluting the stock solution with HPLC grade methanol in appropriate amounts, using a series of volumetric flasks. All the preparations were done in dim light at room temperature (20 °C). The standard solutions were stored in dark at −20 °C immediately after preparation until they were used for HPLC analysis. Triplicates of each standard solution at various concentrations were analyzed and the mean values were used to construct the calibration curves.

2.8. Calibration of standards

The procedure of Scott, Finglas, Scale, Hart, and de Froidmont-Gortz (1996) was followed for the calculation of the concentrations of the standards for the construction of the standard curve. The concentrations of lutein and zeaxanthin were calculated from the absorbance readings at 450 nm using molar extinction coefficients ($E^{1\%}_{1\ cm}$) of 2550 and 2480, respectively, with correction for their purities. Furthermore, absorbance measurements of the individual peaks (from 200 nm to 600 nm) were determined at the up-slope, apex, and down-slope. The matching of the three spectra indicated the degree of peak purity.

2.9. Statistics

Lutein and zeaxanthin values reported are the means of triplicate determination on three batches of each vegetable ($n = 9$) with their standard deviations (±SD), except for the effect of cooking experiment, where triplicate analysis of two batches ($n = 6$) were used. Tests for analysis of variance were carried out using SPSS version 12.0 (SPSS Inc., Chicago, USA) to determine the significant differences between the lutein or zeaxanthin values reported. Values differing at the confidence level of $p < 0.05$ are considered significantly different.

3. Results and discussion

3.1. Calibration curves of standards

The selected reference standards were used as external standards for the identification and quantification of the HPLC peaks. The retention time and the wavelength of maximum absorption of each standard are listed in Table 2. The chromatogram of all the standards at 450 nm is

Table 2
Retention time and maximum absorption wavelength (λ_{max}) of standard peaks

Standard Peaks	Retention time (min)	Wavelengths of maximum absorption (nm)
Lutein	13.9	451.4, 480.6
Zeaxanthin	15.3	448.0, 475.8
Chlorophyll *b*	16.9	461.3
β-Cryptoxanthin	22.4	451.1, 481.9
Chlorophyll *a*	22.7	431.2

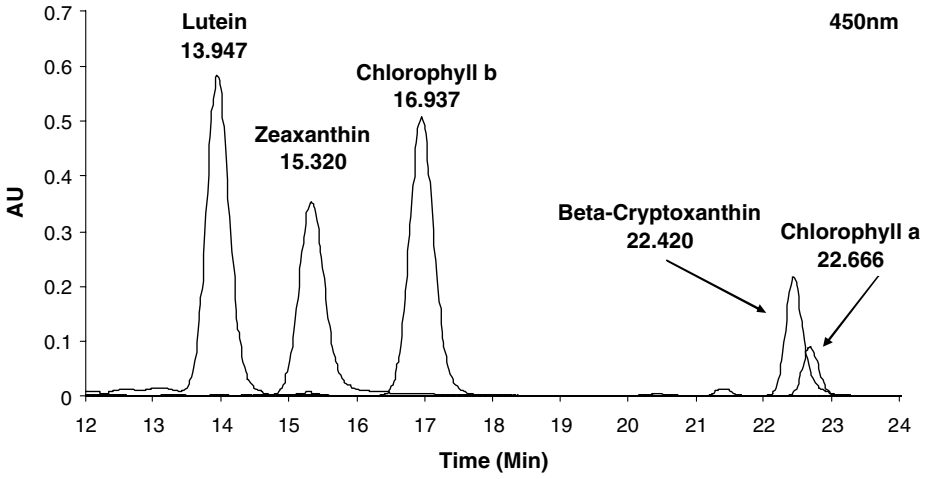

Fig. 1. Chromatogram of external standards and their retention time.

shown in Fig. 1. It was clear from the chromatogram that the lutein and zeaxanthin peaks were well separated, which means that the common resolution problem between these two peaks during carotenoid analyses has been well resolved using the modified HPLC method employed in this study. The calibration curves of absorbance responses at 450 nm (peak area) against solution concentrations of lutein and zeaxanthin standard were linear in the concentration range studied, and the fitted lines showed very good correlations of $R^2 = 0.9959$ and $R^2 = 0.9806$ for lutein and zeaxanthin, respectively.

3.2. Lutein and zeaxanthin content in leaves

Fig. 2 shows a typical chromatogram of the lutein, zeaxanthin, and other carotenoids of West Indian Pea tree leaves. Due to the poor-separation between β-cryptoxanthin and chlorophyll *a*, the retention time of β-cryptoxanthin was confirmed by spiking the sample with that compound. All the chromatographic peaks were found to be pure as confirmed by checking the wavelength of maximum absorption λ_{max}, and the characteristics of the absorption spectra of each of the peaks at their up-slope, apex, and down-slope. Among those peaks identified, only lutein and zeaxanthin contents were quantified. Table 3 summarizes the lutein and zeaxanthin content found in the three tree leaves as well as other common vegetables from South East Asia, expressed on the basis of dry matter and fresh weight. On the basis of fresh weight, West Indian pea tree leaves had the highest level of lutein, followed by drumstick leaves and chekup manis. All other green leafy vegetables investigated in this study, were lower compared to these three tree leaves. When expressed on the basis of dry matter however, red and green-leafy amaranth had the highest level of lutein, followed by green-leafy amaranth. West Indian pea tree leaves, drumstick leaves and chekup manis had significantly higher levels of lutein than Chinese kailan, mini kailan, or green-stemmed Ceylon spinach and red stemmed Ceylon spinach. Recently, Lakshminarayana, Raju, Krishnakantha, and Baskaran

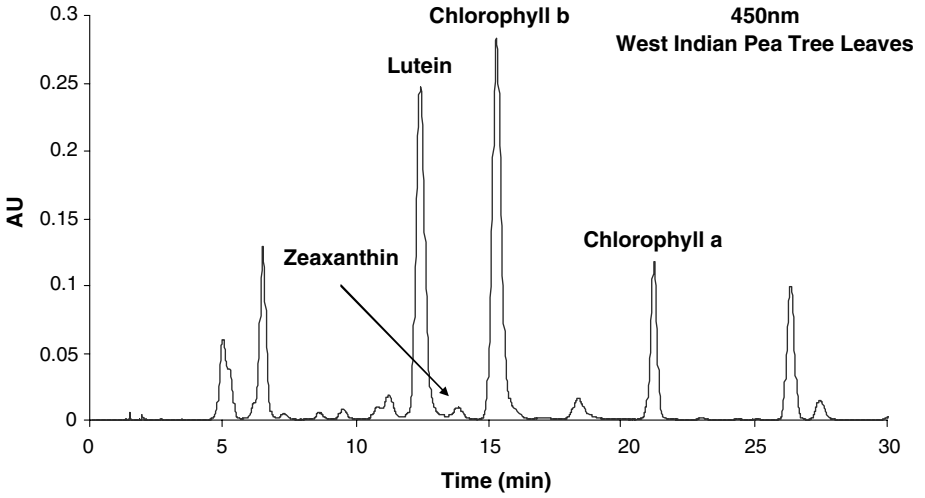

Fig. 2. Chromatogram of West Indian pea tree leaf extract.

Table 3
Lutein and zeaxanthin content in selected leafy vegetables

Sample	% Dry matter[a]	Lutein content		Zeaxanthin content	
		mg/100 g Of dry matter[b]	mg/100 g Of fresh weight [b]	mg/100 g Of dry matter[b]	mg/100 g Of fresh weight[b]
West Indian Pea tree leaves	19.5 ± 0.4	145.2 ± 5.1a	28.3 ± 1.1a	7.1 ± 1.7a	1.4 ± 0.3a
Drumstick tree leaves	17.8± 0.5	139.7 ± 11.2a	24.8 ± 2.1a	7.3 ± 1.2a	1.3 ± 0.2a
'Chekup manis'	17.6 ± 0.3	110.6 ± 4.1b	19.5 ± 0.8b	48.1 ± 3.7b	8.5 ± 0.7b
Red and green-leaf amaranth	8.2 ± 0.3	180.0 ± 6.7c	14.7 ± 0.7c	3.0 ± 2.7c	0.3 ± 0.2c
Green-leaf amaranth	9.5 ± 0.3	151.6 ± 7.6a	14.3 ± 0.9c	NQ	NQ
Local kailan	9.3 ± 0.4	139.4 ± 3.8a	13.06 ± 0.7d	NQ	NQ
Spinach	8.9 ± 0.2	133.7 ± 5.4a	11.9 ± 0.6d	30.7 ± 4.2d	2.7 ± 0.4d
Chinese kailan	13.3 ± 0.5	88.5 ± 9.8d	11.8 ± 1.4d	69.9 ± 12.8e	9.3 ± 1.7b
Mini kailan	13.2 ± 0.6	45.1 ± 1.7e	6.0 ± 0.4e	NQ	NQ
Green-stemmed Ceylon spinach	10.9 ± 1.9	52.0 ± 1.9e	5.7 ± 1.0e	59.0 ± 4.1e	6.5 ± 1.2e
Red-stemmed Ceylon spinach	4.9 ± 0.2	99.5 ± 3.0d	4.9 ± 0.3e	3.7 ± 4.3c	0.2 ± 0.2c

Values expressed as mean ± standard derivation ($n = 9$).
(*Note*: NQ means the zeaxanthin peak in the corresponding HPLC chromatograph was not large enough for quantification.)
[a] Dry mater obtained from vacuum oven moisture determinations.
[b] Means followed by a different letter within a column are significantly different at ($p < 0.05$).

(2005) reported lutein values for West Indian pea tree leaves, drumstick leaves, red-stemmed Ceylon spinach and spinach, on the basis of 100 g of dry matter. Their results were significantly lower than those that we report here for similar products. They however did not analyse chekup manis. The probable reason for this different may be the different cultivars that they obtained from India, the soil conditions and other environmental effects. Such difference are known to occur in a number of natural products of the same species. Also they reported poor separation between lutein and zeaxanthin peaks in HPLC chromatogram. However, our lutein content in fresh spinach is comparable to the value reported by Humphries and Khachik (2003).

In Table 3, the lutein and zeaxanthin contents in the three tree leaves were compared with those found in other common green leafy vegetables from the region. The dry matter was calculated from the moisture determination. The three types of tree leaves under investigation had significantly higher levels of L & Z than those found in other common vegetables when expressed on a wet weight basis. Perhaps, it is more meaningful to express the L & Z values on the basis of wet weight rather than dry weight basis, as they are generally consumed fresh of just cooked and not in the dried form. L & Z were expressed in both dry basis and wet basis in order to compare with literature values.

It was estimated that the average daily dietary L & Z intake in European populations is about 2 mg (O'Neill et al., 2001). Mares-Perlman, Fisher, and Klein (2001) reported in their analysis of the third National Health and Nutrition Examination Survey that American adults on average consume l–2 mg lutein/day. These values of dietary intake of lutein are far below the amounts required to reduce the risk of AMD or cataract. Seddon et al. (1994) found that 6 mg/day of lutein lead to a 43% lower risk of AMD. Chasan-Taber et al. (1999) conducted a prospective study of 77,466 female nurses 45–71 years old from 1980 through 1992 and found that those with the highest intake of lutein and zeaxanthin had 22% lower risk of cataract extraction compared to those in the lowest quintile of intake. A recent study (Richer et al., 2004) shows that 10 mg L & Z per day is required to improve the vision in AMD risk patients. Thus it is clear that, despite the lutein's continuously emerging role as an important nutrient for human health, the daily dietary intake of L & Z in US and Europe is far below the levels purported to reduce the risk of eye diseases, including cataracts and AMD.

It is clear from Fig. 3, that consuming just 100 g (wet weight) of West Indian pea tree leaves will provide nearly 30 mg of L & Z per day. Similarly, 100 g of drumstick leaves and chekup manis leaves (less than half a cup) will also provide nearly 26 mg and 28 mg of L & Z per day, respectively. These values are nearly three times higher than the levels needed to improve vision in AMD patients (Richer et al., 2004). The high L & Z contents in the three leafy vegetables studied may help to increase the L & Z intake of the populations in South East Asia. Besides, the three leafy vegetables studied, other leafy vegetables consumed by South East Asians are; amaranth (Chinese spinach) kailan (Chinese Kale), and Ceylon spinach (Malabar spinach). Knowing their lutein content is also very important to understand the dietary lutein intake of the Asian population. Amaranth, including green-leafy amaranth and red and green-leafy amaranth, contains relatively high amount of lutein (14.3–14.7 mg/100 g). Kailan, including local kailan, Chinese kailan, and mini kailan, has medium amount of lutein. The zeaxanthin levels in chekup manis (8.5 ± 0.7 mg/100 g) and Chinese kailan (9.3 ± 1.7 mg/100 g) were the highest among the vegetables in this study (Table 3). Ceylon spinach was found to contain relatively low levels of lutein, but the green-stemmed Ceylon spinach was high in zeaxanthin content (6.5 ± 1.2 mg/100 g).

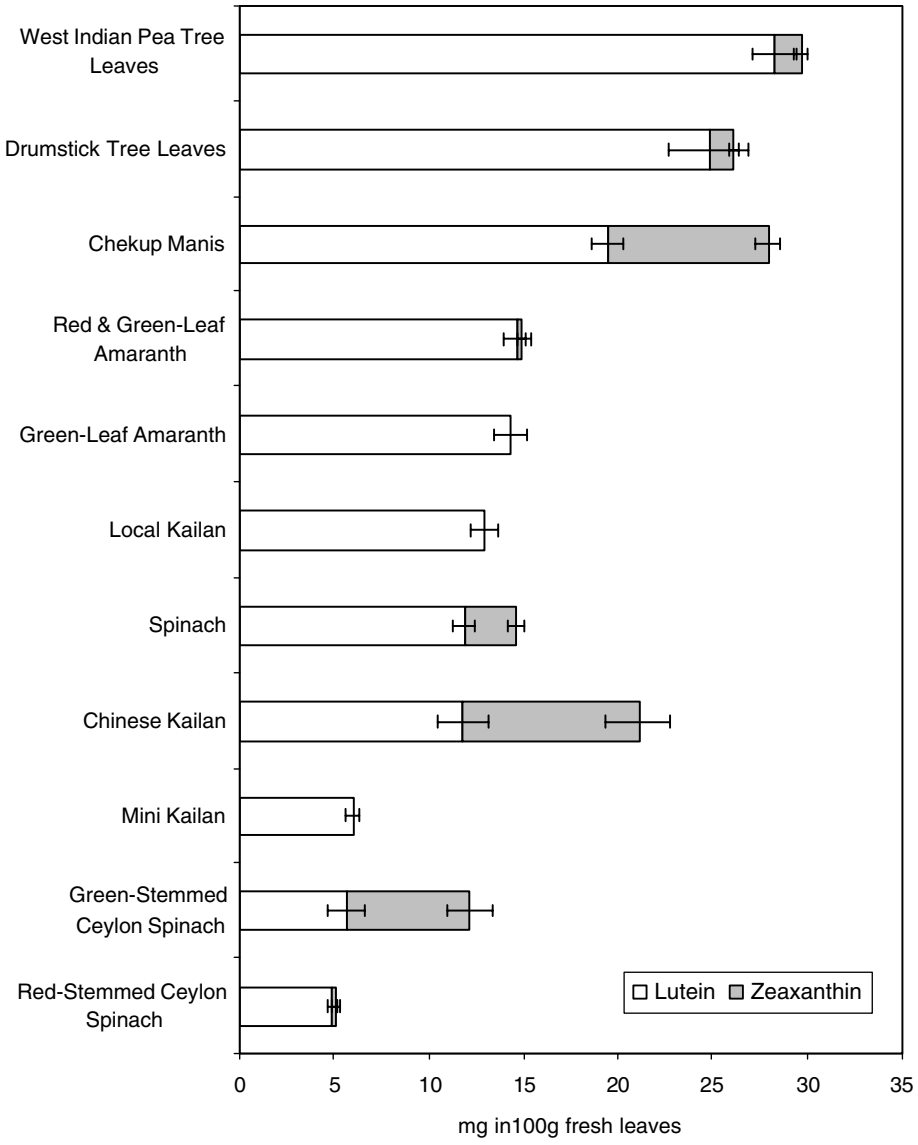

Fig. 3. Comparison of lutein and zeaxanthin content between selected leafy vegetables.

3.3. Effect of cooking on lutein

In this study, chekup manis was chosen to test the effect of cooking on total analyzable lutein. The results showed that the solids content decreased from an initial level of 17.1% to the 12.2% after cooking (Table 4). Meanwhile, the analyzed lutein content (both on dry basis and wet basis) increased significantly ($p < 0.05$). Cooking for 20 min increased the total lutein content detected by 28%, compared to that found in the raw product, which indicated that the lutein was released from the food matrix during the cooking process. Lutein content in the aqueous phase after cooking was also analyzed but none was detected, suggesting that no leaching of lutein into the water during the cooking process had occurred. A number of previous studies have shown that cooking increases the carotenoid content of various vegetables and tomatoes (Granado, Olmedilla, Blanco, & Rojas-Hidalgo, 1992; Khachik et al., 1992) and our findings confirm this effect. It was reported by Alves-Rodrigues and Shao (2004) that the lutein from cooked spinach showed a absorption of 21% whereas less than 1%

Table 4
Change of lutein content in 'chekup manis' during cooking (100 °C)

Processing time (min)	% Dry matter	Mean lutein content[a]	
		mg/100 g Of dry matter[b]	mg/100 g Of fresh weight[b]
0	17.1 ± 0.1	139.2 ± 3.3a	23.8 ± 0.6a
4	12.0 ± 0.2	237.5 ± 7.8bc	28.5 ± 1.0bc
8	11.9 ± 0.1	223.5 ± 9.6c	26.6 ± 1.2c
12	12.0 ± 0.2	240.5 ± 10.2bc	29.0 ± 1.3bc
16	11.9 ± 0.1	241.1 ± 6.9b	28.7 ± 0.8b
20	12.2 ± 0.2	248.7 ± 9.8b	30.3 ± 1.3b

[a] Values reported as mean ± SD ($n = 6$).
[b] Means with a common letter within a column are not significantly different at ($p < 0.05$).

of lycopene was absorbed from fresh tomatoes. For the lutein in the vegetable to be absorbed, it must be released from its food matrix and be accessible to the brush-boarder of the small intestine in the human body in a form that can be absorbed by the enterocytes (Alves-Rodrigues & Shao, 2004). This process is dependent on time of residence in the small intestines, and other factors, such as the composition of the meal, the presence and efficiency of digestive enzymes and other endogenous digestants, and the status of food. The bioavailability of carotenoids appear to be promoted by various means of processing, such as cooking, chopping, grating, and juicing (McEligot et al., 1999; van het Hof, West, Weststrate, & Hautvast, 2000).

4. Conclusion

It is clear from this study that the three leafy vegetables (chekup manis, sesbania, and drumstick), which were the focus of this study had significantly higher levels of lutein and zeaxanthin than the other commonly consumed vegetable in South East Asia. Lutein and zeaxanthin are known to be effective in the prevention of AMD and catarach. This may be the scientific retionale in support of the believes of the local populations, that these leafy vegetables are good for eye health and vision.

References

Almela, L., Fernandez-Lopez, J. A., & Lopez-Roca, J. M. (1992). High-performance liquid chromatography-diode-array detection of photosynthetic pigments. *Journal of Chromatography, 607*, 215–219.

Alves-Rodrigues, A., & Shao, A. (2004). The science behind lutein. *Toxicology Letters, 150*, 57–83.

AOAC (2002) (17th ed.). *Official methods of analysis of AOAC international* (Vol. 2). Gaithersburg, MD, USA: Association of Official Analytical Chemists.

Chan, C., Leung, I., Lam, K. W., & Tso, M. O. M. (1998). The occurrence of retinol and carotenoids in human subretinal fluid. *Current Eye Research, 17*, 890–895.

Chasan-Taber, L., Willett, W. C., Seddon, J. M., Stampfer, M. J., Rosner, B., Colditz, G. A., et al. (1999). A prospective study of carotenoid and vitamin A intakes and risk of cataract extraction in US women. *American Journal of Clinical Nutrition, 70*, 509–516.

Dagnelie, G., Zorge, I. S., & McDonald, T. M. (2000). Lutein improves visual function in some patients with retinal degeneration, a pilot study via the Internet. *Optometry, 71*, 147–164.

Duke, J. A. (1983a). *Moringa oleifera* Lam. In *Handbook of energy crops*, http://www.hort.purdue.edu/newcrop/duke_energy/Moringa_oleifera.html Last Visited, January 31, 2004.

Duke, J. A. (1983b). *Sesbania grandiflora* (L.) Pers. In *Handbook of energy crops*, http://www.hort.purdue.edu/newcrop/duke_energy/Sesbania_grandiflora.html Last Visited, January 31, 2004.

Fletcher, R. (1998). *Sauropus androgynus* (sweet leaf bush). *The Australian New Crops Newsletter*, *9*, http://www.newcrops.uq.edu.au/newslett/ncnl9191.htm Last Visited, January 31, 2004.

Gilmore, A. M., & Yamamoto, H. Y. (1991). Resolution of lutein and zeaxanthin using a non-end capped lightly carbon-loaded C_{18} high performance liquid chromatographic column. *Journal of Chromatography, 542*, 137–145.

Granado, F., Olmedilla, B., & Blanco, I. (2003). Nutritional and clinical relevance of lutein in human health. *British Journal of Nutrition, 90*, 487–502.

Granado, F., Olmedilla, B., Blanco, I., & Rojas-Hidalgo, E. (1992). Carotenoid composition in raw and cooked Spanish vegetable. *Journal of Agriculture and Food Chemistry, 40*, 2135–2140.

Harper, A. E. (1999). Defining the essentiality of nutrients. In M. E. Shils, J. A. Olson, M. Shike, & A. C. Ross (Eds.), *Modern nutrition in health and disease* (9th ed., pp. 3–10). Baltimore: Williams and Wilkins.

Hart, D. J., & Scott, K. J. (1995). Development and evaluation of an HPLC method for the analysis of carotenes in vegetables and fruits in the UK. *Food Chemistry, 54*, 101–111.

Humphries, J. M., & Khachik, F. (2003). Distribution of lutein, zeaxanthin, and related geometrical isomers in fruits, vegetables, wheat, and pasta products. *Journal of Agricultural and Food Chemistry, 51*, 1322–1327.

Khachik, F., Goli, M. B., Beecher, G. R., Holden, J., Lusby, W. K., Tenorio, M. D., et al. (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomato and several green vegetables. *Journal of Agricultural and Food Chemistry, 40*, 390–398.

Krinsky, N. L., Landrum, J. T., & Bone, R. A. (2003). Biological mechanism of the protective role of lutein and zeaxanthin in the eye. *Annual Review of Nutrition, 23*, 171–201.

Lakshminarayana, R., Raju, M., Krishnakantha, T. P., & Baskaran, V. (2005). Determination of major carotenoids in a few Indian leafy vegetables by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 53*, 2838–2842.

Landrum, J. T., & Bone, R. A. (2001). Lutein; zeaxanthin; and the macular pigment. *Archives of Biochemistry and Biophysics, 385*, 28–40.

Mares-Perlman, J. A., Fisher, A. I., Klein, R., et al. (2001). Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *American Journal of Epidemiology, 153*, 424–432.

McEligot, A. J., Rock, C. L., Shanks, T. G., Flatt, S. W., Newman, V., Faerber, S., et al. (1999). Comparison of serum carotenoid responses between women consuming vegetable juice and women consuming raw or cooked vegetables. *Cancer Epidemiology Biomarkers and Prevention, 8*, 227–231.

O'Neill, M. E., Carroll, Y., Corridan, B., Olmedilla, B., Granado, F., Blanco, I., et al. (2001). An European carotenoid database to assess carotenoid intakes and its use in a five country comparative study. *British Journal of Nutrition, 85*, 499–507.

Olmedilla, B., Granado, F., Blanco, I., & Vaquero, M. (2003). Lutein; but not α-tocopherol; supplementation improves visual function in patients with age-related cataracts, a 2-y double blind placebo-controlled pilot study. *Nutrition, 19*, 21–24.

Richer, S., Stiles, W., Statkute, L., Pei, K. Y., Frankowski, J., Nyland, J., et al. (2004). Doublemasked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry, 75*, 216–230.

Scott, K. J., Finglas, P. M., Scale, R., Hart, D. J., & de Froidmont-Gortz, I. (1996). Interlaboratory studies of HPLC procedures for the analysis of carotenoids in foods. *Food Chemistry, 57*(1), 85–90.

Seddon, J. M., Ajani, U. A., Sperduto, F. L. D., Hiller, R., Blair, N., Burton, T. C., et al. (1994). Dietary carotenoids, Vitamins C and E, and advanced age-related macular degeneration. *Eye Disease Case–Control Study Group Jam, 272*, 1413–1420.

Semba, R. D., & Dagnelie, G. (2003). Are lutein and zeaxanthin conditionally essential nutrients for eye health? *Medical Hypotheses, 61*(4), 465–472.

Sommerburg, O. G., Siems, W. G., Hurst, J. S., Lewis, J. W., Kliger, D. S., & van Kujik, F. G. J. M. (1999). Lutein and zeaxanthin are associated with photoreceptors in the human retina. *Current Eye Research, 19*, 491–495.

van het Hof, K. H., West, C. E., Weststrate, J. A., & Hautvast, J. G. A. J. (2000). Dietary factors that affect the bioavailability of carotenoids. *Journal of Nutrition, 130*, 503–506.

Yeum, K. J., Taylor, A., Tang, G., & Russell, R. M. (1995). Measurement of carotenoids; retinoids and tocopherols in human lens. *Investigative Ophthalmology and Visual Science, 36*, 2756–2761.