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Carotenoid Composition and Content of Malaysian Vegetables and Fruits by the AOAC and HPLC Methods

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ABSTRACT

The β-carotene contents of forty vegetables and fourteen fruits were determined using the AOAC open-column (magnesia and Hyflo Super Cel mixture) chromatographic method and compared with a newly developed reverse-phase HPLC method, in which carotenoids were separated isocratically on an octadecylsilane (C_{18}) column using a ternary mixture of acetonitrile, methanol and ethyl acetate. Results obtained showed that the AOAC method gave falsely elevated results for samples containing α-carotene, as well as those with very low β-carotene concentrations. On the other hand, the HPLC method successfully separated and quantitated the major carotenoids present; namely, lutein, cryptoxanthin, lycopene, γ- and α-carotenes in addition to β-carotene. The carotenoid composition of most of the green vegetables was rather consistent, comprising only lutein and β-carotene. In contrast, there was no clear pattern of carotenoids present in the other vegetables and fruits, where several other carotenoids were detected in varying proportions. The vitamin A activity, expressed as μg of retinol equivalent (RE), was calculated on the basis of all pro-vitamin A carotenoids (cryptoxanthin, γ-, α- and β-carotenes) detected. Most of the green leafy vegetables, including several local vegetables, had high RE. Several green non-leafy and other vegetables were found to have low and medium RE. None of the fruits studied may be said to have high vitamin A activity. RE calculated

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on the basis of results from the AOAC method was found to be erroneously low for samples with significant proportions of pro-vitamin A carotenoids other than β-carotene, and falsely elevated for those with α-carotene. Total carotenoid concentrations can be estimated by taking absorbance readings of sample extracts directly in a spectrophotometer or by the HPLC method.

The study clearly shows that the HPLC method would give a more complete picture of the carotenoid composition as well as a more accurate quantitation of the vitamin A value of the vegetables and fruits. The nutritional significance of the findings is obvious since these foods are important sources of vitamin A for the majority of the communities in the country.

INTRODUCTION

Vitamin A deficiency remains one of the major public health nutritional problems in many developing countries, and is an important cause of preventable blindness. The compounds pertinent in this deficiency problem, that afflicts particularly young children, are carotenoids, the major contributors of vitamin A in the diet of most communities in the region. Although an extensive literature on these compounds has been built up, carotenoids are still being actively studied all over the world, since many gaps in knowledge exist and new frontiers are being pursued (Tee, 1988).

A basic need in carotenoid research and development is knowledge of the content and composition of this group of pigments, which are widely distributed in nature and found without exception in photosynthetic tissues. In recent years, there has been particular emphasis on an understanding of the types and concentrations of various carotenoids in foods for two main reasons. First, this is of importance in relation to the pro-vitamin A activity of the carotenoids. It is thought that previously reported values of vitamin A activity in food composition tables may have been unreliable since methodologies used were not sufficiently discriminative and thus had included carotenoids that do not possess vitamin A activity (Zakaria *et al.*, 1979; Beecher & Khachik, 1984; Underwood, 1984; Bureau & Bushway, 1986). Some of these carotenoids may occur in higher concentrations than β-carotene, the most potent precursor of vitamin A. Second, carotenoids, including those without vitamin A activity, are now thought to play important roles beyond their classical functions in nutrition and vision. With their highly conjugated double bonds, carotenoids may act as free radical traps or antioxidants, and therefore play important roles in cancer causation and prevention (Peto *et al.*, 1981; Olson, 1986; Temple & Basu, 1988).

The authors have embarked on a systematic study to develop improved

methodologies for the separation and quantitation of retinol and several carotenoids in foods and biological specimens, especially blood serum. A systematic review of the literature on methods for carotenoid analysis was first carried out (Tee & Lim, 1991). Initially, several studies into the physico-chemical characteristics of retinol and several carotenoids in various solvents and laboratory conditions were carried out to assist in the choice of analytical conditions. A combination of UV–vis spectroscopy and HPLC was used in these studies. An HPLC system for the analysis of these compounds was next developed, studying particularly the solvent system, peak detection and quantitation, and sample preparation. The objective was to develop a simple system, workable for routine determination of retinol and several carotenoids in a wide variety of foods of both plant and animal origin. It is hoped that the method developed could be used to re-evaluate the vitamin A value of foods tabulated in the current Malaysian Food Composition Table (Tee *et al.*, 1988).

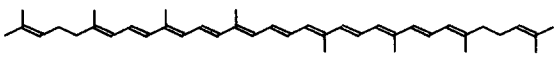
The HPLC method developed was applied to a variety of foods. Each food sample was also simultaneously determined for vitamin A and carotenoids by the open-column chromatographic procedure of the AOAC (Williams, 1984), with the aim of determining whether the difference in β-carotene values thus far reported using the AOAC method were significantly different from the more specific HPLC method. If differences exist, it is hoped that the study will indicate which types of vegetables and fruits show the greatest differences. Such differences have been mentioned by various investigators, but there has not been a detailed comparison for a wide variety of foods as in the present study.

MATERIALS AND METHODS

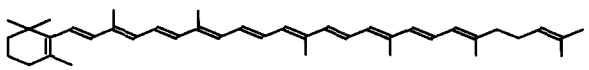
Solvents and carotenoid standards

Solvents used for sample preparation and pre-treatment and for open-column chromatography procedures, were all analytical-grade reagents. Solvents for high-pressure liquid chromatography were of HPLC grade. All solvents for use as the mobile phase in HPLC were filtered through a 0·45 μm regenerated cellulose membrane filter and degassed using an ultra-sonic bath.

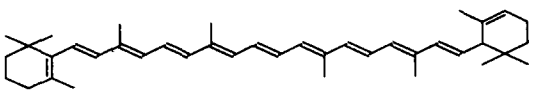
α- and β-Carotenes and lycopene standards were purchased from Sigma Chemical Company. γ-Carotene, *β-apo*-carotenal, cryptoxanthin, zeaxanthin, and lutein were gifts from F. Hoffmann La-Roche, Switzerland. The eight carotenoids used have varying structures, including the acyclic conjugated polyenelycopene; carotenoids with *psi*, *beta* and *epsilon* end-groups; oxygenated carotenoids; and an *apo*-carotenoid (Fig. 1). UV–vis



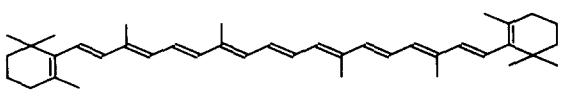
I. Lycopene (ψ, ψ-carotene)



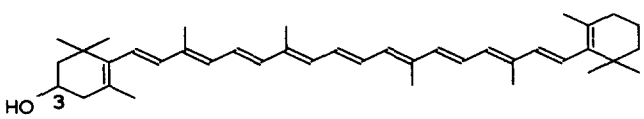
II. γ-Carotene (β, ψ-carotene)



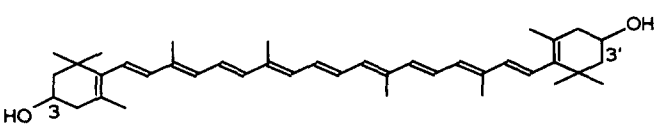
III. α-Carotene (β, ε-carotene)



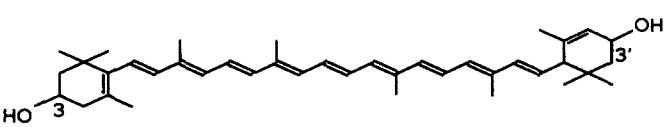
IV. β-Carotene (β, β-carotene)



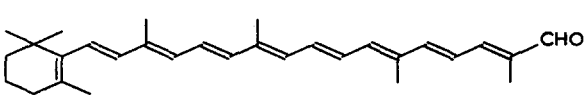
V. β-Cryptoxanthin; 3-hydroxy-β-carotene (β, β-caroten-3-ol)



VI. Zeaxanthin (β, β-carotene-3,3'-diol)



VII. Lutein; xanthophyll; 3,3'-dihydroxy-α-carotene (β,ε-carotene-3,3'-diol)



VIII. β-*apo*-8'-Carotenal (8'-*apo*-β-caroten-8'-al)

Fig. 1. Structures of carotenoid standards (systematic names of carotenoids given in parentheses).

absorption spectra of these standards were determined and are given in Fig. 2. Stock solutions of these carotenoids were prepared in hexane (except that lutein and zeaxanthin were prepared in ethanol and *β-apo*-carotenal in petroleum ether) in concentrations of 100 μg per ml and stored in amber bottles below −20°C. Working solutions of 1 μg per ml of the standards were prepared daily. The appropriate extinction coefficients published in the literature (De Ritter, 1981) were used to calculate the exact concentration of each of the carotenoids. The preparation of all standard carotenoids was carried out with no unnecessary delay, in a room with subdued light and with all windows tinted with a light-protective film. All sample treatment and analytical procedures were also carried out in this room.

Sample preparation and pre-treatment

Commonly consumed vegetables and fruits were purchased from markets and stalls. Samples were chosen from various groups of vegetables and fruits with differing characteristics. Edible portions of the foods were size-reduced in a blender and 2–10 g immediately weighed for analysis.

Sample pre-treatment procedures were essentially those of AOAC (Williams, 1984), except for the introduction of a saponification step. Preliminary studies carried out by the authors have shown that saponification was able to remove other pigments (mainly chlorophyll) from the food samples, which would otherwise interfere in the chromatography process, especially in the HPLC method. The saponification process did not appear to affect the β-carotene content, although there was some loss of lutein and other xanthophylls, which have no vitamin A activity.

To duplicate portions of the test sample were added a volume of 100% (w/v) potassium hydroxide equal to the weight of the food sample used, and 40 ml of ethanol. The mixture was saponified on an electric heating mantle for 30 min. The saponified mixture was cooled and extracted with 25 ml portions of hexane until the extract was colourless. The hexane extracts were pooled, washed until free of alkali, dried over sodium sulphate, and reduced to a small volume by heating over a water-bath with the aid of a stream of oxygen-free nitrogen. The resulting solution was made up immediately to a suitable volume (e.g. 25 ml) with hexane, referred to hereafter as the 'test solution'.

The total carotenoid concentration of the test solution was determined as described in the next section. The solution was next subjected to two chromatographic and subsequent quantitation procedures: (1) by open-column chromatography using magnesia and Hyflo Super Cel mixture and quantitation using absorbance reading at 450 nm (AOAC method); and (2) high-pressure liquid chromatography and detection and quantitation at

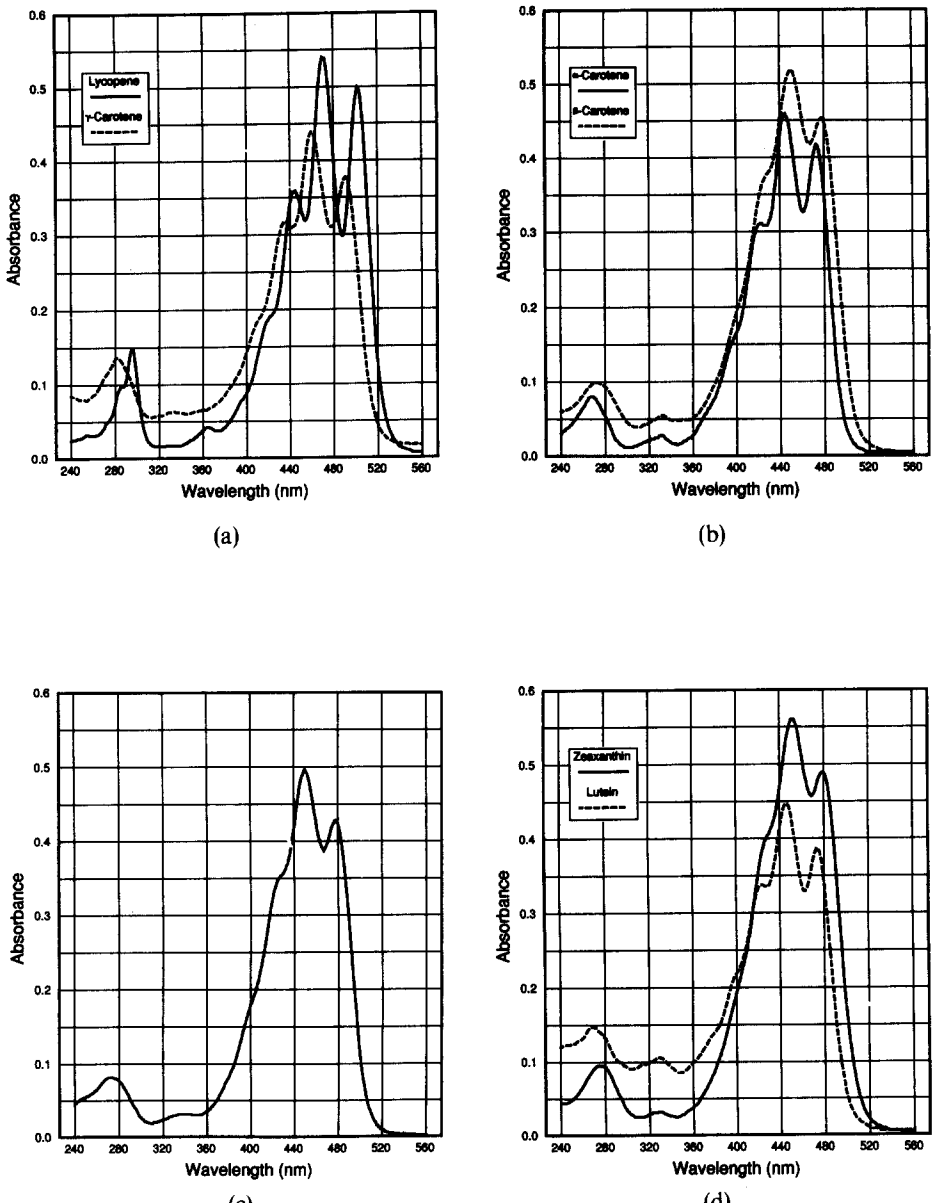
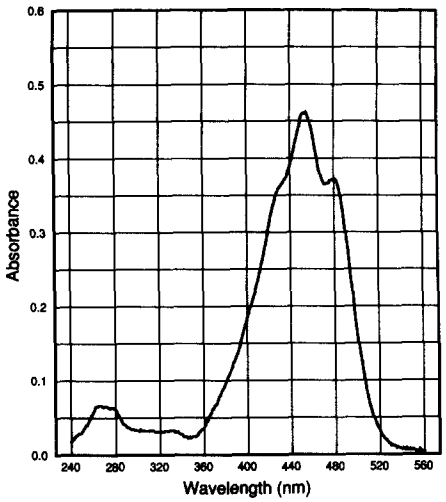


Fig. 2. UV–Vis absorption spectra of (a) lycopene and γ-carotene in hexane (2 μg/ml); (b) α- and β-carotene in hexane (2 μg/ml); (c) cryptoxanthin in hexane (2 μg/ml); (d) zeaxanthin and lutein in ethanol (4 μg/ml).



(e)

Fig. 2—*contd.*(e) β-apo-carotenal in petroleum ether (2 μg/ml).

436 nm (HPLC method). The analytical procedures are summarised in Fig. 3.

Determination of total carotenoid concentration

The test solution was read directly in a spectrophotometer at 450 nm and a β-carotene standard curve was used to calculate the total carotenoid content. The results obtained are referred to as having been obtained by the 'direct spectrophotometric method'.

The total carotenoid concentration was also determined by the high-pressure liquid chromatographic (HPLC) method described below.

Open-column chromatography (AOAC method)

A suitable volume (e.g. 10 ml) of the test solution was pipetted into a glass column prepacked with a mixture of activated magnesia (Sea Sorb 43) (Fisher Scientific Co. or Sigma Chemical Co.) and diatomaceous earth (Hyflo Super Cel) (Fisher Scientific Co.), in the ratio of 1:1, for chromatography using the AOAC method (Williams, 1984). β-Carotene was eluted from the column with approximately 80 ml of 10% (v/v) acetone in hexane. The eluate was evaporated on a water-bath with the aid of a stream of nitrogen and made up to a suitable volume (e.g. 10 ml) with hexane. The absorbance of the solution was read in a spectrophotometer at 450 nm and the concentration of β-carotene calculated by comparison against a calibration curve prepared with the β-carotene standard.

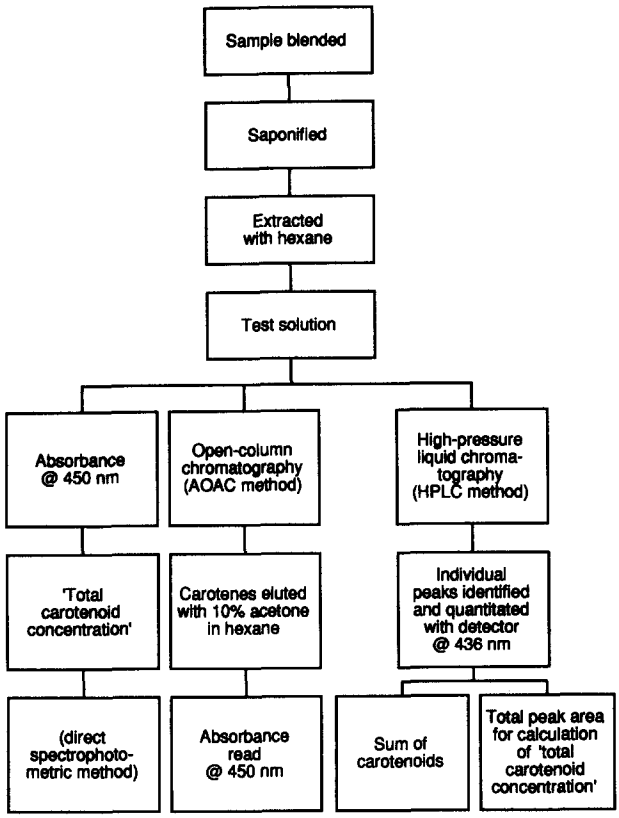


Fig. 3. Summary of analytical procedures.

High-pressure liquid chromatography (HPLC method)

HPLC conditions

A Waters high-pressure liquid chromatograph equipped with a 440 nm fixed-wavelength detector was used. A 436 nm wavelength kit was fitted onto the detector and an attenuation of 0·02 absorbance units full scale (AUFS) was set. Another detector with a 313 nm wavelength kit and connected in series to the first, was found to be useful in assisting monitoring of carotenoids, particularly the *cis*-isomers of carotenes. A stainless steel 30 cm × 3·9 mm i.d. 10 μm μBondapak C_{18} column was used for the chromatographic separation. This was preceded by a Waters Guard-PAK pre-column module housing a disposable Guard-PAK pre-column insert packed with the same material as that in the analytical column. Sample injection volumes, dispensed using a Rheodyne 7125 injector, were usually 50 to 100 μl. A Waters 6000A solvent delivery system was used to deliver the mobile phase (acetonitrile–methanol–ethyl acetate, 88:10:2, v/v) at the rate of 2·0 ml/min. Peak areas were quantified with a Waters 730 Data Module.

Chromatography of carotenoids

Hexane in the test solution was first evaporated off on a water-bath with the aid of nitrogen gas. The residue was immediately redissolved in a suitable volume of the mobile phase. After passing through a 0·45 μm regenerated cellulose membrane filter, suitable volumes were injected into the chromatograph. Identification and quantitation of the carotenoids were carried out by comparing with reference carotenoids similarly chromatographed. Some food samples were found to contain a few carotenoids which could not be identified. The concentrations of these carotenoids were estimated as β-carotene. The concentrations of individual carotenoids were summed to give 'sum of carotenoids'. Total peak areas from the chromatograms were used to determine total carotenoid content, calculated using a β-carotene standard curve.

To assist in the identification of carotenoids in the samples, the pigments were eluted from the magnesia column (by the AOAC method) using a stepwise increase in the proportion of acetone in hexane. The absorption spectrum of each eluate was obtained and an aliquot injected into the HPLC to determine its purity and retention time. These data were compared with those given by authentic carotenoid standards. Details of these procedures were reported in Tee & Lim (1990).

RESULTS AND DISCUSSION

This paper presents results for forty vegetables and fourteen fruits, listed in Tables 1 and 2, respectively. The English names of the foods are first listed, followed by the local names (in the Malay and other languages) and the scientific names. Where the English names are not available, the scientific names of the foods are used in the tables as well as the figures in this paper.

Total carotenoid content

The total carotenoid concentration of each vegetable or fruit was determined by two methods: (1) taking the absorbance reading of the hexane extract at 450 nm previous to any chromatographic separation ('direct spectrophotometric method'); and (2) calculating the concentration from total peak area of HPLC chromatograms (HPLC method). There are two major limitations of total carotenoid concentrations determined by either method. Detection at a single wavelength (450 nm for the 'direct spectrophotometric method' and 436 nm for the HPLC method) is not adequate since absorption maxima for the various carotenoids differ considerably. The calculation based on β-carotene as the reference standard

TABLE 1
Names of Vegetables Studied

English name	*Local name*	*Scientific name*
Green, leafy		
Cashew leaves	*Pucuk gajus*	*Anacardium occidentale*
Ceylon spinach	*Remayong*	*Basella rubra*
C. griffithii	*Cemperai*	*Champereia griffithii*
Chinese cabbage	*Pak-coy*	*Brassica chinensis*
Chinese chives	*Kucai*	*Allium odorum*
Chinese kale	*Kai-lan-coy*	*Brassica alboglabra*
Chinese mustard leaves	*Sawi*	*Brassica juncea*
Coriander leaves	*Daun ketumbar*	*Coriandrum sativum*
Curry leaves	*Karrupillay*	*Murray koenigii*
Drumstick leaves	*Daun kelor*	*Moringa oleifera*
Fern shoots	*Pucuk paku*	*Diplazium esculentum*
H. javanica	*Pegaga gajah*	*Hydrocotyle javanica*
Lettuce	*Sang-coy*	*Lactuca sativa*
Mint leaves	*Daun pudina*	*Mentha arvensis*
M. citrifolia	*Daun Mengkudu*	*Morinda citrifolia*
N. oleracea	*Tanki*	*Neptunia oleracea*
Papaya shoots	*Daun betik*	*Carica papaya*
Salted vegetable	*Hum-coy*	—
S. androgynus	*Cekur manis*	*Sauropus androgynus*
Sesbania	*Daun turi*	*Sesbania grandiflora*
S. nigrum	*Ranti*	*Solanum nigrum*
Spinach	*Bayam putih*	*Amaranthus viridis*
Spinach, red	*Bayam merah*	*Amaranthus gangeticus*
Spring onion	*Daun bawang*	*Allium fistulosum*
Swamp cabbage	*Kangkung*	*Ipomoea aquatica*
Tapioca shoots	*Pucuk ubi kayu*	*Manihot utilissima*
Wolfberry leaves	*Kau-kei-coy*	*Lycium chinense*
Green, non-leafy		
Chilli, green	*Lada hijau*	*Capsicum annuum*
Four-angled bean	*Kacang botor*	*Psophocarpus tetragonolobus*
French bean	*Kacang buncis*	*Phaseolus vulgaris*
Long bean (dark green)	*Kacang panjang*	*Vigna sinensis*
Long bean (light green)	*Kacang panjang*	*Vigna sinensis*
Paprika/Bell pepper	*Lada hijau besar*	*Capsicum annuum*
Snake gourd	*Ketola ular*	*Tricosanthes anguina*
S. torvum	*Terung pipit*	*Solanum torvum*
Others		
Carrot	*Lobak merah*	*Daucus carota*
Chilli, red	*Lada merah*	*Capsicum annuum*
Pumpkin	*Labu merah*	*Cucurbita maxima*
Tomato	*Tomato*	*Lycopersicum esculentum*
Yam stalks	*Batang keladi*	—

TABLE 2
Names of Fruits Studied

English name	*Local name*	*Scientific name*
Banana (var. 1)	*Pisang emas*	*Musa sapientum*
Banana (var. 2)	*Pisang tanduk*	*Musa sapientum*
B. macrophylla	*Buah kundang*	*Bouea macrophylla*
Jackfruit	*Nangka*	*Artocarpus heterophyllus*
Mandarin orange	*Limau Cina*	*Citrus reticulata*
Mango (Black-gold)	*Mangga*	*Mangifera indica*
Musk lime	*Limau kesturi*	*Citrus microcarpa*
Orange	*Limau manis*	*Citrus nobilis*
Papaya	*Betik*	*Carica papaya*
Papaya exotica	*Betik eksotika*	*Carica papaya*
Plum, red	*Buah plum*	*Prunus* spp.
Starfruit/Carambola	*Belimbing manis*	*Averrhoa carambola*
Tree tomato	*Tomato pokok*	*Cyphomandra betacea*
Watermelon, red	*Tembikai*	*Citrullus vulgaris*

is also an approximation since the different carotenoids have different extinction coefficients.

Tables 3 and 4 show total carotenoid concentrations in the vegetables and fruits obtained by the 'direct spectrophotometric' and HPLC methods. Figures 4 and 5 show the ratios of results obtained by the former method to those by the HPLC method, while Fig. 6 shows similar results obtained for the fruits. A ratio of unity indicates similar results were given by the two methods. For most of the vegetables and fruits (forty-six out of fifty-four samples studied), the ratios obtained were between 0·6 and 1·0, i.e. varying within $\pm 20\%$ from 0·8. The results indicate that the HPLC method tended to give slightly higher total carotenoid concentrations. In spite of the limitations mentioned above, the data obtained could provide useful estimations of total carotenoid concentrations of vegetables and fruits.

Carotenoid composition

Only the HPLC method was able to give the carotenoid composition of the vegetables and fruits studied. The HPLC conditions employed gave satisfactory separation for lutein (retention time, RT = 3·6 min), cryptoxanthin (RT = 6·0 min), lycopene (RT = 7·5 min), γ-carotene (RT = 9·0 min), α-carotene (RT = 10·2 min), and β-carotene (RT = 10·8 min). Zeaxanthin, structurally very similar to lutein (Fig. 1) was minimally separated from the latter. β-*apo*-8′-Carotenal, with the carbon skeleton shortened to 30, was also minimally separated from lutein. However, all three carotenoids had

TABLE 3
Total Carotenoid Content[a] of Selected Vegetables Determined by Direct Spectrophotometric and HPLC Methods

Name of vegetable	*Direct spectrophotometric method*	*HPLC method*
Green, leafy		
Cashew leaves	1778	1989
Ceylon spinach	3432	4556
C. griffithii	11833	17310
Chinese cabbage	2703	3728
Chinese chives	3688	4369
Chinese kale	4395	5299
Chinese mustard leaves	3237	3741
Coriander leaves	4050	4344
Curry leaves	11569	14170
Drumstick leaves	12986	14565
Fern shoots	1944	2278
H. javanica	4281	4832
Lettuce	151	162
Mint leaves	5412	6242
M. citrifolia	7047	12272
N. oleracea	14788	16708
Papaya shoots	2120	2517
Salted vegetable	7312	9635
S. androgynus	31822	39910
Sesbania	21366	37896
S. nigrum	8330	9462
Spinach	5481	7058
Spinach, red	6111	6788
Spring onion	1885	1547
Swamp cabbage	2083	2078
Tapioca shoots	5738	6975
Wolfberry leaves	10976	12773
Green, non-leafy		
Chilli, green	678	810
Four-angled bean	555	589
French bean	525	784
Long bean (dark green)	776	1070
Long bean (light green)	368	792
Paprika/Bell pepper	300	453
Snake gourd	302	338
S. torvum	179	215
Others		
Carrot	9468	9997
Chilli, red	5678	6077
Pumpkin	1830	2212
Tomato	1276	1224
Yam stalks	92	131

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.

TABLE 4
Total Carotenoid Content[a] of Selected Fruits Determined by Direct Spectrophotometric and HPLC Methods

Name of fruit	*Direct spectrophotometric method*	*HPLC method*
Banana (var. 1)	99	119
Banana (var. 2)	239	332
B. macrophylla	1 029	1 353
Jackfruit	193	197
Mandarin orange	669	916
Mango (Black-gold)	540	615
Musk lime	406	419
Orange	334	515
Papaya	2 489	3 664
Papaya exotica	3 107	4 057
Plum, red	230	286
Starfruit/Carambola	726	1 483
Tree tomato	1 604	1 583
Watermelon (red)	4 748	4 365

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.

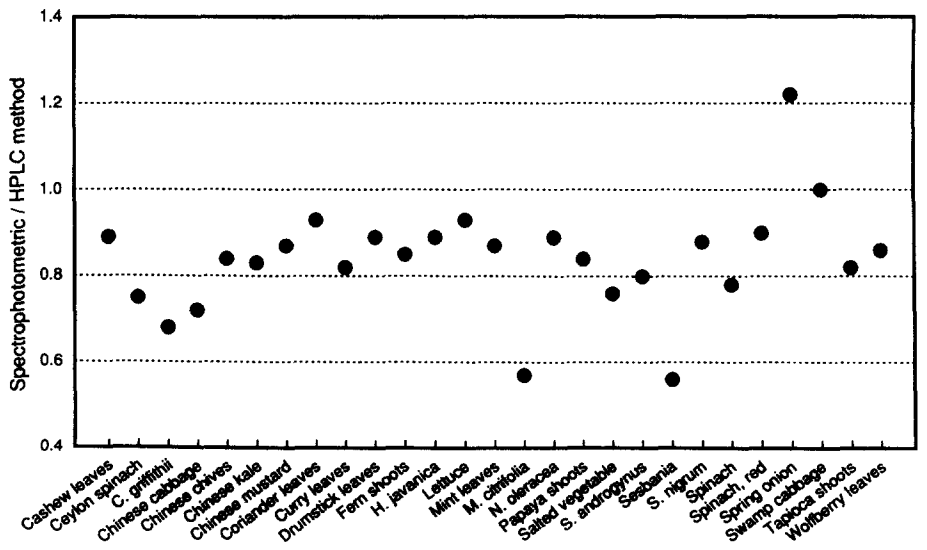


Fig. 4. Ratio of total carotenoid concentrations of green, leafy vegetables determined by direct spectrophotometric and HPLC methods.

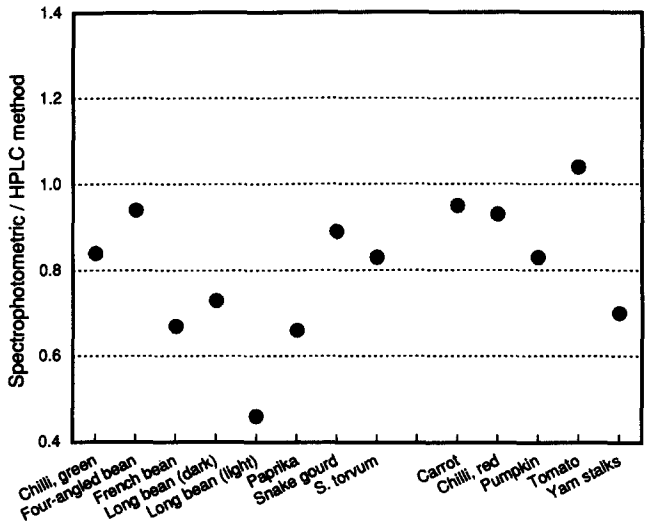


Fig. 5. Ratio of total carotenoid concentrations of green non-leafy and other vegetables determined by direct spectrophotometric and HPLC methods.

slightly different absorption spectra (Fig. 2). When vegetable extracts were fractionated on the magnesia column using step-wise increase of acetone in hexane as the eluant, the fraction eluted from the column with an RT of 3·3 min in the HPLC chromatogram was found to have an absorption spectrum similar to that of lutein. α- and β-Carotenes, differing only in the position of the double-bond in one of the two end groups (Fig. 1) were not completely separated. However, there was no difficulty in accurate identification and quantitation of these two pigments.

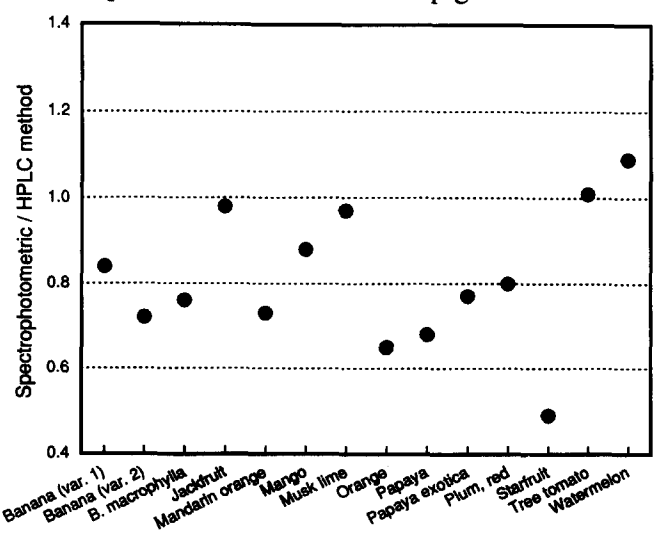


Fig. 6. Ratio of total carotenoid concentrations of fruits determined by direct spectrophotometric and HPLC methods.

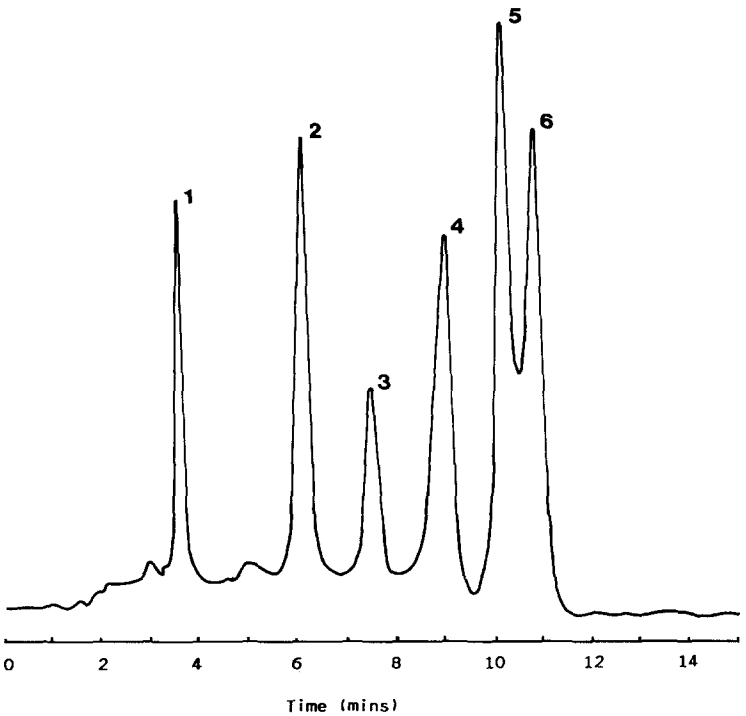


Fig. 7. HPLC chromatogram of carotenoid standards. Detector 436 nm, 0·02 AUFS. Other chromatography conditions as given in text. Concentrations of lutein, cryptoxanthin and lycopene were 0·5 μg/ml, and of α-, β- and γ-carotenes were 1·0 μg/ml. 100 μl used for injection. 1 = Lutein; 2 = cryptoxanthin; 3 = lycopene; 4 = γ-carotene; 5 = α-carotene; 6 = β-carotene.

A chromatogram of a mixture of these carotenoids is given in Fig. 7. It can be seen that elution order of the carotenoids on the reverse-phase C_{18} column was as expected, i.e. the more polar compounds were eluted earlier. As can be seen from the chromatogram, the oxygenated carotenoids or xanthophylls were eluted early. Lutein and zeaxanthin, the dihydroxy pigments, were eluted first, followed by the hydroxy carotenoid cryptoxanthin, and then the straight-chain carotenoid lycopene. The non-polar carotenoid hydrocarbons, γ-, α- and β-carotenes were eluted last from the column.

The concentrations of the major carotenoids quantitated are given in Tables 5 and 6 for the vegetables and fruits, respectively. The carotenoids are tabulated in the order of their elution from the HPLC column, except for 'other carotenoids' not identified. Zero values in the tables refer to levels of carotenoids which were not detected or could not be quantitated accurately using the procedure as described. Figures 8–10 give the composition of the carotenoids, expressed as the percentage of each carotenoid to the sum of all carotenoids.

TABLE 5
Content[a] of Major Carotenoids in Selected Vegetables

Name of vegetable	*Lutein*	*Cryptoxanthin*	*Lycopene*	*gamma-Carotene*	*alpha-Carotene*	*beta-Carotene*	*Others*[b]	*Sum*[c]
Green, leafy								
Cashew leaves	773	0	0	0	0	1 342	0	2 115
Ceylon spinach	1 299	0	0	0	0	3 533	0	4 832
C. griffithii	9 871	0	0	0	3 677	3 218	0	16 766
Chinese cabbage	963	0	0	0	0	3 022	0	3 985
Chinese chives	1 083	0	0	0	0	3 509	0	4 592
Chinese kale	1 540	0	0	0	0	4 092	0	5 631
Chinese mustard leaves	1 019	0	0	0	0	2 928	0	3 947
Coriander leaves	1 339	0	0	0	0	3 167	0	4 506
Curry leaves	5 252	0	0	0	0	9 328	0	14 579
Drumstick leaves	7 128	0	0	0	0	7 536	0	14 663
Fern shoots	1 002	0	0	0	0	1 438	0	2 440
H. javanica	1 305	0	0	0	0	3 840	0	5 145
Lettuce	73	0	0	0	0	97	0	170
Mint leaves	1 701	0	0	0	0	4 836	0	6 537
M. citrifolia	8 842	0	0	0	0	3 108	1 296	13 246
N. oleracea	6 236	0	0	0	0	11 395	0	17 631
Papaya shoots	821	0	0	0	0	1 829	0	2 650
Salted vegetable	7 318	0	0	0	0	3 395	0	10 713
S. androgynus	29 913	0	0	0	0	13 351	3 292	46 556
Sesbania	20 213	0	0	0	0	13 611	5 890	39 714
S. nigrum	2 888	0	0	0	0	7 048	0	9 936

Spinach	4175	0	0	0	0	3177	0	7352
Spinach, red	2047	0	0	0	0	5088	0	7134
Spring onion	323	0	0	0	0	1282	0	1605
Swamp cabbage	335	0	0	0	0	1895	0	2229
Tapioca shoots	1676	0	0	0	0	5720	0	7396
Wolfberry leaves	7591	0	0	0	0	5867	0	13458
Green, non-leafy								
Chilli, green	386	0	0	0	0	468	0	854
Four-angled bean	142	0	0	0	0	476	0	617
French bean	460	0	0	0	0	236	154	849
Long bean (dark green)	423	0	0	0	0	569	153	1144
Long bean (light green)	300	0	0	0	0	412	146	857
Paprika/Bell pepper	223	0	0	0	0	267	0	490
Snake gourd	225	0	0	0	0	148	0	372
S. torvum	154	0	0	0	0	74	0	228
Others								
Carrot	0	0	0	0	3410	6769	0	10179
Chilli, red	941	1754	0	0	0	1663	1971	6328
Pumpkin	940	0	0	0	756	578	0	2273
Tomato	130	0	723	0	0	365	0	1218
Yam stalks	80	0	0	0	0	61	0	141

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.
[b] Unidentified carotenoids.
[c] Summation of all carotenoids tabulated.

TABLE 6
Content[a] of Major Carotenoids in Selected Fruits

Name of fruit	*Lutein*	*Cryptoxanthin*	*Lycopene*	*gamma-Carotene*	*alpha-Carotene*	*beta-Carotene*	*Others*[b]	*Sum*[c]
Banana (var. 1)	27	0	0	0	62	40	0	128
Banana (var. 2)	37	0	0	0	157	92	0	286
B. macrophylla	457	155	0	52	0	301	514	1 477
Jackfruit	95	17	0	0	0	56	56	223
Mandarin orange	113	688	0	59	0	81	151	1 091
Mango (Black-gold)	0	0	0	0	0	615	0	615
Musk lime	65	446	0	0	0	12	0	522
Orange	30	332	0	0	0	25	218	605
Papaya	0	1 483	2 003	118	0	228	294	4 125
Papaya exotica	0	615	2 333	189	0	321	304	3 760
Plum, red	149	40	0	0	0	127	0	316
Starfruit/Carambola	66	1 066	0	0	0	28	551	1 710
Tree tomato	0	1 236	0	0	0	599	0	1 834
Watermelon (red)	0	457	5 301	90	0	324	0	6 171

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.
[b] Unidentified carotenoids.
[c] Summation of all carotenoids tabulated.

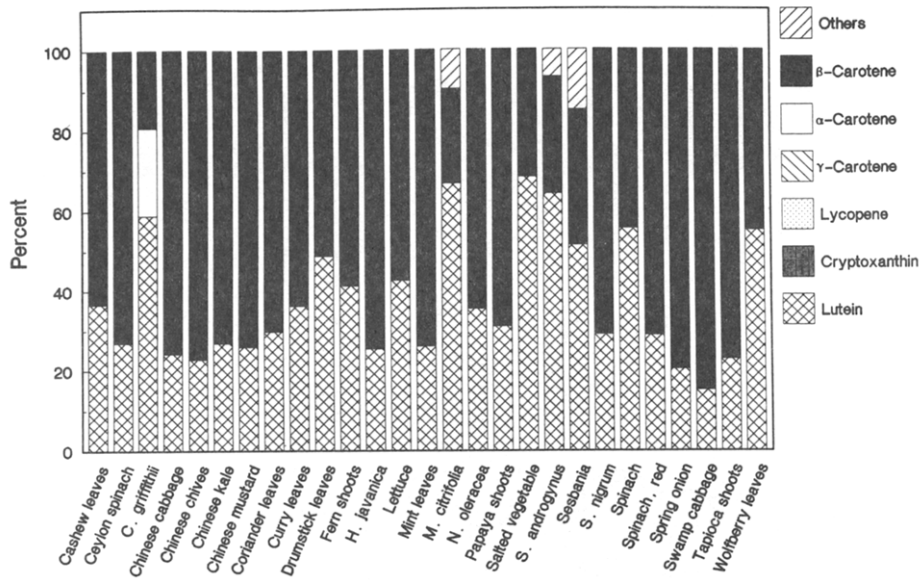


Fig. 8. Carotenoid composition of green, leafy vegetables.

For most of the green leafy vegetables, simple HPLC chromatograms were obtained and the major carotenoids detected were rather consistent (Table 5 and Fig. 8). In most cases, only β-carotene and lutein were obtained. The former was found in all the vegetables studied, and was clearly the major carotenoid in most of the vegetables. In twenty of the green leafy vegetables studied, β-carotene made up over 50% of the sum of all carotenoids

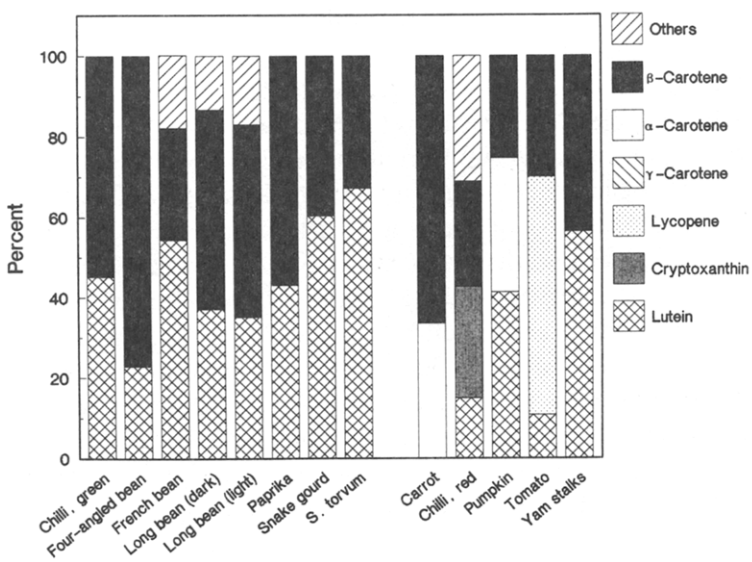


Fig. 9. Carotenoid composition of green, non-leafy and other vegetables.

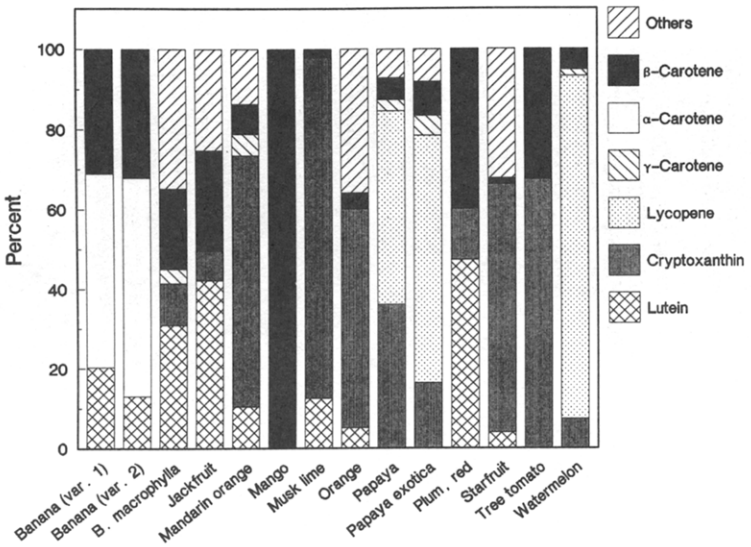


Fig. 10. Carotenoid composition of fruits.

quantitated. For the remaining seven samples, at least 20% of the carotenoids was β-carotene. Lutein was also detected in all vegetables in fairly high proportions. Except for five samples, lutein made up over 25% of the sum of all carotenoids in these vegetables. The other carotenoids were encountered infrequently. α-Carotene was found only in *C. griffithii*, whilst γ-carotene, lycopene and cryptoxanthin were not detected under the conditions employed. For three of the vegetables in these two groups, a small proportion (<15%) of the carotenoids was contributed by a few unidentified carotenoids.

Carotenoid compositions of the green non-leafy vegetables were similar to those for the green leafy varieties (Table 5 and Fig. 9). Seven of the eight vegetables in the former group were found to have over 25% of lutein and over 30% of β-carotene. A few unidentified carotenoids were detected (constituting about 15% of the sum of carotenoids) in three vegetables; namely, French bean and the two varieties of long beans. These carotenoids appear to be characteristic of these leguminous vegetables.

In contrast to the green vegetables, the carotenoid composition of the other vegetables was rather different (Table 5 and Fig. 9). Although β-carotene and lutein were found in all these fruit and root vegetables, several other carotenoids were encountered. α-Carotene was detected in carrot and pumpkin, while cryptoxanthin was found in red chilli. Lycopene was detected only in tomato, and made up about 60% of the carotenoids quantitated.

The fruits also presented rather different carotenoid compositions from

those obtained for the green vegetables (Table 6 and Fig. 10). There was no clear pattern of carotenoids present in the samples studied. β-Carotene was detected in all the fruits, but its proportion varied considerably, ranging from 100% in mango to less than 10% for seven other fruits. Lutein was found in nine of the fourteen fruits studied, but in smaller proportions than in green vegetables. Cryptoxanthin was found in most of the fruits studied, and contributed to over 50% of the carotenoids in five of the fruits. As for the vegetables, α-carotene was infrequently encountered, having been detected only in the two banana species, contributing about 50% of total carotenoids. Lycopene also occurred infrequently, detected only in papaya (including the cultivar Exotica) and watermelon (red variety). In the last named, it constituted over 80% of all the carotenoids. γ-Carotene was found in small proportions (< 5%) in five of the fruits studied. Seven of the fruits studied also had significant proportions of the unidentified carotenoids.

β-Carotene content

β-Carotene concentrations determined by the AOAC and HPLC methods are tabulated in Tables 7 and 8. Figures 11–13 show the ratios of β-carotene determined by the two methods for the green leafy vegetables, green non-leafy and other vegetables and fruits, respectively.

For the green leafy vegetables, the ratios of β-carotene determined by the AOAC and HPLC methods clustered between 0·8–1·2, i.e. varying within

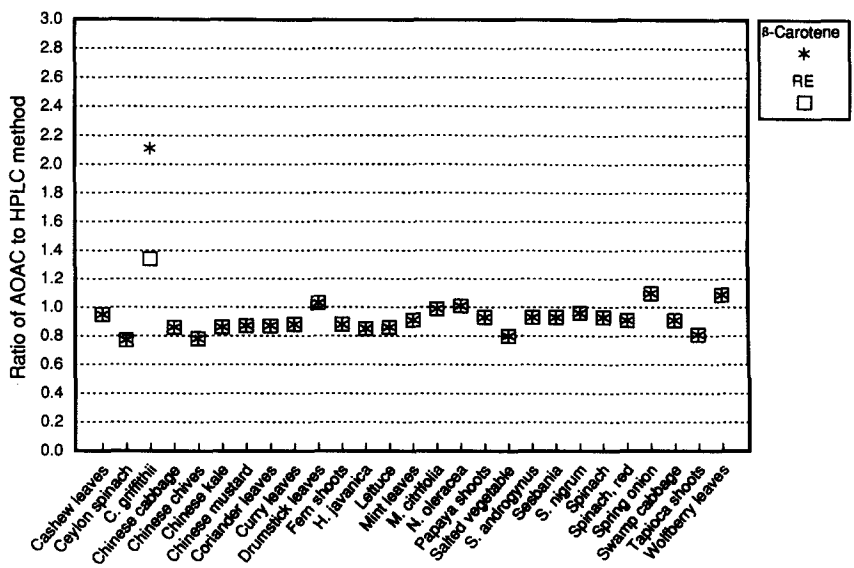


Fig. 11. Ratio of β-carotene and retinol equivalent (RE) of green, leafy vegetables determined by AOAC and HPLC methods.

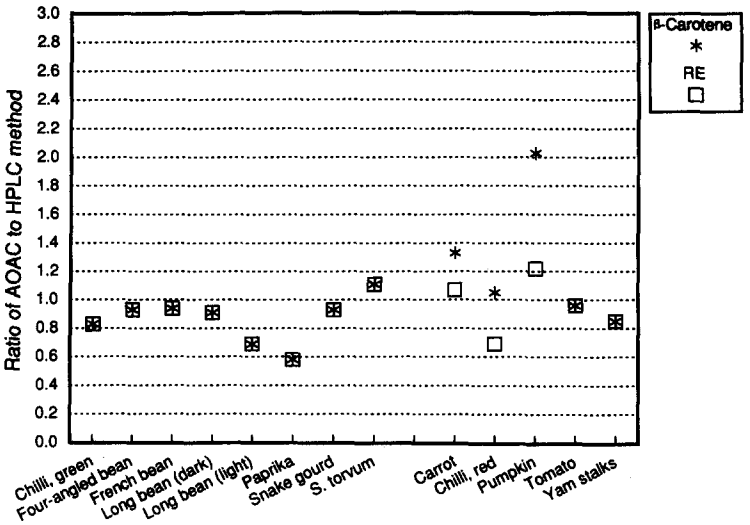


Fig. 12. Ratio of β-carotene and retinol equivalent (RE) green, non-leafy and other vegetables determined by AOAC and HPLC methods.

$\pm 20\%$ from unity (Table 7 and Fig. 11). Only in one vegetable was the ratio outside this range; *C. griffithii* was found to have an exceptionally high ratio of 2·1. This high ratio was due to the presence of α-carotene in this vegetable which was eluted together with β-carotene from the magnesia column and erroneously estimated together with it in the AOAC method. This was confirmed by injecting the eluate from the magnesia column into the high-pressure liquid chromatograph and observing the two carotenes in the

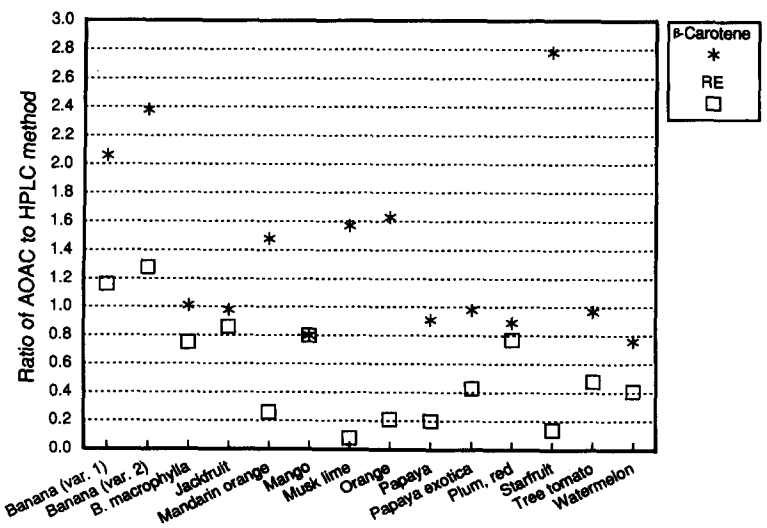


Fig. 13. Ratio of β-carotene and retinol equivalent (RE) of fruits determined by AOAC and HPLC methods.

TABLE 7
β-Carotene Content[a] of Selected Vegetables Determined by the AOAC and HPLC Methods

Name of vegetable	*AOAC method*	*HPLC method*
Green, leafy		
Cashew leaves	1 278	1 342
Ceylon spinach	2 714	3 533
C. griffithii	6 788	3 218
Chinese cabbage	2 604	3 022
Chinese chives	2 739	3 509
Chinese kale	3 500	4 092
Chinese mustard leaves	2 536	2 928
Coriander leaves	2 745	3 167
Curry leaves	8 241	9 328
Drumstick leaves	7 724	7 536
Fern shoots	1 273	1 438
H. javanica	3 266	3 840
Lettuce	83	97
Mint leaves	4 411	4 836
M. citrifolia	3 074	3 108
N. oleracea	11 459	11 395
Papaya shoots	1 709	1 829
Salted vegetable	2 704	3 395
S. androgynus	12 363	13 351
Sesbania	12 697	13 611
S. nigrum	6 760	7 048
Spinach	2 947	3 177
Spinach, red	4 646	5 088
Spring onion	1 404	1 282
Swamp cabbage	1 729	1 895
Tapioca shoots	4 607	5 720
Wolfberry leaves	6 414	5 867
Green, non-leafy		
Chilli, green	388	468
Four-angled bean	443	476
French bean	221	236
Long bean (dark green)	520	569
Long bean (light green)	285	412
Paprika/Bell pepper	154	267
Snake gourd	138	148
S. torvum	82	74
Others		
Carrot	9 027	6 769
Chilli, red	1 743	1 663
Pumpkin	1 170	578
Tomato	352	365
Yam stalks	52	61

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.

TABLE 8
β-Carotene Content[a] of Selected Fruits Determined by the AOAC and HPLC Methods

Name of fruit	*AOAC method*	*HPLC method*
Banana (var. 1)	82	40
Banana (var. 2)	219	92
B. macrophylla	303	301
Jackfruit	55	56
Mandarin orange	120	81
Mango (Black-gold)	495	615
Musk lime	18	12
Orange	40	25
Papaya	208	228
Papaya exotica	314	321
Plum, red	113	127
Starfruit/Carambola	77	28
Tree tomato	582	599
Watermelon (red)	246	324

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.

chromatogram. For most of the other vegetables, the ratio tended to be less than 1·0, indicating that the HPLC method gave slightly higher results.

The ratios of β-carotene content determined by the AOAC and HPLC methods for the green non-leafy and other vegetables were more varied (Table 7 and Fig. 12). However, most of the ratios were within the narrow range of 0·8 to 1·2. For pumpkin, the ratio was exceptionally high at 2·1, due to the presence of α-carotene which was giving erroneously high results by the AOAC method.

For the fruits, there was considerable variation in the ratios of β-carotene concentration given by the two methods (Table 8 and Fig. 13). For half of the fruits studied, the ratios were between 0·8 and 1·2. For the only two fruits with α-carotene, i.e. the two species of banana, the ratios were greater than 2·0. The reason for this over-estimation by the AOAC method has been explained above. Three other fruits with ratios of about 1·5 were musk lime, orange and starfruit. These fruits were found to have low levels of β-carotene (less than 100 μg per 100 g edible portion), which made up only a small proportion of all the carotenoids detected. The relatively insensitive and non-specific nature of the AOAC method, especially for foods with low β-carotene, could be the reason for the over-estimation by this method.

Results obtained from the HPLC method showed that highest concentrations of β-carotene were found in green leafy vegetables, particularly *S. androgynus*, sesbania and *N. oleracea* (Table 7). All these three

local vegetables had a β-carotene content of over 11 000 μg per 100 g of edible portion. Six other green leafy vegetables were found to have a β-carotene content from 5000–10 000 μg per 100 g of vegetable. With the exception of carrot (β-carotene about 9000 μg per 100 g), none of the green non-leafy and other vegetables was found to have high β-carotene content. All the fruits studied were not rich in β-carotene, with concentrations of less than 1000 μg per 100 g of sample. The sample of mango studied was interesting in that all the carotenoids were solely β-carotene, and the concentration was the highest of all the fruits.

Vitamin A activity (retinol equivalent)

Conventionally, the nutritional significance of carotenoids is related to the pro-vitamin A activity of these compounds. Although over 500 carotenoids have been reported to occur naturally, only a few are known to possess pro-vitamin A activity and occur in significant amounts in natural foods (Bauernfeind, 1972; Goodwin, 1986). For vitamin A activity, a carotenoid must have at least one unsubstituted β-ionone ring with an attached polyene side chain of at least eleven carbon atoms. Consistent with these important structural requirements, the following carotenoids identified in this study have been known to possess pro-vitamin A activity: β-carotene, α-carotene, γ-carotene, and cryptoxanthin. The vitamin A activity of β-carotene, expressed as μg retinol equivalent (RE) was calculated as RE = (μg β-carotene)/6 (NAS, 1980). The other three carotenoids mentioned, possessing only one unsubstituted β-ionone ring, may be expected to have about 50% of the biological activity of β-carotene. The formula used for these pro-vitamin A carotenoids was therefore RE = (μg carotenoid)/12.

The RE values calculated from β-carotene determined by the HPLC method and those calculated based on all carotenoids with pro-vitamin A activity by the same analytical method are given in Table 9 for the vegetables. The differences in RE between the two methods of calculation, expressed as a percentage of RE from all carotenoids, are also given in the table. For the green leafy vegetables, with the exception of *C. griffithii*, since no other carotenoids with pro-vitamin A activity were detected, there was no difference in RE calculated by the two methods. For *C. griffithii*, excluding α-carotene from the calculation would result in a 36% error in RE content for the vegetable. There was also no difference in RE calculations for green non-leafy vegetables. For the other vegetables; namely, carrot, red chilli and pumpkin, differences in RE ranged from 20 to 40%.

Since other pro-vitamin A carotenoids besides β-carotene were detected in all the fruits studied except mango, the RE values calculated on the basis of all these carotenoids were higher than those calculated based on β-

TABLE 9

Retinol Equivalent (RE)[a] of Selected Vegetables Determined by the AOAC and HPLC Methods

Name of vegetable	*AOAC method*[b]	*HPLC method*		
		β-carotene[c]	*total*[d]	*% difference*[e]
Green, leafy				
Cashew leaves	213	224	224	0
Ceylon spinach	452	589	589	0
C. griffithii	1 131	536	843	36·4
Chinese cabbage	434	504	504	0
Chinese chives	457	585	585	0
Chinese kale	583	682	682	0
Chinese mustard leaves	423	488	488	0
Coriander leaves	457	528	528	0
Curry leaves	1 374	1 555	1 555	0
Drumstick leaves	1 287	1 256	1 256	0
Fern shoots	212	240	240	0
H. javanica	544	640	640	0
Lettuce	14	16	16	0
Mint leaves	735	806	806	0
M. citrifolia	512	518	518	0
N. oleracea	1 910	1 899	1 899	0
Papaya shoots	285	305	305	0
Salted vegetable	451	566	566	0
S. androgynus	2 061	2 225	2 225	0
Sesbania	2 116	2 269	2 269	0
S. nigrum	1 127	1 175	1 175	0
Spinach	491	530	530	0
Spinach, red	774	848	848	0
Spring onion	234	214	214	0
Swamp cabbage	288	316	316	0
Tapioca shoots	768	953	953	0
Wolfberry leaves	1 069	978	978	0
Green, non-leafy				
Chilli, green	65	78	78	0
Four-angled bean	74	79	79	0
French bean	37	39	39	0
Long bean (dark green)	87	95	95	0
Long bean (light green)	47	69	69	0
Paprika/Bell pepper	26	45	45	0
Snake gourd	23	25	25	0
S. torvum	14	12	12	0
Others				
Carrot	1 504	1 128	1 412	20·1
Chilli, red	290	277	423	34·5
Pumpkin	195	96	159	39·5
Tomato	59	61	61	0
Yam stalks	9	10	10	0

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.
[b] Calculated as RE = (μg carotene)/6.
[c] Based on β-carotene only; RE = (μg β-carotene)/6.
[d] Based on β-carotene and all other pro-vitamin A carotenoids, i.e. α-carotene, γ--carotene, cryptoxanthin; RE = [(μg β-carotene)/6] + [(μg other carotenoids)/12].
[e] Calculated as: $\left[\frac{\text{total RE} - \text{RE from } \beta\text{-carotene}}{\text{total RE}}\right] \times 100.$

TABLE 10
Retinol Equivalent (RE)[a] of Selected Fruits Determined by the AOAC and HPLC Methods

Name of fruit	*AOAC method*[b]	*HPLC method*		
		β-carotene[c]	*total*[d]	*% difference*[e]
Banana (var. 1)	14	7	12	43·8
Banana (var. 2)	36	15	28	46·0
B. macrophylla	51	50	67	25·6
Jackfruit	9	9	11	12·8
Mandarin orange	20	14	76	82·2
Mango (Black-gold)	82	103	103	0
Musk lime	3	2	39	95·1
Orange	7	4	32	87·1
Papaya	35	38	171	77·8
Papaya exotica	52	54	120	55·6
Plum, red	19	21	24	13·7
Starfruit/Carambola	13	5	93	95·1
Tree tomato	97	100	203	50·8
Watermelon (red)	41	54	99	45·8

[a] Mean of duplicate analysis; expressed as μg per 100 g edible portion of sample.
[b] Calculated as RE = (μg carotene)/6.
[c] Based on β-carotene only; RE = (μg β-carotene)/6.
[d] Based on β-carotene and all other pro-vitamin A carotenoids, i.e. α-carotene, γ-carotene, cryptoxanthin; RE = [(μg β-carotene)/6] + [(μg other carotenoids)/12].
[e] Calculated as: $\left[\frac{\text{total RE} - \text{RE from } \beta\text{-carotene}}{\text{total RE}}\right] \times 100.$

carotene only (Table 10). The vitamin A activity calculated on the basis of β-carotene alone would result in RE values which were 12–87% lower.

The RE values calculated from carotene determined by the AOAC method and all carotenoids with pro-vitamin A activity by the HPLC method are given in Table 9 for the vegetables. As can be expected, with the exception of *C. griffithii*, the ratios of RE calculated by the two methods were exactly the same as ratios obtained for β-carotene (Fig. 11), because β-carotene was the only pro-vitamin A carotenoid detected for these vegetables. Owing to the presence of α-carotene in *C. griffithii*, the RE for this vegetable was falsely elevated when calculated from carotene determined by the AOAC method since α-carotene possesses only half the pro-vitamin A activity of β-carotene.

Similarly, for the green non-leafy vegetables, since no other pro-vitamin A carotenoids besides β-carotene were detected, the ratios of RE calculated from carotene determined by the AOAC method and all carotenoids with pro-vitamin A activity by the HPLC methods were exactly the same as the

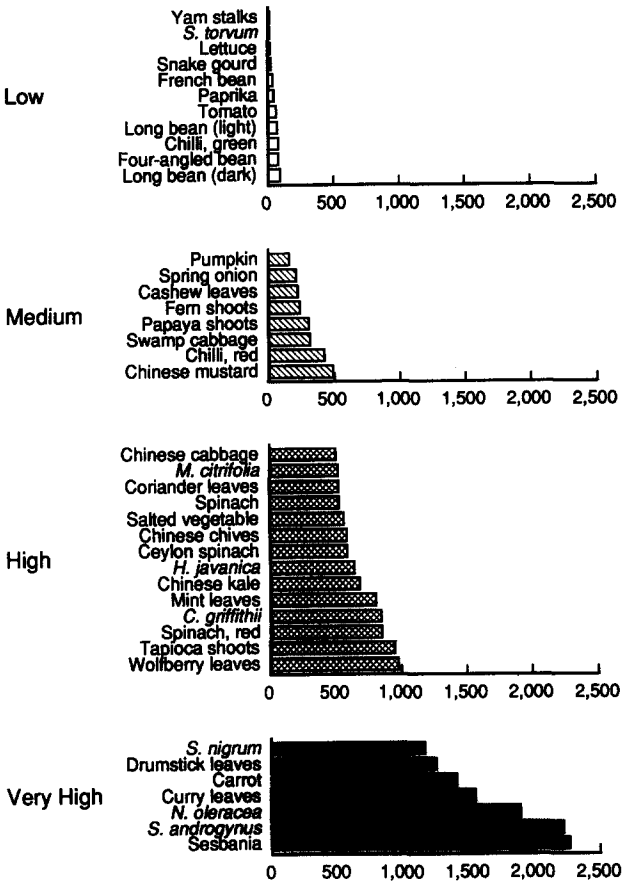


Fig. 14. Classification of vegetables according to retinol equivalent (RE).

ratios obtained for β-carotene (Table 9 and Fig. 12). With the exception of two vegetables, the ratios were found to be between 0·8 and 1·2.

For three of the other vegetables studied, the ratios for RE were between this range (Fig. 8). The ratio for pumpkin was exceptionally high at about 2·0, indicating a falsely elevated RE given by the AOAC method. The reason for this is as given for *C. griffithii* above. On the other hand, RE for red chilli given by the AOAC method would be falsely low since the procedure was not able to quantitate cryptoxanthin.

Similarly, because of the presence of other pro-vitamin A carotenoids in fruits (except for mango), the ratios for RE determined by the AOAC method to that by the HPLC method were different from the β-carotene ratios. Ratios for RE were all lower than those for β-carotene, and were generally below 1·0 (Table 10 and Fig. 13). These data show that the AOAC method was underestimating the vitamin A activity of the fruits, since the method was not able to quantitate the other pro-vitamin A carotenoids.

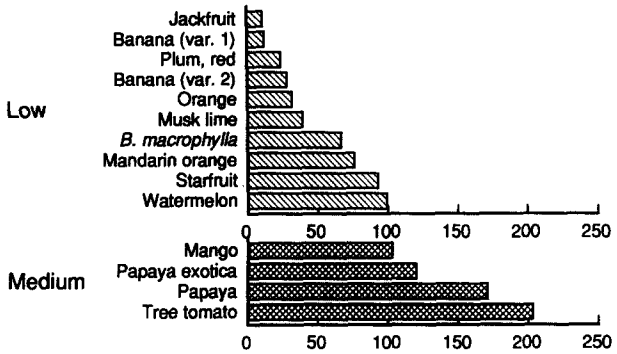


Fig. 15. Classification of fruits according to retinol equivalent (RE).

To facilitate easy identification of vegetables which are good sources of vitamin A activity, these foods were grouped into four categories; namely, low ($<100\,\mu$g RE per 100 g edible portion), medium (100–499 μg RE), high (500–999 μg RE) and very high ($>1000\,\mu$g RE) (Fig. 14). Vegetables with high and very high RE were all green leafy vegetables, with the exception of carrot, a root vegetable. Of particular interest are four local vegetables with over 1000 μg RE; namely, sesbania, *S. androgynus*, *N. oleracea*, and *S. nigrum*. Several other local vegetables were in the high RE category. A few other green leafy vegetables and red chilli and pumpkin made up the group with medium RE. All the green non-leafy vegetables, as well as yam stalk and tomato were found to be poor sources of vitamin A.

A similar grouping was made for the fruits studied (Fig. 15). None of the fruits may be considered as having high or very high vitamin A activity. The two species of papaya, tree tomato and mango were found to have medium RE, while the other fruits were poor sources of vitamin A, particularly the first variety of banana and jackfruit.

CONCLUSION

A non-aqueous reverse-phase HPLC method for the determination of carotenoids in various vegetables and fruits has been developed. The method uses the basic configuration of an HPLC system, and would thus be useful for routine determinations. A ternary mixture of acetonitrile, methanol and ethyl acetate was used to separate the carotenoids isocratically in an octadecylsilane (C_{18}) column. A fixed wavelength detector at 436 nm was used to detect the carotenoids, the peaks being monitored and quantitated in an integrator. The method gave satisfactory separation and quantitation of lutein, cryptoxanthin, lycopene, γ-, α- and β-carotenes. The emphasis has

been on major carotenoids that occur in sufficient amounts to contribute significantly to dietary intake.

This is the first report on the concentration of major carotenoids in relation to total carotenoids in a number of Malaysian vegetables and fruits. It is also the first report of a parallel study of carotenoid determination by the AOAC and the HPLC methods for a fairly large number of samples. Findings from the study have clearly shown that the HPLC method would give a more complete picture of the carotenoid composition as well as a more accurate quantitation of the pro-vitamin A activity of vegetables and fruits. Depending on the composition of the carotenoids present, the AOAC method could under- or over-estimate the β-carotene concentration and, therefore, the RE activity. The HPLC procedure reported could be useful for updating the vitamin A activity of plant materials in the current Malaysian Food Composition Table, thereby providing the correct identification of foods rich in pro-vitamin A activity. The nutritional significance of the findings is clear since these foods are important sources of vitamin A for the majority of the communities in the country. Furthermore, there is currently a great deal of interest in carotenoids not possessing vitamin A activity as they may be associated with lower cancer risk.

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