High–pressure liquid chromatography – a powerful tool for the analysis of carotenoids in vegetables and fruits

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The major source of vitamin A in the diet of rural communities is carotenoids, a group of yellow and red colour pigments present in vegetables and fruits. There is a large number of naturally occurring carotenoids, but only some of them are precursors of vitamin A. Thus, accurate data on sources of vitamin A–active carotenoids are needed for various programmes aimed at overcoming and preventing the vitamin A deficiency problem. Unfortunately, previous data on vitamin A value of foods, determined mainly by the AOAC method, are now considered unreliable. Studies were therefore carried out to develop a high–pressure liquid chromatographic (HPLC) method for more accurate determination of carotenoid composition and content of vegetables and fruits. Each sample was also simultaneously determined by the column chromatographic procedure of the Association of Official Analytical Chemists (AOAC), with the objective of determining differences in results given by the two methods. In the reversed–phase HPLC method developed, carotenoids were separated isocratically on an octadecylsilane (C18) column using a ternary mixture of acetonitrile, methanol and ethyl acetate. Although the AOAC method has been designed to quantitate only ß–carotene, it was observed that a–carotene was eluted and quantitated together with the former. Results obtained for samples containing α–carotene were therefore falsely elevated. On the other hand, the HPLC method successfully separated and quantitated the major carotenoids present, namely lutein, cryptoxanthin, lycopene, τ– and α–carotenes in addition to ß–carotene. Vitamin A activity of each sample studied, expressed as μg retinol equivalent (RE), was calculated based on concentrations of all carotenoids with provitamin A activity determined by the HPLC method. Due to the limitations of the AOAC method, RE calculated was found to be erroneously low for samples with significant proportions of provitamin A carotenoids other than ß–carotene, and falsely elevated for those α–carotene. The study clearly shows that the HPLC method would give a more complete picture of the carotenoid composition as well as a more accurate quantitations of the vitamin A value of the vegetables and fruits. This report discuses the methodology of the study and highlights some results to illustrate the capability of the HPLC method.

INTRODUCTION

Although vitamin A deficiency has long been recognized, it remains one of the major public health nutritional problems in many developing countries, and is an important cause of preventable blindness. Since preformed vitamin A in meat, liver and eggs is out of reach of the economically deprived, the main source of vitamin A in the diet of the rural communities is carotenoids. The term "carotenoids" embrances a large number of naturally occurring fat–soluble compounds that are coloured yellow to red.[1] Carotenoids are synthesized exclusively by photosynthetic microorganisms and by members of the plant kingdom where they play major roles in metabolism. In relation to human nutrition, the carotenoids serve as sources of vitamin A activity. Over 500 carotenoids have been reported to occur naturally, but only a few of them both have vitamin A activity and occur in significant amounts in natural foods.[2-3] It is thus important to have accurate data on the sources of vitamin A–active carotenoids for the implementation of various programmes aimed at overcoming or preventing the vitamin A deficiency problem.

In recent years, there has been particular emphasis on understanding the types and concentrations of various carotenoids in foods. It has been pointed out that previously reported values of vitamin A activity in food composition tables may have been unreliable since the methodologies used were not sufficiently discriminative.[4-6] In addition, carotenoids are now thought to play important roles beyond their classical functions in nutrition and vision. With their highly conjugated double bonds, carotenoids, including those

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without vitamin A activity, may act as free radical traps or antioxidants, and therefore play important roles in cancer causation and prevention.[7-9]

This laboratory has embarked on a systematic study to develop improved methodologies based on high-pressure liquid chromatography for the separation and quantitation of carotenoids in foods and biological specimens, especially serum. The method should preferably be simple, workable for routine determination of several carotenoids. It is hoped that the method developed could be used to re-evaluate the vitamin value of foods tabulated in the current Malaysian Food Composition Table.[10] A high-pressure liquid chromatography (HPLC) method for the analysis of these compounds has been developed and applied to a variety of vegetables and fruits. Each food sample was also simultaneously determined by the open column chromatographic procedure of the Association of Official Analytical Chemists (AOAC),[11] the procedure widely used for determination of vitamin A value in food composition tables. The objective was to determine if the difference in carotene values obtainable 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 type of vegetables and fruits show the greatest differences.

This report discusses the methodology of the study, and highlights some results obtained to illustrate the capability of the HPLC method. Complete results for all the 40 vegetables and 14 fruits studied are being analysed for reporting at a later date.

MATERIALS AND METHODS

Solvents and Carotenoid Standards Solvents used for sample preparation and pretreatment, 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. τ-Carotene, β-apo-carotenal, cryptoxanthin, zeaxanthin, and lutein were gifts from F. Hoffmann La-Roche, Switzerland. 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 1μg per ml of the standards were prepared daily. UV-visible absorption spectra of these standards were determined and used in assisting the identification of carotenoids in food samples. The appropriate extinction coefficients published in the literature[12] were used to calculate the exact concentration of each of the carotenoids. The preparation of all standard carotenoids were carried out with no unnecessary delay, in a room with subdued light and all windows tinted with a light-protective film. All sample treatment and analytical procedures were also carried out in this room.

Sample Preparation and Pretreatment 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 blended and 2-10 g immediately weighed for analysis.

Sample pretreatment procedures were essentially those of the AOAC,[11] 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.

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 minutes. The saponified mixture was cooled and extracted with 50-ml portions of hexane until the extract was colourless. The hexane extracts were pooled, washed till 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 test solution was next subjected to the following analytical procedures: (1) 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 436 nm (HPLC method). Details of the two procedures are as 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.[11] 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 concentration of β-carotene calculated using a calibration curve prepared with the β-carotene standard.

High-pressure Liquid Chromatography (HPLC method)

HPLC conditions A Waters high-pressure liquid chromatograph equipped with a 440 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. A Waters 6000A solvent delivery system was used to deliver the mobile phase (acetonitrile-methanol-ethyl acetate, 88:10:2, v/v) at a rate of 2.0 ml/min. A stainless steel 30 cm x 3.9 mm I.D. 10 µm µB ondapak C_{18} column was used for the chromatographic separation. This was preceded by a Waters Guard-PAK precolumn module housing a disposable Guard-PAK precolumn insert packed with the same material as that in the analytical column. Sampel injection volumes, dispensed using a Rheodyne 7125 injector, were usually 50 to 100 µl. Peak areas were quantitated 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 concentration of individual carotenoids were summed to give "sum of carotenoids".

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.

RESULTS AND DISCUSSION

This preliminary report presents results for 20 vegetables and 10 fruits, listed in Table I. Results obtained for all the 40 vegetables and 14 fruits studied are being analysed for publication. The English names of the foods are first listed, followed by the names in Bahasa Malaysia (or other local names) and scientific names. Where the English names are not available, the local names of the foods are given.

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), τ-carotene (RT = 9.0 min), α-carotene (RT = 10.2 min), and β-carotene (RT = 10.8 min). Zeaxanthin, structurally very similar to lutein 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 caroteinoids had slighdy different absorption spectra. 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 a RT of 3.3 minutes 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 were not completely separated. However, there was no difficulty in accurate identification and quantitation of these two pigments.

TABLE I : Names of vegetables and fruits studied

English name	Local name	Scientific name
Green, leafy vegetables		
Cekur manis	*Cekur manis*	*Sauropus androgynus*
Cemperai	*Cemperai*	*Champereia griffithii*
Chinese cabbage	*Pak-coy*	*Brassica chinensis*
Drumstick leaves	*Daun kelor*	*Moringa oleifera*
Fern shoots	*Pucuk paku*	*Diplazium esculentum*
Papaya shoots	*Daun betik*	*Carica papaya*
Pegaga gajah	*Pegaga gajah*	*Hydrocotyl javanica*
Ranti	*Ranti*	*Solanum nigrum*
Spinach	*Bayam putih*	*Amaranthus viridis*
Swamp cabbage	*Kangkung*	*Ipomoea aquatica*
Tanki	*Tanki*	*Neptunia oleracea*
Tapioca shoots	*Pucuk ubi kayu*	*Manihot utilissima*
Green, non-leafy vegetables		
French bean	*Kacang buncis*	*Phaseolus vulgaris*
Long bean, dark green	*Kacang panjang*	*Vigna sinensis*
Paprika/Bell pepper	*Lada hijau besar*	*Capsicum annuum*
Snake gourd	*Ketola ular*	*Tricosanthes anguina*
Other vegetables		
Carrot	*Lobak merah*	*Daucus carota*
Chilli, red	*Lada merah*	*Capsicum annuum*
Pumpkin	*Labu merah*	*Cucurbita maxima*
Tomato	*Tomato*	*Lycopersicum esculentum*
Fruits		
Banana	*Pisang emas*	*Musa sapientum*
Banana	*Pisang tanduk*	*Musa sapientum*
Buah kundang	*Buah kundang*	*Bouea macrophylla*
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*
Tree Tomatao	*Tomato pokok*	*Cyphomandra betacea*
Watermelon, red	*Tembikai*	*Citrullus vulgaris*

A chromatogram of a mixture of carotenoid standards is given in Figure 1. It can be seen that elution order of the carotenoids on the reversed-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,

τ–, α– and β–carotenes were eluted last from the column.

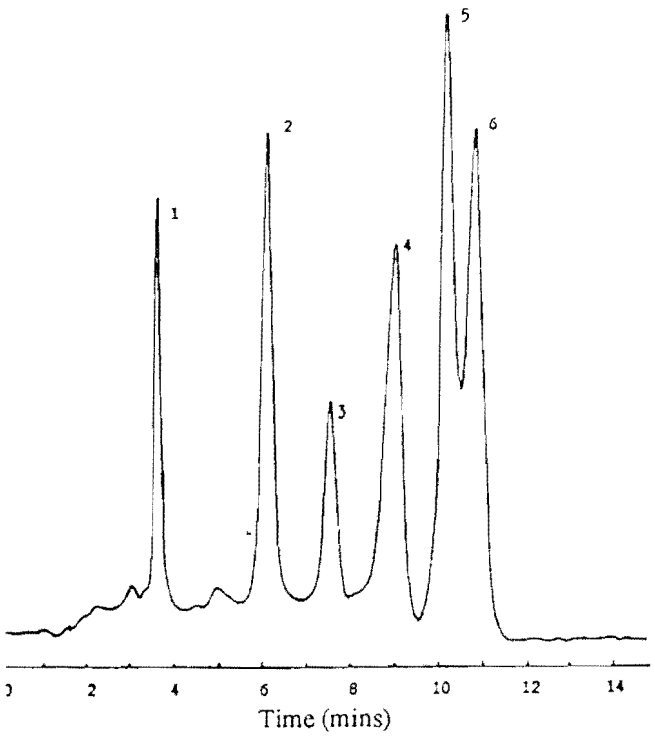

FIG 1 – HPLC Chromatogram of Carotenoid Standards. Detector • 436 nm, 0.02 AUFS. Other chromatography conditions as given in text. Concentrations of lutein, cryptoxanthin and lycopene were 0.5 μg/ml, and of α–, β– and τ–carotenes were L0 μg/ml. 100 μl used for injection. 1 = Lutein; 2 = cryptoxanthin; 3 = lycopene; 4 = τ–carotene; 5 = α–carotene; 6 = β–carotene

The concentrations of the major carotenoids quantitated are given in Tables 2 and 3 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. Figures 2 and 3 give the composition of the carotenoids in the vegetables and fruits respectively, expressed as the percentage of each carotenoid to the sum of all carotenoids.

For most of the green vegetables (leafy and non–leafy), the major carotenoids detected were rather consistent, and simple HPLC chromatograms were obtained (Table 2 nd Figure 2). 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 13 of the green vegetables studied, β–carotene made up over 40% of the sum of all carotenoids. For the remaining three samples, at least 20% of the carotenoids was β–carotene. Lutein was also detected in all vegetables in fairly high proportions. Except for two 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 *cemperai*, whilst τ–carotene, lycopene and cryptoxanthin were not encountered. For three of the vegetables in these two groups, a small proportion (<20%) of the carotenoids was contributed by a few unidentified carotenoids.

In contrast to the green vegetables, the carotenoid composition of the other vegetables was rather different (Table 2 and Figure 2). 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 605 of the carotenoids quantitated.

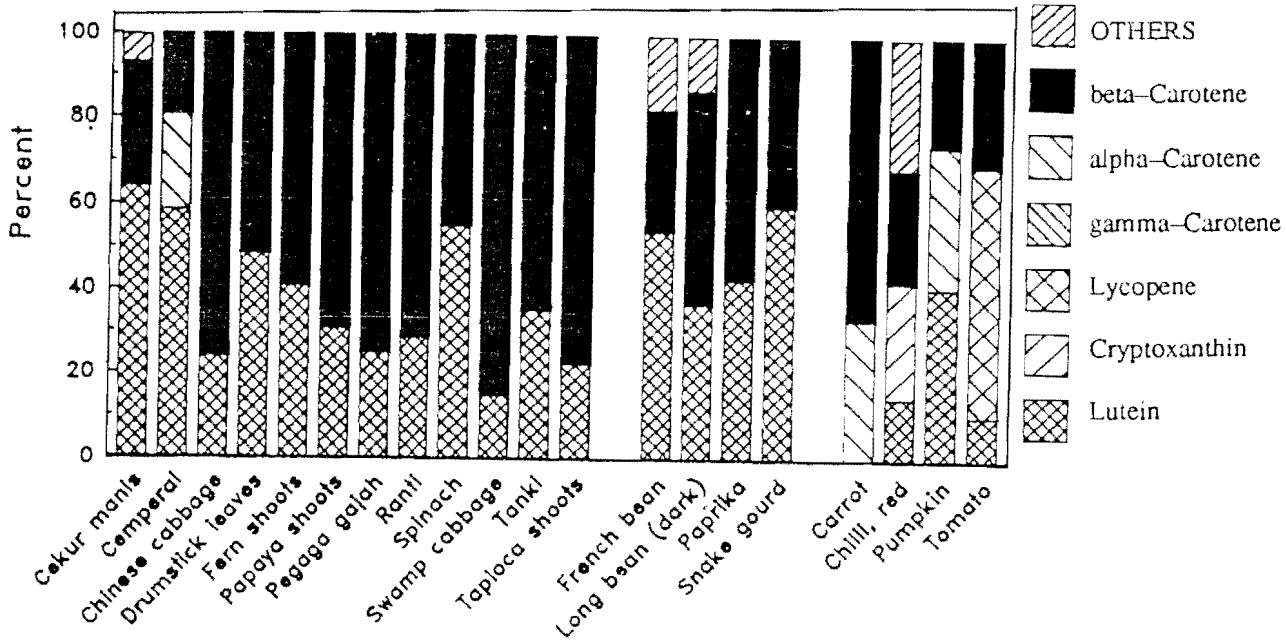

FIG 2 – Carotenoid Composition of Vegetables

TABLE II – Content[1] of Major Carotenoids in Vegetables

Name of Vegetable	Lutein	Crypto–xanthin	Lyco-pene	gamma–Carotene	alpha–Carotene	beta–Carotene	Others[2]	Sum[3]
Green, leafy vegetables								
Cekur manis	29913	0	0	0	0	13351	3292	46556
Cemperai	9871	0	0	0	3677	3218	0	16766
Chinese cabbage	963	0	0	0	0	3022	0	3985
Drumstick leaves	7128	0	0	0	0	7536	0	14663
Fern shooots	1002	0	0	0	0	1438	0	2440
Papaya shoots	821	0	0	0	0	1829	0	2650
Pegaga gajah	1305	0	0	0	0	3840	0	5145
Ranti	2888	0	0	0	0	7048	0	9936
Spinach	4175	0	0	0	0	3177	0	7352
Swamp cabbage	335	0	0	0	0	1895	0	2229
Tanki	6236	0	0	0	0	11395	0	17631
Tapioca shoots	1676	0	0	0	0	5720	0	7396
Green, non–leafy vegetables								
French bean	460	0	0	0	0	236	154	849
Long bean (dark green)	423	0	0	0	0	569	153	1144
Paprika/Bell pcPper	223	0	0	0	0	267	0	490
Snake gourd	225	0	0	0	0	148	0	372
Other vegetables								
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
Tomata	130	0	723	0	0	365	0	1218

[1] Mean of duplicate analyses; expressed as μg per 100 g of edible portion of sample
[2] Unidentified carotenoids
[3] Summation of all carotenoids tabulated

The fruits also presented rather different carotenoid compositions from those obtained for the green vegetables (Table 3 and Figure 3). 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 five other fruits. Lutein was found in five of the ten 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 three of the fruits. As for the vegetables, α–carotene was infrequently encountered, having been detected only in the two banana species, contributing to 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 four of the fruits studied. Four of the fruits studied also had siginificant proportions of the unidentified carotenoids.

β–Carotene Content β–Carotene concentrations determined by the AOAC and HPLC methods are tabulated in Table 4. Figure 4 shows the ratio of the carotene determined by the two methods.

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 ±20% from unity. Only in one vegetable was the ratio outside this range; *cemperai* 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. 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 slightly more varied, with most of the ratios betwen 0.6–1.4. For pumpkin, the ratio was exceptionally high at 2.0, due to the presence of α–carotene which was giving erroneously high result by the AOAC method.

For the fruits, there was considerable variation in the ratios of β–carotene concentration given by the two methods. For six of the fruits studied, the ratios were between 0.7 and 1.0. 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. Two other fruits with ratios of about 1.6 were musk lime and orange. 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

TABLE III – Content[1] of Major Carotenoids in Fruits

Name of Fruit	Lutein	Crypto–xanthin	Lyco–pene	gamma–Carotene	alpha–Carotene	beta–Carotene	Others[2]	Sum[3]
Pisang emas	27	0	0	0	62	40	0	128
Pisang tanduk	37	0	0	0	157	92	0	286
Buah kundang	457	155	0	52	0	301	514	1477
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	1483	2003	118	0	228	294	4125
Papaya exotica	0	615	2333	189	0	321	304	3760
Tree tomato	0	1236	0	0	0	599	0	1834
Watermelon (red)	0	457	5301	90	0	324	0	6171

[1] Mean of duplicate analyses; expresseed as μg per 100 g of edible portion of sample
[2] Unidentified carotenoids
[3] Summation of all carotenoids tabulated

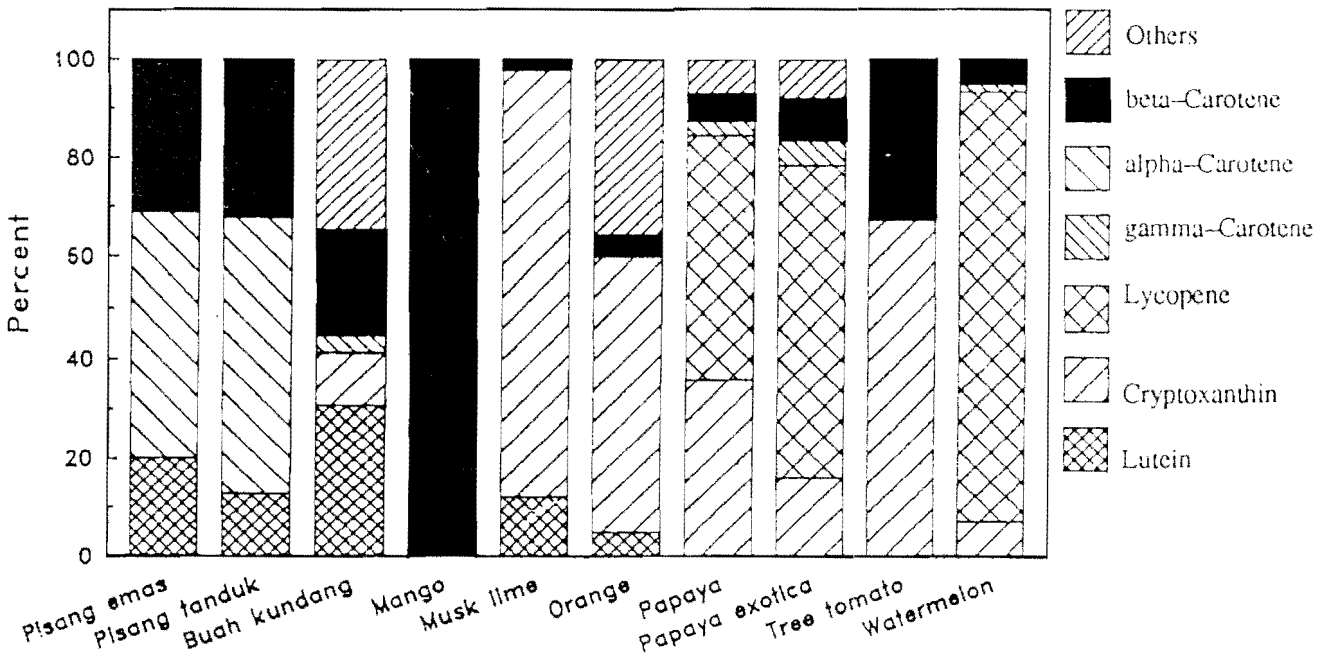

FIG 3– Carotenoid Composition of Fruits

concentrations of β–carotene were found in green leafy vegetables, particularly *cekor manis* and *tanki* (Table 4). Both local vegetables had a β–carotene content of over 11,000 µg per 100 g of edible portion. Seven other green leafy vegetables were found to have a β–carotene content of between 3,000–10,000 µg per 100 g of vegetable. With the exception of carrot (β–carotene about 7,000 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 100 µg per 100 g of sample. The sample of mango studied was interesting in that all the carotenoids was β–carotene, and the concentration was the highest of all the fruits (about 600 µg per 100g).

Vitamin A Activity (Retinol Equivalent) Conventionally, the nutritional significance of carotenoids is related to the provitamin A acitivity of these compounds. For vitamin A activity, a carotenoid must have at least one unsubstituted β–

TABLE IV – β–carotene Content[1] of Vegetables and Fruits determined by the AOAC and HPLC Methods

Name of Vegetable/Fruit	AOAC method	HPLC method
Green, leafy vegetables		
Cekur manis	12363	13351
Cemperai	6788	3218
Chinese cabbage	2604	3022
Drumstick leaves	7724	7536
Fern shoots	1273	1438
Papaya shoots	1709	1829
Pegaga gajah	3266	3840
Ranti	6760	7048
Spinach	2947	3177
Swamp cabbage	1729	1895
Tanki	11459	11395
Tapioca shoots	4607	5720
Green, non–leafy vegetables		
French bean	221	236
Long bean (dark green)	520	569
Paprika/Bell pepper	154	267
Snake gourd	138	148
Other vegetables		
Carrot	9027	6769
Chilli, red	1743	1663
Pumpkin	1170	578
Tomato	352	365
Fruits		
Pisang emas	82	40
Pisang tanduk	219	92
Buah kundang	303	301
Mango (Black–gold)	495	615
Musk lime	18	12
Orange	40	25
Papaya	208	228
Papaya exotica	314	321
Tree tomato	582	599
Watermelon (red)	246	324

[1] Mean of duplicate analyses; expressed as µg per 100 g of edible portion of sample

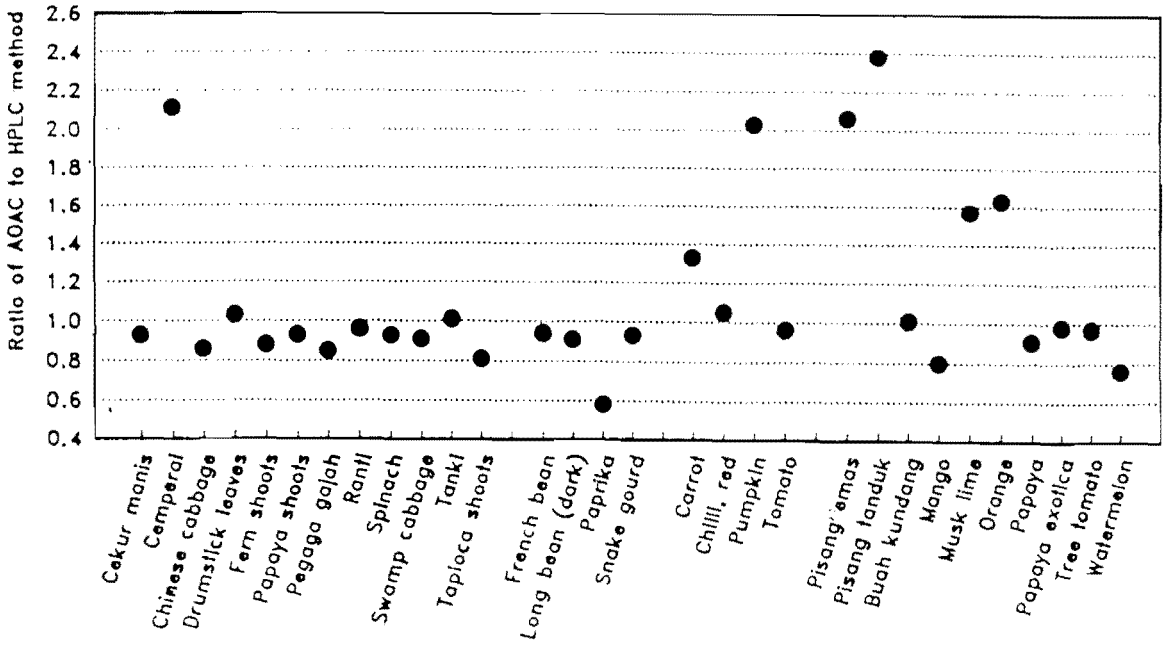

FIG 4–Ratio of beta–Carotene of Vegetables and Fruits Determined by the AOAC and HPLC Methods

ionone ring with an attached polyene side of at least 11 carbon atoms. Consistent with these important structural requirements, the following carotenoids identified in this study have been known to possess provitamin A activity: β–carotene, α–carotene, τ–carotene, and cryptoxanthin. The vitamin A activity of β–carotene, expressed as μg retinol equivalent (RE) was calculated as RE = (μg β–carotene)/6 (National Academy of Sciences, 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 provitamin A carotenoids was therefore RE = (μg carotenoid)/12.

Data on concentration of individual carotenoids obtained by the HPLC method can therefore be used for the calculation of RE using the formula:

$$RE = \frac{\mu g\ \beta\text{–carotene}}{6} + \frac{\mu g\ \text{other provitamin A carotenoids}}{12}$$

Results obtained are given in Tables 5 and 6 for the vegetables and fruits respectively.

On the other hand, results obtained from the AOAC method could not be used satisfactorily for the calculation of RE. Firstly, this is because the method determines both α– and β–carotenes together as the latter. RE calculated would be falsely elevated since α–carotene possesses only half the vitamin A activity of β–carotene. Secondly, the AOAC method is not able to quantitate the other provitamin A carotenoids present. In foods where these carotenoids are present, especially fruits, RE calculated would be erroneously low (Tables 5 and 6).

To facilitate easy identification of vegetables which are good sources of vitamin A activity, results obtained by the HPLC method were used to group the foods studied into four categories, namely low (<100 μg RE per 100 g edible portion), medium (100–499 μg RE), high (500–999 μg RE) and very high (>1,000 μg RE). These are illustrated graphically in Figure 5. Vegetables with high and very high RE were all green leafy vegetables, with the exception of carrot, a root vegetable. Of particular interest are three local vegetables with over 1,000 μg RE, namely *cekur manis*, *tanki*, and *ranti*. 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 tomato were found to be poor sources of vitamin A.

A similar grouping was made for the fruits studied (Figure 6). 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 *pisang emas*.

CONCLUSION

A non–aqueous reversed–phase HPLC method for the determination of carotenoids in various vegetables and fruits has been developed. The method uses the basic configurations of a 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, τ–, α– and β–carotenes. The emphasis has been on major carotenoids that occur in sufficient amounts to contribute significantly to dietary intake.

Findings from the study have clearly shown that the HPLC is a powerful tool for the analysis of carotenoids in vegetables and fruits. The method would give a more complete picture of the carotenoid composition as well as a more accurate quantitation of the provitamin A activity of the 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 up–dating the vitamin A activity of plant materials in the current Malaysian Food Composition Table, thereby providing the correct identification of foods rich in provitamin A activity. The nutritional significance of the findings are clear since these foods are important sources of vitamin A for the majority of the rural communities in the country.

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TABLE V – Retinol Equivalent (RE)[1] of Vegetables determined by the AOAC and HPLC Methods

Name of Vegetable	AOAC method[2]	HPLC method[3]
Green, leafy vegetables		
Cekur manis	2061	2225
Cemperai	1131	843
Chinese cabbage	434	504
Drumstick leaves	1287	1256
Fern shoots	212	240
Papaya shoots	285	305
Pegaga gajah	544	640
Ranti	1127	1175
Spinach	491	530
Swamp cabbage	288	316
Tanki	1910	1899
Tapioca shoots	768	953
Green, non–leafy vegetables		
French bean	37	39
Long bean (dark green)	87	95
Paprika/Bell pepper	26	45
Snake gourd	23	25
Other vegetables		
Carrot	1504	1412
Chilli, red	290	423
Pumpkin	195	159
Tomato	59	61

[1] Mean of duplicate analayses; expressed as μg per 100 g of edible portion of sample
[2] Calculated as RE = (μg carotene)/6
[3] Based on β–carotene and all other provitamin A carotenoids, i.e. α–carotene, τ–carotene, cryptoxanthin; RE = [(μg β–carotene)/6] + [(μg other carotenoids)/12]

TABLE VI – Retinol Equivalent (RE)[1] of Fruits determined by the AOAC and HPLC Methods

Name of Fruit	AOAC method[2]	HPLC method[3]
Pisang emas	14	12
Pisang tanduk	36	28
Buah kundang	51	67
Mango (Black–gold)	82	103
Musk lime	3	39
Orange	7	32
Papaya	35	171
Papaya exotica	52	120
Tree tomato	97	203
Watermelon (red)	41	99

[1] Mean of duplicate analayses; expressed as μg per 100 g of edible portion of sample
[2] Calculated as RE = (μg carotene)/6
[3] Based on β–carotene and all other provitamin A carotenoids, i.e. α–carotene, τ–carotene, cryptoxanthin; RE = [(μg β–carotene)/6] + [(μg other carotenoids)/12]

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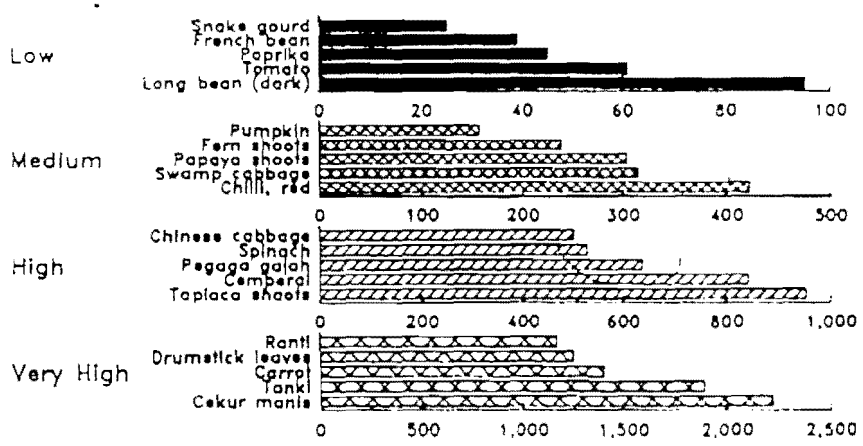

FIG 5 – Classification of Vegetables According to Retinol Equivalent (RE)

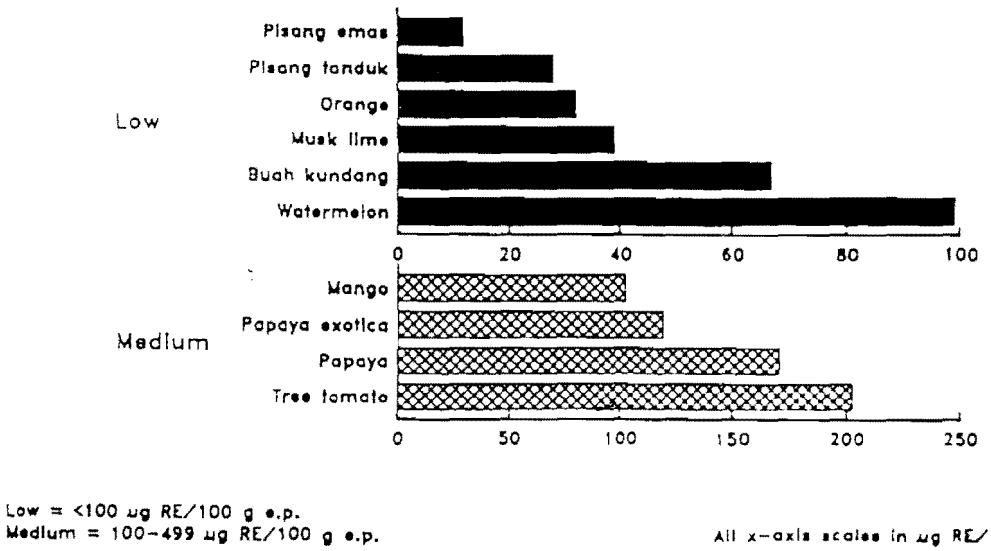

FIG 6 – Classification of Fruits According to Retinol Equivalent (RE)