Food Chemistry 45 (1992) 289–296
Analytical Methods Section

Re-analysis of vitamin A values of selected Malaysian foods of animal origin by the AOAC and HPLC methods

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(Received 12 November 1990; revised version received and accepted 16 October 1991)

Vitamin A values of 40 foods of animal origin from various food groups and several processed foods were studied using a newly developed, reverse-phase, high-performance liquid chromatography (HPLC) method. Carotenoids and retinol were separated isocratically on an octadecylsilane (C_{18}) column using a ternary mixture of acetonitrile, methanol and ethyl acetate. Two detectors connected in series were used to detect and quantify carotenoids simultaneously at 436 nm and retinol at 313 nm in a single chromatographic run. All samples were also simultaneously determined using the Association of Official Analytical Chemists (AOAC) open-column (alumina) chromatographic method. The AOAC method was found to give significantly higher retinol contents in the foods studied, due to the presence of other pigments that gave falsely elevated absorbance readings. Although there was no statistically significant difference in β-carotene contents given by the HPLC and AOAC methods, there were more foods with higher results given by the latter method. β-Carotene contents were generally low; only in seven foods did the carotene contribute more than 50% of the total vitamin A value. The contribution of other provitamin A carotenoids is probably insignificant. Thus, the total vitamin A activity of these foods was mainly contributed by retinol.

The proposed HPLC method has been shown to be applicable to the determination of carotenoids in vegetables and fruits, as well as to the determination of carotenoids and retinol in foods of animal origin.

INTRODUCTION

In recent years, there has been greater emphasis on obtaining more accurate data on vitamin A values of foods. Rapid advances have taken place in the development of methodologies for more accurate quantification of various carotenoids and retinoids in foods. Since the late 1970s, high-performance liquid chromatography (HPLC) has become a widely used procedure for these purposes, mainly because of its ability to effect rapid separation, its non-destructiveness and, more importantly, the better resolution that is achieved (Taylor, 1983). However, most of the reports in the literature deal with the analysis of a limited number of foods. Some studies deal with the characterisation of a large number of carotenoids, but the procedures employed were necessarily complicated and unsuitable for routine analytical use. Not many studies dealing with the simultaneous determination of retinol and carotenoids of foods have been reported (Tee & Lim, 1991*a*). Because of the complicated nature of the required analyses, the application of HPLC to the analysis of foods is still being developed and improved.

The authors embarked on a systematic study to develop improved methodologies for the separation and

Food Chemistry 0308-8146/92/$05.00

quantification of retinol and several carotenoids in foods. A simple HPLC method was developed, workable for the routine analysis of a wide variety of foods of both plant and animal origins. The method was also applicable to the analysis of blood samples for the assessment of vitamin A status of communities. Such a system would then obviate the use of different and complicated chromatographic conditions for different food samples, as well as for blood. In all the studies carried out, the samples were simultaneously determined by the newly developed HPLC method and the open-column chromatographic methods of the Association of Official Analytical Chemists (AOAC) (Deutsch, 1984), to enable a systematic comparison of results obtained by the two methods. Few parallel studies of this nature have been carried out, although the AOAC methods, widely used for the generation of data for food composition tables, have been said to be insufficiently discriminative.

The HPLC method developed has been applied to the study of carotenoid composition of various Malaysian vegetables and fruits for more accurate quantification of vitamin A values (Tee & Lim, 1991*b*). Results obtained from the HPLC method were compared with those given by the AOAC method. This paper reports the suitability of the HPLC method for the simultaneous determination of retinol and several carotenoids in foods of animal origin. Differences in results, if any, obtained from the HPLC and AOAC methods are also reported.

MATERIALS AND METHODS

Solvents and standards

Analytical-grade solvents were used for sample preparation and pretreatment, and for open-column chromatographic procedures. Solvents for HPLC 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 ultrasonic bath.

α-Carotene, β-carotene, lycopene and retinol standards were purchased from Sigma Chemical Company. Stock solutions of the carotenoids were prepared in hexane and those of retinol in ethanol, in concentrations of 100 μg ml^{-1}, and stored in amber bottles below 20°C. Working solutions of 1 μg ml^{-1} 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. Absorbance readings and absorption spectra of all standard solutions were monitored daily. The preparation of all standard solutions was carried out rapidly, in a room with subdued light and with all windows tinted with a light-protective film. All sample treatments and analytical procedures were also carried out in this room.

Sample preparation and pretreatment

Some common foods of animal origin from various food groups and several processed foods were obtained for study. Edible portions of the samples were homogenised in a blender and 20 g immediately weighed for analysis. Liquid foods were well mixed before taking an aliquot for analysis.

Sample pretreatment procedures were as described by AOAC (Deutsch, 1984). To duplicate portions of each food sample were added 20 ml of 100% (w/v) potassium hydroxide and 80 ml of ethanol. After saponification on an electric heating mantle for 30 min, the mixture was cooled and extracted four times with 30-ml portions of hexane. The hexane extracts were pooled, washed with water till free of alkali, and dried over sodium sulphate. The extract was reduced to a small volume over a water-bath and made up immediately to a suitable volume (e.g. 25 ml) with hexane.

The hexane extract was then subjected to two chromatographic and subsequent quantification procedures:

(1) open-column chromatography using alumina and quantification by absorbance reading at 450 nm for the carotene fraction and 325 nm for the retinol fraction (AOAC method); and

(2) HPLC and simultaneous detection and quantification at 436 nm for carotenes and 313 nm for retinol (HPLC method).

Alumina-column chromatography (AOAC method)

A suitable volume (e.g. 10 ml) of the hexane extract was pipetted into a glass column packed with alumina (Merck, aluminium oxide 90, neutral, activity grade I), deactivated by adding 5 ml of water to 95 g of the adsorbent. Carotenes were eluted from the column with approximately 10 ml of 4% (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 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 from a calibration curve prepared with a β-carotene standard.

Retinol was next eluted from the alumina column using 15% (v/v) acetone in hexane. The eluate was similarly evaporated and made up to suitable volume (e.g. 10 ml) in ethanol and the absorbance reading taken at 325 nm. The concentration of retinol in the solution was calculated using a retinol standard. For eluates with a yellow colour (due to the presence of xanthophylls), a correction factor was applied to the absorbance obtained at 325 nm. The correction factor was determined as described in AOAC (Deutsch, 1984).

The purities of both the 4% and the 15% acetone-in-

hexane eluates from the alumina column were examined by obtaining the absorption spectra from 250 to 600 nm, as well as by HPLC.

High-performance liquid chromatography (HPLC method)

HPLC conditions

The HPLC conditions used were similar to those used by the authors for the analysis of carotenoids in fruits and vegetables (Tee & Lim, 1991*b*). The Waters HPLC was equipped with two Model-440 fixed-wavelength detectors connected in series. A 436-nm wavelength kit was fitted onto the first detector, whilst the second detector was fitted with one of 313 nm (or 340 nm). The attenuation on both detectors was set at 0·02 absorbance units full scale (AUFS). The analytical column, a 30 cm × 3·9 mm i.d. stainless-steel column packed with 10-μm μBondapack C_{18}, was preceded by a Waters Guard-PAK pre-column module. Sample injection volumes, dispensed using a Rheodyne 7125 injector, were usually 50–100 μl. The mobile phase consisted of a ternary mixture of acetonitrile, methanol and ethyl acetate (88:10:2, v/v) pumped at a rate of 2·0 ml min^{-1} using a Waters 6000A solvent delivery system. Eluted carotenoid peaks were plotted using pen 1 of a Waters 730 Data Module and the peak areas quantified. The retinol peak was plotted using pen 2 and the peak height was used for quantification, since the Data Module is capable of integrating peak area only on one pen.

Chromatography of carotenoids and retinol

Hexane in the extract was first evaporated off on a water-bath and the residue immediately redissolved in a suitable volume of the mobile phase. After filtering through a 0·45-μm regenerated cellulose membrane, suitable volumes were injected into the chromatograph. Identification and quantification of carotenoids and retinol were carried out by comparing peak areas obtained with areas given by authentic samples of these compounds similarly chromatographed.

Statistical analyses

All statistical tests for significant differences between mean values for two sets of data were carried out using the Wilcoxon signed-rank test (two-tailed), the non-parametric method for not normally distributed observations, using the SPSS/PC+ statistical package.

RESULTS AND DISCUSSION

Simultaneous determination of carotenoids and retinol

Several investigators have reported the determination of both carotenes and retinol in various food samples using HPLC (Tee & Lim, 1991*a*). However, in most of these studies, carotene and vitamin A were eluted and detected using two separate sets of chromatographic conditions. Examples of such studies are van de Weerdhof *et al.* (1973), Thompson and Maxwell (1977), Thompson *et al.* (1980), Scott *et al.* (1984), Heinonen *et al.* (1988), and van de Dokkum *et al.* (1990). There would be considerable savings in time if β-carotene and retinol could be determined in a single chromatographic run. Since the absorption maxima of these two compounds differ widely, detection would have to be carried out at two wavelengths. One way to do this would be to change the wavelength in the middle of a chromatographic separation. The switch could be done manually, as was carried out by Stancher and Zonta (1982), but would require close monitoring of the run and could be rather tedious. Alternatively, wavelength change could be effected at a predetermined time using programmable detectors. Simultaneous detection could also be carried out by using dual-wavelength detectors. If only fixed-wavelength detectors were available, continuous monitoring of the eluate could be carried out by connecting two such detectors (with different wavelength filters) in series. Landen and co-workers (Landen & Eitenmiller, 1979; Landen, 1980, 1982) reported the continuous monitoring of β-carotene at 436 nm and retinyl esters at 313 nm using two fixed-wavelength detectors.

In this study, two Model-440 fixed-wavelength detectors connected in series effected the simultaneous detection and quantification of carotenoids and retinol. A 436-nm wavelength kit was used for carotenoids, while retinol was detected at 313 nm. The latter wavelength is also useful for detecting the presence of *cis*-isomers of β-carotene, which will appear as a larger than usual peak at this wavelength. Figure 1 shows a chromatogram of three carotenoids and retinol detected in the same chromatographic run and plotted by both pens in the Waters 730 Data Module. The polarity of pen 2 was reversed to enable clear viewing of the chromatogram.

Carotenoid content

The mean β-carotene content of 40 types of foods studied by the AOAC and the HPLC methods are tabulated in Table 1 according to food groups. Ratios of results given by the AOAC method to those given by the HPLC method are given in the last column of the table. A ratio of unity indicates that similar results were given by the two methods. Marginal differences in results, with ratios varying within ± 20% from unity (ratio between 0·8 and 1·2) were observed for 16 of the food studied. For another 6 foods, results given by the HPLC method were higher, with ratios of 0·1–0·7 times those of the AOAC method. The latter method could be underestimating in these foods, all of which had very low levels of β-carotene, due to the non-specific

Table 1. Mean β-carotene content of selected foods of animal origin and processed foods[a]

Name of food	AOAC method	HPLC method	AOAC: HPLC
Eggs			
Century egg	11·0	3·5	3·1
Duck egg[b]	24·0	31·8	0·8
Hen egg	16·0	1·0	16·0
Quail egg	20·0	5·0	4·0
Salted egg	18·5	9·5	2·0
Fish and seafoods			
Anchovies	7·0	6·0	1·2
Black pomfret	0·4	0·1	4·0
Canned sardine	1979	62·5	31·7
Cockles[b]	157	194	0·8
Crab meat	33·0	44·0	0·8
Cuttlefish	0·0	0·0	1·0
Dried cuttlefish	9·5	1·0	9·5
Dried oyster	208	138	1·5
Dried prawn	33·0	34·5	1·0
Fermented shrimp	1·5	0·1	15·0
Indian mackerel	1·5	0·1	15·0
Lobster	136	149	0·9
Pink prawn	41·0	49·5	0·8
Scallop	55·0	39·0	1·4
Shrimp paste	14·5	7·5	1·9
Spanish mackerel	0·3	0·1	3·0
Meat and meat products			
Beef, local	6·5	9·5	0·7
Chicken burger	0·7	3·5	0·2
Chicken frankfurter	0·2	1·4	0·2
Chicken heart	1·5	0·1	15·0
Chicken liver[b]	29·3	17·7	1·7
Chicken thigh	0·0	0·0	1·0
Mutton, local	0·0	0·0	1·0
Milk and milk products			
Butter	563	675	0·8
Cheese[c]	173	221	0·8
Malted milk powder[b]	14·7	7·5	2·0
Full cream milk powder[b]	128	137	0·9
Sweetened condensed filled milk[b]	2·5	5·5	0·5
Sweetened condensed milk	37·2	52·8	0·7
Ultra-heat-treated milk	8·5	20·0	0·4
Oils			
Ghee	452	549	0·8
Margarine	907	897	1·0
Other processed foods			
Canned baked bean	2053	127	16·2
Cornflakes	78·5	51·5	1·5
Wheat infant cereal	18·5	20·5	0·9

[a] Mean, in micrograms per 100 g of edible portion, of duplicate analyses (except as indicated below).
[b] 6 analyses.
[c] 4 analyses.

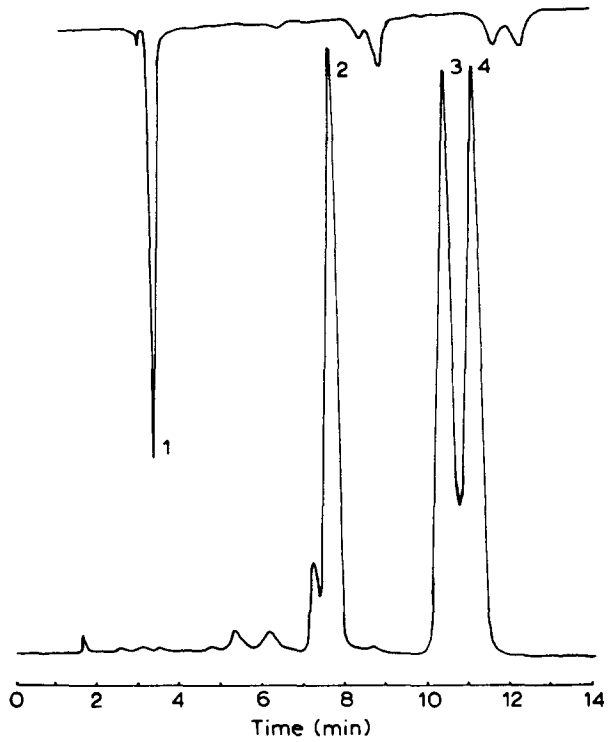

Fig. 1. HPLC chromatogram of a mixture of retinol, lycopene, α-carotene and β-carotene. Concentrations (per millilitre): 0·3 μg retinol; 2 μg lycopene; 1 μg α-carotene; 1 μg β-carotene. 100 μl used for injection.

nature of the method. There were more foods with high results given by the AOAC method. For 7 of the foods, results were between 1·3 and 2 times those of the HPLC method. In another 4 foods, results were between 2·1 and 5 times higher, while the remaining 7 foods showed AOAC results more than 5 times higher than those by the HPLC method. Nevertheless, when results obtained by the two methods were compared using the Wilcoxon signed-rank test (two-tailed), the non-parametric method for not normally distributed observations, it was found that there was no statistically significant difference ($Z = 0{\cdot}26$; $P = 0{\cdot}79$; $n = 40$).

When the β-carotene values given by the AOAC method were much higher than those given by the HPLC method, the UV–vis absorption spectra of the 4% acetone-in-hexane eluate from the alumina column were seen to be not characteristic of β-carotene. It is clear that other pigments, possibly other carotenoids, were present in the eluate, thereby contributing to the elevated absorbance reading at 450 nm. Examination of the HPLC chromatograms of the 4% acetone-in-hexane eluates confirmed the presence of other peaks absorbing at around 450 nm. Therefore, results given by the AOAC method were overestimated.

Further examination of the differences in results given by the two methods showed that, although the AOAC method gave much higher results (over 5 times that of the HPLC method) for some of the foods studied, the exaggerated values may not be of much nutritional significance, because, for most of these foods, β-carotene values were low, less than 10 μg per 100 g of edible portion. The only two foods that contain significant amounts of β-carotene (50–100 μg per 100 g of food), and in which the AOAC method gave results which were over 15 times that of the HPLC method,

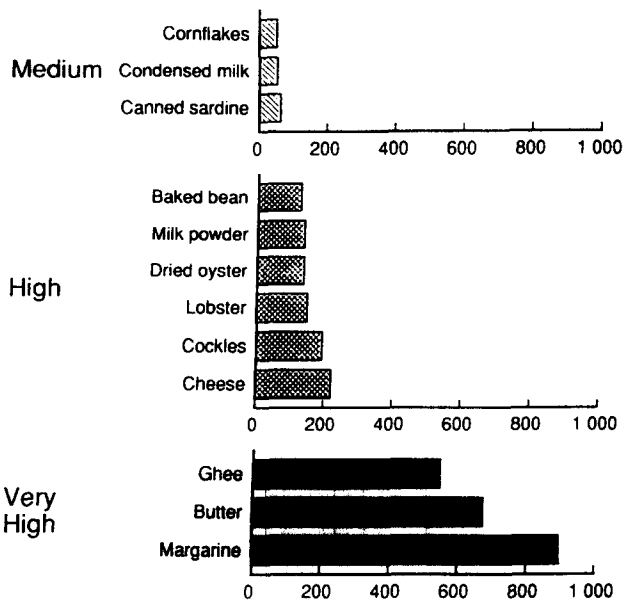

Fig. 2. Classification of foods according to β-carotene content as determined by HPLC. All *x*-axis scales in micrograms per 100 g of edible portion.

were canned sardine in tomato sauce and canned baked bean. The erroneously high carotene values for these two processed foods were found to be caused by the elution of lycopene into the 4% acetone-in-hexane fraction and estimated as β-carotene.

On the basis of results given by the HPLC method, the foods studied were grouped into those with low (< 50 μg per 100 g of edible portion), medium (50–99 μg per 100 g), high (100–499 μg per 100 g) and very high (> 500 μg per 100 g) β-carotene contents (Fig. 2). Twenty-eight foods (70%) in the low category are probably of little significance as contributors of dietary β-carotene intake. These foods may be considered as poor sources of provitamin A, contributing less than 10 μg of retinol equivalent (assuming 6 μg of β-carotene is equal to 1 μg of retinol equivalent (NAS, 1989)).

α-Carotene was not detected in all the foods studied. Lycopene was infrequently encountered, but it was found in high concentrations (> 1 500 μg per 100 g of edible portion) in canned baked bean and canned sardine, contributed by the tomato sauce used in the preparation of the foods.

Retinol content

Table 2 gives the mean retinol content of the foods studied by the two methods. As for carotene, the ratio between results given by the AOAC and HPLC methods provided an indication of the differences between results given by the two methods. Marginal differences in results were obtained for 12 of the foods studied, with ratios between 0·8 and 1·2. For the remaining 28 foods, results given by the AOAC method were all higher than those given by the HPLC method. For 7 of the foods, the ratio was between 1·3 and 2. The AOAC method gave results that were higher by 2·1–5 times in 14 of the foods and more than 5 times for the remaining 7 foods. Using the signed-rank test, it was found that results obtained by the AOAC method were

Table 2. Mean retinol content of selected foods of animal origin and processed foods[a]

Name of food	AOAC method	HPLC method	AOAC: HPLC
Eggs			
Century egg	118	96·0	1·2
Duck egg[b]	196	141	1·4
Hen egg	140	90·5	1·6
Quail egg	209	97·0	2·2
Salted egg	136	54·0	2·5
Fish and seafoods			
Anchovies	50·5	14·5	3·5
Black pomfret	52·0	62·5	0·8
Canned sardine	76·5	13·5	5·7
Cockles[b]	69·3	8·3	8·3
Crab meat	7·5	2·0	3·8
Cuttlefish	16·5	9·5	1·7
Dried cuttlefish	36·0	12·0	3·0
Dried oyster	132	32·5	4·1
Dried prawn	28·0	1·0	28·0
Fermented shrimp	143	98·5	1·5
Indian mackerel	25·5	8·0	3·2
Lobster	9·5	2·5	3·8
Pink prawn	8·5	3·0	2·8
Scallop	74·0	16·0	4·6
Shrimp paste	203	52·5	3·9
Spanish mackerel	34·0	7·5	4·5
Meat and meat products			
Beef, local	12·0	1·0	12·0
Chicken burger	79·5	15·5	5·1
Chicken frankfurter	46·5	11·5	4·0
Chicken heart	45·0	15·5	2·9
Chicken liver[b]	6264	8235	0·8
Chicken thigh	24·5	16·5	1·5
Mutton, local	23·5	4·5	5·2
Milk and milk products			
Butter	176	140	1·3
Cheese[c]	138	129	1·1
Malted milk powder[b]	586	552	1·1
Full cream milk powder[b]	661	672	1·0
Sweetened condensed filled milk[b]	265	281	0·9
Sweetened condensed milk	274	257	1·1
Ultra-heat-treated milk	38·5	20·5	1·9
Oils			
Ghee	169	151	1·1
Margarine	246	243	1·0
Other processed foods			
Canned baked bean	28·0	1·0	28·0
Cornflakes	764	803	1·0
Wheat infant cereal	363	363	1·0

[a] Mean, in micrograms per 100 g of edible portion, of duplicate analyses (except as indicated below).
[b] 6 analyses.
[c] 4 analyses.

Table 3. Total RE in selected foods of animal origin and processed foods determined by the HPLC method[a]

Name of food	Total RE (μg per 100 g of edible portion)	Percentage of total RE from retinol
Eggs		
Century egg	96·6	99·4
Duck egg	146	96·4
Hen egg	90·5	100
Quail egg	97·8	99·1
Salted egg	55·6	97·2
Fish and seafoods		
Anchovies	15·5	93·5
Black pomfret	62·5	100
Canned sardine	23·9	56·4
Cockles	40·6	20·5
Crab meat	9·3	21·4
Cuttlefish	9·5	100
Dried cuttlefish	12·0	100
Dried oyster	55·5	58·6
Dried prawn	5·8	0·0
Fermented shrimp	98·5	100
Indian mackerel	8·0	100
Lobster	27·3	9·1
Pink prawn	11·3	26·7
Scallop	22·5	71·1
Shrimp paste	53·8	97·7
Spanish mackerel	7·5	100
Meat and meat products		
Beef, local	2·6	38·7
Chicken burger	16·1	96·4
Chicken frankfurter	11·7	98·1
Chicken heart	15·5	100
Chicken liver	8238	100
Chicken thigh	16·5	100
Mutton, local	4·5	100
Milk and milk products		
Butter	252	55·5
Cheese	166	77·8
Malted milk powder	554	99·8
Full cream milk powder	694	96·7
Sweetened condensed filled milk	281	99·7
Sweetened condensed milk	266	96·7
Ultra-heat-treated milk	23·8	86·0
Oils		
Ghee	243	62·3
Margarine	392	61·9
Other processed foods		
Canned baked bean	21·1	0·0
Cornflakes	812	98·9
Wheat infant cereal	366	99·1

[a] RE = retinol equivalent, computed as $\text{retinol } (\mu g) + \frac{\beta\text{-carotene } (\mu g)}{6}$.

significantly higher than those given by the HPLC method ($Z = 4{\cdot}18$; $P < 0{\cdot}001$; $n = 40$).

UV–vis absorption spectra of the 15% acetone-in-hexane eluates that contained the retinol were examined to assist in determining the reason for the higher values obtained by the AOAC method. For these foods, the 15% acetone-in-hexane eluates were usually coloured, owing to the presence of other pigments. Thus, the retinol peaks were usually falsely raised, thereby giving elevated absorbance readings at 325 nm. The HPLC chromatograms of these fractions from the alumina column also confirmed the presence of several pigments absorbing at around 325 nm. For several foods, although there was a substantial absorbance reading, the HPLC chromatogram showed no peak at the retention time for retinol (2·4 min).

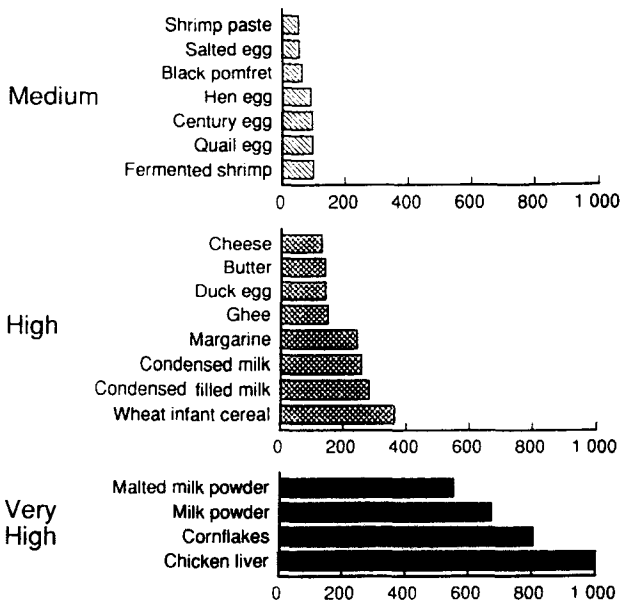

Fig. 3. Classification of foods according to retinol content as determined by HPLC. All *x*-axis scales in micrograms per 100 g of edible portion. The retinol content of chicken liver was 8235 μg per 100 g.

As for β-carotene content, the foods were grouped into four categories according to their retinol concentration as determined by the HPLC method. Figure 3 shows foods with medium (50–99 μg per 100 g of edible portion), high (100–499 μg per 100 g) and very high (> 500 μg per 100 g) retinol contents. Twenty-one foods with low (< 50 μg per 100 g) contents were not included in the chart.

Vitamin A activity (retinol equivalent)

The vitamin A activities of the foods studied were calculated by summing the retinol content and one-sixth of the β-carotene content and expressing the results in micrograms of retinol equivalent (RE) (NAS, 1989). Table 3 gives the RE calculated, together with the percentage contribution from retinol for each food. When grouped into various levels of RE content as above, the foods in each group were identical to those previously obtained for the grouping based on retinol content alone. The only exception was the dried oyster, which could now be classified as having medium RE, with additional contribution from β-carotene. It would appear

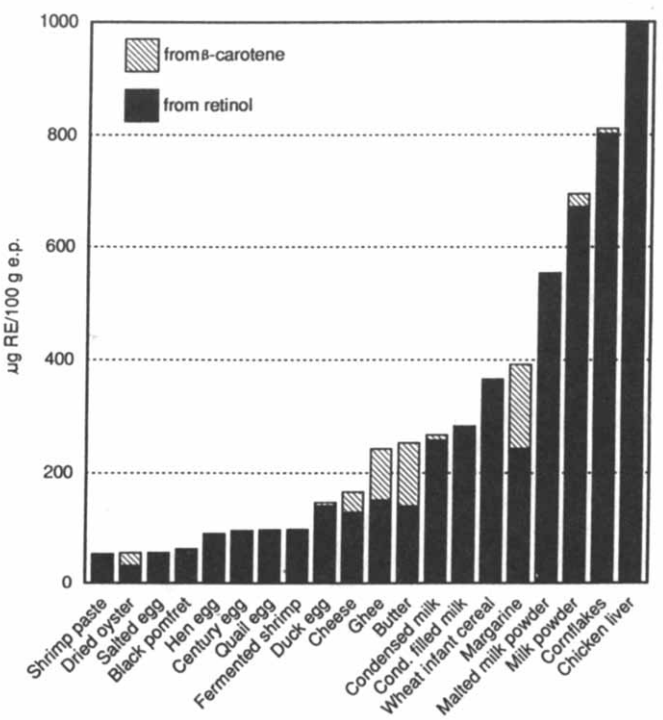

Fig. 4. Contribution of β-carotene and retinol to total vitamin A activity. Only foods with RE > 50 µg per 100 g plotted. The RE for chicken liver was 8235 µg per 100 g, all contributed by retinol.

that β-carotene contribution to total RE was low in many foods. Only in 7 foods did β-carotene contribute more than 50% of the total RE, and these were all foods with 'low' vitamin A content. Figure 4 shows that, of those with more than 50 µg of RE per 100 g of food, only 4 foods contained β-carotene that contributed more than one-third of the total RE.

CONCLUSIONS

The proposed HPLC method, using basic configurations of liquid chromatography, has been shown to be useful for the determination of vitamin A values in fruits and vegetables, as well as in foods of animal origin. For the latter group of foods, two fixed-wavelength detectors connected in series effected the simultaneous detection and quantification of carotenoids and retinol in a single chromatographic run. The method can be used for the analysis of vitamin A values of foods for updating and improvement of food composition databases.

The AOAC and HPLC methods were found to give statistically significant differences in β-carotene and retinol contents in the 40 foods of animal origin and processed food studied. The former method tended to give higher results for both nutrients, owing to the presence of other pigments that gave falsely elevated absorbance readings. UV-vis absorption spectra of the eluates from the alumina column, as well as HPLC chromatograms, were used to detect the presence of interfering pigments.

The β-carotene contents in the foods of animal origin studied were generally low; only in 7 foods did the carotene contribute more than 50% of the total vitamin A value, and these were all foods with low vitamin A contents. The contribution of other carotenoids is probably insignificant. Thus, the total vitamin A activities of these foods were contributed mainly by retinol.

ACKNOWLEDGEMENTS

The authors thank Dr M. Jegathesan, Director of the Institute for Medical Research, for granting permission to publish this paper. The technical assistance of Ms Rajamanohari and Ms S. C. Khor is also acknowledged. The project was funded under the Intensification of Research Priority Areas (IRPA) Programme of the Ministry of Science, Technology and Environment Malaysia (Project No. IMR 53).

REFERENCES

De Ritter, E. (1981). Absorption maxima and E ($1_{\%}/1_{cm}$) values for carotenoids. In *Carotenoids as Colorants and Vitamin A Precursors*, ed. J. C. Bauernfeind. Academic Press, New York, USA, pp. 883–923.

Deutsch, M. J. (1984). Vitamins and other nutrients. In *Official Methods of Analysis of the AOAC* (14th edn), ed. S. Williams. Association of Official Analytical Chemists, Arlington, VA, USA, pp. 830–6.

Heinonen, M., Ollilainen, V., Linkola, E., Varo, P. & Koivistoinen, P. (1988). Carotenoids and retinoids in Finnish foods: ready-to-eat foods. *J. Food Comp. Anal.*, **1**, 221–30.

Landen Jr, W. O. (1980). Application of gel permeation chromatography and nonaqueous reverse phase chromatography to high pressure liquid chromatographic determination of retinyl palmitate in fortified breakfast cereals. *J. AOAC*, **63**, 131–6.

Landen Jr, W. O. (1982). Application of gel permeation chromatography and nonaqueous reverse phase chromatography to high performance liquid chromatographic determination of retinyl palmitate and α-tocopheryl acetate in infant formulas. *J. AOAC*, **65**, 810–6.

Landen Jr, W. O. & Eitenmiller, R. R. (1979). Application of gel permeation chromatography and nonaqueous reverse phase chromatography to high pressure liquid chromatographic determination of retinyl palmitate and β-carotene in oil and margarine. *J. AOAC*, **62**, 283–9.

NAS (1989). Fat-soluble vitamins: Vitamin A. In *Recommended Dietary Allowances* (10th edn). National Academy Press, Washington, DC, USA, pp. 78–114.

Scott, K. J., Bishop, D. R., Zechalko, A., Edwards-Webb, J. D., Jackson, P. A. & Scuffam, D. (1984). Nutrient content of liquid milk. I. Vitamin A, D3, C and of the B complex in pasteurized bulk liquid milk. *J. Dairy Res.*, **51**, 37–50.

Stancher, B. & Zonta, F. (1982). High-performance liquid chromatographic determination of carotene and vitamin A and its geometric isomers on foods Application to cheese analysis. *J. Chromatogr.*, **238**, 217–25.

Taylor, R. F. (1983). Chromatography of carotenoids and retinoids. *Adv. Chromatogr.*, **22**, 157–213.

Tee, E.-S. & Lim, C.-L. (1991*a*). The analysis of carotenoids and retinoids; a review. *Food Chem*, **41**, 147–93.

Tee, E.-S. & Lim, C.-L. (1991*b*). Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. *Food Chem*, **41**, 309–39.

Thompson, J. N. & Maxwell, W. B. (1977). Reverse phase high pressure liquid chromatography of vitamin A in margarine, infant formula, and fortified milk. *J. AOAC*, **60**, 766–71.

Thompson, J. N., Hatina, G. & Maxwell, W. B. (1980). High performance liquid chromatographic determination of vitamin A in margarine, milk, partially skimmed milk, and skimmed milk. *J. AOAC*, **63**, 894–8.

van de Weerdhof, T., Wiersum, M. L. & Reissenweber, H. (1973). Application of liquid chromatography in food analysis. *J. Chromatogr.*, **83**, 455–60.

van Dokkum, W., de Vos, R. H. & Schrijver, J. (1990). Retinol, total carotenoids, β-carotene, and tocopherols in total diets of male adolescents in the Netherlands. *J. Agric. Food Chem.*, **38**, 211–6.