Carotenoid composition and content of legumes, tubers and starchy roots by HPLC

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ABSTRACT

As part of a series of studies on the analytical and nutritional aspects of carotenoids and retinoids in foods, seventeen types of legumes and their products and ***9*** tubers and starchy roots were studied for their carotenoid composition and content by HPLC. All samples were saponified and subsequently chromatographed using a reverse-phase HPLC method previously developed in this laboratory in which carotenoids were separated isocratically on an octadecylsilane (C18) column using a ternary mixture of acetonitrile, methanol and ethyl acetate (88:10:2) as the mobile phase. Carotenoid peaks obtained were tentatively identified using 6 reference standards similarly chromatographed. The HPLC method used enabled the separation and quantitation of the major carotenoids present, namely, lutein, cryptoxanthin, lycopene, **g**-, ***a***- and **b**-carotenes. For most of the legumes, the major carotenoids detected were **b**-carotene, lutein and cryptoxanthin. Lutein was found in all the legumes studied, and was clearly the major carotenoid in most of the legumes, followed by unidentified carotenoids, **b**-carotene and cryptoxanthi. The other carotenoids were encountered infrequently and at low levels. The starchy roots and tubers gave a different carotenoid composition from those obtained for the legumes: lycopene and **b**-carotene were detected in all the samples except in sago. There was no clear pattern of carotenoids present in the samples studied. Compared with the vegetables and fruits, the carotenoid concentration in legumes, tubers and roots were found to be much lower. None of the items studied can be said to be good sources of vitamin A. Nevertheless, they are still of nutritional import flee, if consumed in significant amounts.

INTRODUCTION

Dietary improvement has been recognised as the major long-term solution to controlling vitamin A deficiency in a community. In those parts of the world where vitamin A deficiency is prevalent, vegetable products are the main source of dietary vitamin A in the form of carotenes. Thus, horticultural and related activities to increase the

availability of carotene-rich vegetable products have been emphasised. These would include not only matters related to production (varieties for promotion, pest and disease control), but also problems of transport, marketing, storage, and preservation. These activities should be coordinated with the aspects of nutrition education programmes since availability does not necessarily mean consumption of these foods by the vulnerable segments of the population, especially young children. The reasons why these foods are not consumed in significant amounts, even in areas where they are abundant, should be understood (Tee, 1992).

In order to carry out nutrition education activities to promote the consumption of carotene-rich products, more accurate data on the composition and content of carotenoids in these foods are required. The international Vitamin A Consultative Group (IVACG) has called on national laboratories to develop the ability to determine accurately the provitamin A content of their fruits and vegetables in order to support vitamin A deficiency prevention and control programmes (Simpson, Tsou & Chichester, 1987). IVACG also emphasised that because carotenoids may be protective against some forms of cancer, the quantitation uf these compounds in foods assumes even greater Importance. In a subsequent report, WACO again highlighted the lack of information on the amount and form of both retinoids and carotenoids in existing food composition databases (Underwood *et al.*, 1989). High-pressure liquid chromatography (HPLC) has been proposed as an efficient tool for the separation and quantitation of carotenoids and its application to the analysis of foods is still being developed and improved (Tee & Lim, 199 la).

In cognizance of the need to develop expertise in retinol and carotenoid analysis and to obtain more accurate data of these vitamins in local foods, a systematic project was initiated by this laboratory. A simple HPLC method was developed, workable for the routine analysis of a wide variety of foods of both plant and animal origins as well as blood. The method was applied to the study of carotenoid composition of various locally available vegetables and fruits (Tee & Lim, 1991b) as well as the simultaneous determination of retinol and several carotenoids in foods of animal origin (Tee & Lim, 1992). The method was also applied to the simultaneous determination of carotenoid profile and retinol content of human serum (Tee, Lim & Chong, 1994). The system developed does not require the use of different and complicated chromatographic conditions for different food samples, as well as for blood.

Legumes are important sources of nutrients for several communities in Malaysia. Starchy roots and tubers, on the other hand are consumed less frequently and in lesser amounts. Several types of these foods, however, could provide significant amounts of carotenoids, if consumed in large amounts. A study was thus carried out to determine the carotenoid composition and content of selected legumes, starchy roots and tubers.

MATERIALS AND METHOD

Solvent and carotenoid standards

Solvents for liquid chromotography were of HPLC grade. All solvents for use as the mobile phase in HPLC were filtered through a 0.45 um regenerated cellulose membrane filter and degassed using an ultra-sonic bath.

a- and **b**-Carotenes and lycopene standards were purchased from Sigma Chemical Company. **g**-Carotene, cryptoxanthin and lutein were gifts from F. Hoffmann La-Roche, Switzerland. These six carotenolds used have varying structures including the acyclic conjugated polyenelycopene, carotenoids with psi, beta and epsilon end groups and oxygenated carotenolds. UV-vis absorption spectra of these standards were determined. Structures and absorption spectra of these compounds have been given in Tee & Lim (1991b). Stock solutions of these carotenoids were prepared differently. **a**-Carotene, 13-carotene and lycopene were prepared in hexane whereas cryptoxanthin and y-carotene were prepared In petroleum ether and lutein in ethanol. All stock solutions were in concentrations of 100 ug per ml and stored in amber bottles below – 20 0 C. Working solutions of 2 ug per ml of the standards were prepared daily in the solvent in which it is prepared and its absorbance reading taken with a UV-visible spectrophotometer. The appropriate extinction coefficients published in the literature (De Ritter, 1981) were used to calculate the exact concentration of each of the carotenoids. For HPLC, the solvents of a ternary mixture of acetonitrile, methanol and ethyl acetate (88: 10:2, v / v), as developed by Tee **&** Lim (199 lb), was prepared fresh daily as the mobile phase. Standard solutions of the above carotenoids mixture (0.25 to 1 ug / ml) were then prepared in the mobile phase and 50 ul injected into HPLC. The preparation of all standard carotenoids was carried out in a room with subdued light and with all windows tinted with a light-protective film. All sample treatment and analytical proce-dures were also carried out in this room.

Sample preparation and pretreatment

Studies were carried out on selected legumes, tubers and starchy roots purchased from local markets and stalls. Edible portions of the foods were comminuted in a blender and 10 g immediately weighed for analysis.

Sample pre-treatment procedures were essentially those of AOAC (Deutsch, 1984), except for the introduction of a saponication step. This process would remove other pigments (mainly chlorophyll) from the food samples which would otherwise intefere in the HPLC chromatography process. As has been reported earlier (Tee & Lim, 1991b), 13-carotene appeared not affected by the saponification process, although there was some loss of lutein and other xanthophylls, which have no vitamin A activity.

To 10 g of the well-blended test sample were added a volume of 60 ml of ethanol and saturated potassium hydroxide equal to the

weight of the food sampled used. The mixture was saponified by heating on an electric heating mantle for 30 minutes. The saponified mixture was cooled and extracted with 40 ml portions of hexane until the extract was colourless. The hexane extracts were pooled, washed with distilled water until free of alkali and then dried over anhydrous sodium sulphate. It was heated to a small volume over a water-bath with the aid of a stream of oxygen-free nitrogen and made up immediately to a suitable volume (eg. 10 ml) with mobile phase, referred to hereafter as the "test solution". After passing through a 0.45-um regenerated cellulose membrane filter, suitable volumes were chromatographed using the conditions described below.

High-pressure liquid chromatography (HPLC)

Carotenoids in the test solutions prepared were studied by the HPLC method developed by Tee & Lim (1991b). A stainless steel 30 cm x 3.9 mm I.D. 10-um μBondapak C18 column was used for the chromatographic separation. This was preceded by a Supelco guard column holder housing a disaposable pre-column insert which was packed with the same material as that in the analytical column. Sample injection volumes, dispensed with a Rheodyne 7125 injector, were usually 50 to 100 ul. A Gilson 305 piston pump was used to deliver the mobile phase (acetonitrile-methanol-ethyl acetate, 88: 10:2, v/v) at the rate of 2.0 ml/min. The peaks were detected using a Waters 440 fixed-wavelength detector, fitted with a 436 nm wavelength kit at an attenuation of 0.01 absorbance units full scale (AUFS). Peak areas were then quantitated with a Gilson 714 System Controller Software operating in an IBM-compatible microcomputer. All chromatograms and results analysed were then recorded on a Panasonic KX-P1081 printer. Quantitation and identification of the carotenoids were carried out by comparing with reference standards similarly chromatographed. Peak areas of samples and standards used for calculation were based on mean values obtained from at least four injections. Some food samples were found to contain a few carotenoids which could not be identified. The concentration of these carotenoids were estimated based on **b**-Carotene standard. The concentrations of individual carotenoids were summed to give "sum of carotenoids".

RESULTS AND DISCUSSION

The legumes, tubers and starchy roots studied are listed in Table 1. The English names of the foods are first listed, followed by the local names (in the Malay and other languages) and their scientific names.

Carotenoid composition

The HPLC conditions employed gave satisfactory separation for lutein (retention time, RT = 3.6 mm), cryptoxanthin (RT = 6.0 mm), lycopene (RT = 7.5 min), **g**-Carotene (RT = 9.0 mm), **a**-carotene (RT = 10.2 mm) and **a**-carotene (RT = 10.8 mm). The elution order of a mixture of these carotenoids on the

Table 1. Names of legumes, tubers and starchy roots studied

English name	*Local name*	*Scientific name*
Legumes and legume products		
Kidney bean/Haricot bean	*Kacang buah pinggang*	-
Black eye bean/Cowpea	*Kacang mata hitam*	*Vigna catjang*
Green gram bean/Mung bean	*Kacang hijau*	*Phaseolus aureus*
Red gram bean	*Kacang merah*	*Phaseolus angularis*
Black gram bean	*Kacang hitam*	*Phaseolus mungo*
Black gram dhall	*Dal, Kacang hitam*	*Phaseolus mungo roxb*
Chickpea (small, brown)/ Common gram	*Kacang kuda*	*Cicer arietinum*
Chickpea (big, yellow)/ Common gram	*Kacang kuda*	*Cicer arietinum*
Bengal gram dhall	-	*Cicer arietinum*
Egyptian kidney bean/ Hyacinth bean	*Kacang mochai/ Kacang sepat*	*Dolichos lablab*
Mysore dhall (orange)	*Dal, Mysor/Lentil*	*Lens esculenta*
Australian dhall	*Dal Australia, kuning*	-
Soya bean	*Kacang soya*	*Glycine max/G. soja*
Soya bean curd	*Tim-cok*	-
Soya bean curd, sheets	*Fucok*	-
Soya bean, fermented	*Tempeh*	-
Appalam	-	-
Starchy Roots And Tubers		
Breadfruit	*Sukun*	*Artocarpus communis*
Potato (yellow variety)	*Ubi kentang*	*Salanum tuberosum*
Sago (large)	*Sagu*	*Metroxylon spp.*
Sago (small)	*Sagu*	*Metroxylon spp.*
Sweet potato (yellow variety)	*Ubi keledek*	*Ipomoea batatas*
Sweet potato (orange variety)	*Ubi keledek*	*Ipomoea batatas*
Tapioca, fresh tuber	*Ubi kayu*	*Manihot utilissima*
Tannia	*Keladi telur*	*Xanthosoma sagittifolium*
Taro	*Ubi keladi Cina*	*Colocasia esculentum*

reverse phase C18 column is that the more polar compounds are eluted earlier. As can be seen from the chromatogram presented by Tee & Lim (1991b), the oxygenated carotenoids or xanthophylls were eluted early. Lutein, the dihydroxy pigment, was eluted first, followed by the hydroxy carotenoid cryptoxanthin, and then the straight-chain carotenoid lycopene. The non-polar carotenoid hydro-carbons, **g**-, **a** - and **b**-carotenes were eluted last from the column.

The concentration of the

major carotenoids quantitated are given in Tables 2 and 3 for the legumes and starchy roots and tubers, 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 quntitated accurately. Figures 1 and 2 give the composition of the carotenoids, expressed as the percentage of each carotenoid to the sum of all carotenoids.

For most of the legumes, the major carotenoids detected were **b**-carotene, lutein and cryptoxanthin (Table 2 and Figure 1). Lutein was found in all the legumes studied, and was clearly the major carotenoid in most of the legumes, followed by the unidentified carotenoids, **b**-carotene and cryptoxanthin. in 14 of the legume-based products studied, lutein made up over 60% of the sum of the total carotenoids quantitated. For the remaining three samples, at least 25% of the carotenoids was lutein. **b**-Carotene and cryptoxanthin were detected in all legumes in low proportions (<20%). The other carotenoids were encountered infrequently and at low levels. Lycopene was found in fermented soya bean and black gram dhall, and y-carotene was found in black gram dhall, chickpea (brown) and soya bean curd (sheet), whilst **a** -carotene was not detected under the condition employed.

The starchy roots and tubers give a different carotenoid composition from those obtained for the legumes (Table 3 and Figure 2). There was no clear pattern of carotenoids present in the samples studied. Lycopene and **b**-carotene was detected in all the starchy roots and tubers except sago. Out of the nine tubers and starchy roots studied, **b**-carotene was detected in all except saga, and contributed over 50% of the total carotenoids in

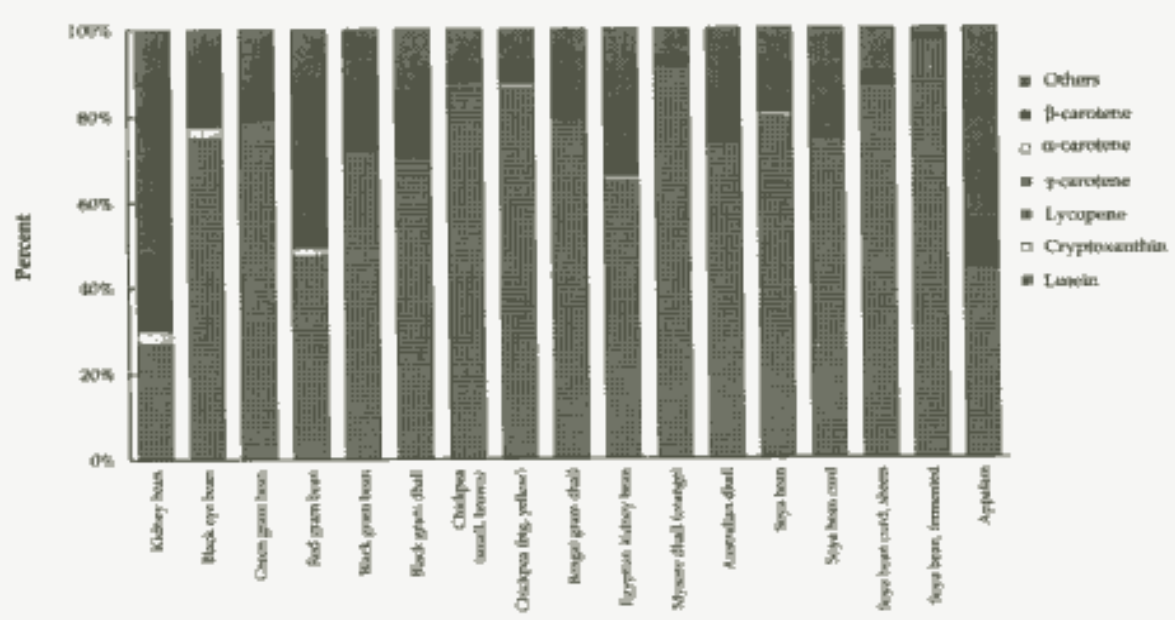

Figure 1. Caroteinoid composition of legumes.

Table 2. Content[a] of Major Carotenoids in Selected Legumes

Name of legume	*Luten*	*Cryptoxanthin*	*Lycopene*	*γ-Carotene*	*α-Carotene*	*β-Carotene*	*Others*[b]	***Sum***[c]
Kidney bean/Harlcot bean	17	2	0[d]	0	0	11	33	63
Black eye bean/Cow pea	51	2	0	0	0	6	9	68
Green gram bean/Mung bean	1342	13	0	0	0	118	243	1716
Red gram bean	45	2	0	0	0	4	44	95
Black gram bean	811	7	0	0	0	150	168	1136
Black gram dhall	212	2	6	6	0	46	46	322
Chickpea (small, brown)	1284	12	0	1	0	62	121	1480
Chickpea (big, yellow)	683	8	0	0	0	40	58	789
Bengal gram dhall	1406	7	0	0	0	32	325	1770
Egyptian kidney bean	169	3	0	0	0	7	81	260
Mysore dhall (orange)	819	5	0	0	0	18	59	901
Australian dhall	1120	0	0	0	0	50	342	1512
Soya bean	229	3	0	0	0	10	36	288
Soya bean curd	187	1	0	0	0	4	59	251
Soya bean curd,sheets	365	3	0	1	0	11	44	424
Soya bean, fermented	297	2	30	0	0	5	5	339
Appalam	4	0	0	0	0	1	4	9

[a]Mean of duplicate analysis: expressed as ug per 100 g edible portion of sample
[b]Unidetified carotenoids
[c]Summation of all carotenoids tabulated
[d]Not detected under the present HPLC conditions (see methods)

Table 3. Content [a] of Major Carotenoids in Selected Starchy Roots and Tubers.

Name of root and tuber	*Luten*	*Cryptoxanthin*	*Lycopene*	*γ-Carotene*	*α-Carotene*	*β-Carotene*	*Others*[b]	*Sum*[c]
Breadfruit	16	1	1	0[d]	0	2	6	26
Potato (yellow)	23	1	1	0	0	4	47	76
Sago (large)	1	0	0	0	0	0	0	1
Sago (small)	0	0	0	0	0	0	0	0
Sweet potato (yellow)	25	0	42	0	0	19	290	376
Sweet potato (orange)	7	27	147	0	0	1140	331	1652
Tapioca	2	3	1	0	0	20	13	39
Tannia	31	1	1	3	0	2	8	46
Taro	3	1	3	1	0	16	11	35

[a]Mean of duplicate analysis: expressed as ug per 100 g edible portion of sample

[b]Unidentified carotenoids

[c]Summation of all carotenoids tabulated

[d]Not deleted under the present HPLC conditions (see methods)

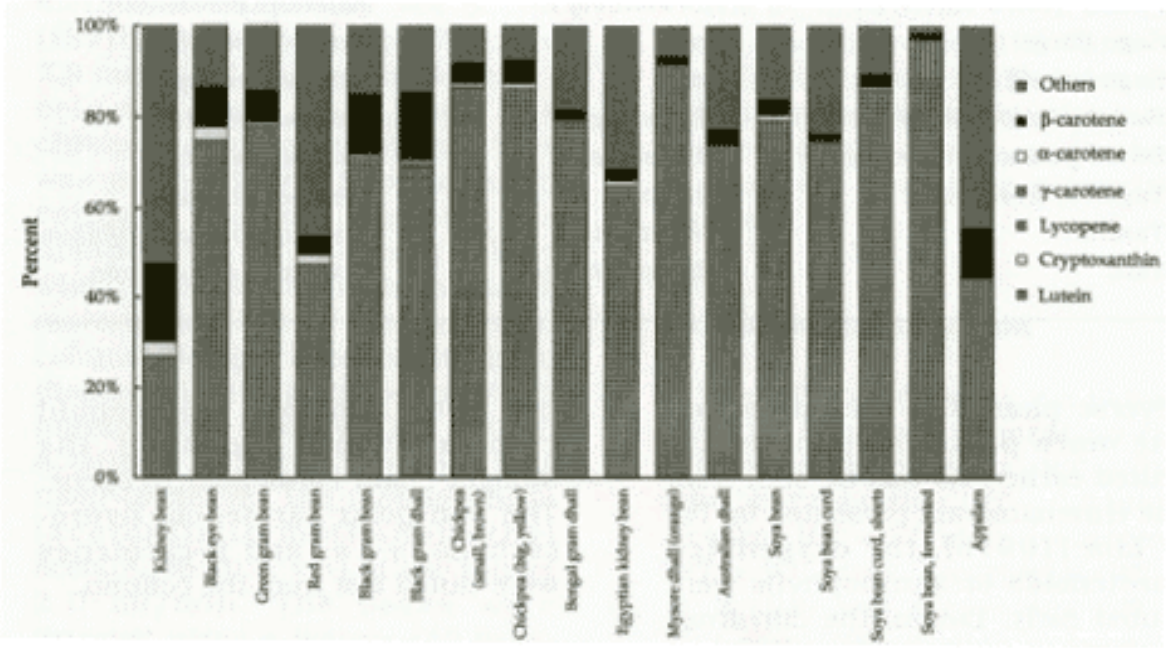

Figure 2. Carotenoid composition of starchy roots and tubers.

sweet potato (orange) and tapioca. However in seven of the starchy roots and tubers, the content of lycopene was less than 10%. Lutein was found in varying proportions in eight out of the nine tubers and starchy roots studied whereas cryptoxanthin was found in six of the starchy roots and tubers in low proportion. No carotenoids were detectable in saga of the small variety; however in the large variety, only lutein was found. a-Carotene was also not detected under the conditions employed.

Sum of carotenoids

The sum of all carotenoids for each legume, tuber and starchy root are tabulated in the last column of Tables 2 and 3. Of the 26 food Items studied, only 6 of them had a total carotenoid content of over 1000 ug per 100 g sample. Compared with the vegetables and fruits, the carotenoid concentration in legumes, tubers and roots are much lower. Many of the green leafy vegetables reported by Tee & Lim (1991b) had carotenoid concentrations several times higher than in the legumes, tubers and roots, and several of them were higher by almost 10 folds.

Vitamin A activity (retinol equivalent)

Conventionally, the nutritional significance of carotenoids is related to the pro-vitamin A activity of these compounds. For vitamin A activity, a carotenoid must have at least one unsubstituted **b** -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 are known to possess pro-vitamin A activity: **b**-carotene, **a** -carotene, **g**-carotene and cryptoxanthin. The vitamin A activity of **b**-carotene, expressed as ug retinal equivalent (RE) was

calculated as RE = (ug 13-carotene)/6 (NAS, 1980). The other three carotenoids mentioned, possessing only one unsubstituted **b**-ionone ring may be expected to have about 50% of the biological activity of **b**-carotene. The formula used for these pro-vitamin A carotenoids was therefore RE = (ug 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:

As can be seen from the RE values tabulated in Table 4, none of the legumes, tubers and starchy roots studied can be said to be good sources of vitamin A. Using the same classification previously adopted for RE values (Tee & Lim, 1991b), all the food items studied will have to be classed as having "low" vitamin A activity (<100 ug RE per 100 g edible portion), except orange-coloured sweet potato which may be termed as "medium" (100-499 ug RE). In contrast, all green leafy vegetables were found to have "high" (500-599 ug RE) or "very high" RE (> 1000 ug RE) (Tee & Lim, 1991b). 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 of "medium" RE. All the green non-leafy vegetables, as well as yam stalk and tomato were found to be poor sources of vitamin A. All the fruits studied were also either in the "medium" or "low" categories. A scatter plot of the RE of these groups of plant foods studied (Figure 3) clearly shows the wide variations in vitamin A activity of these foods.

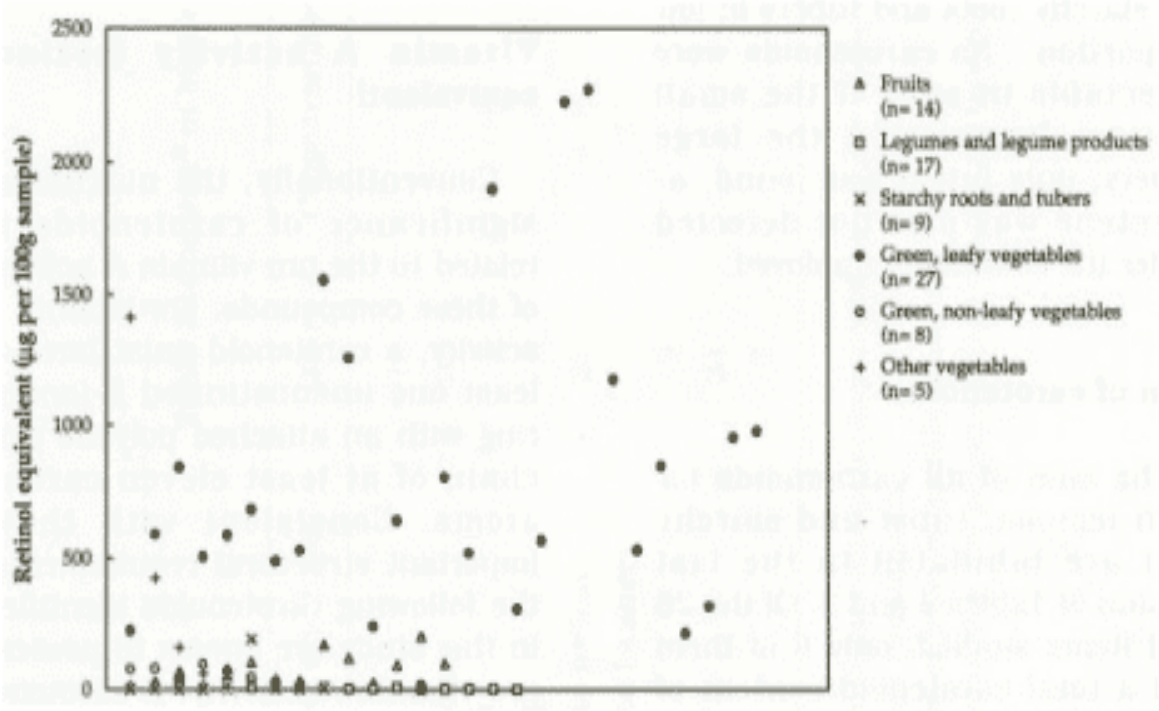

Figure 3. Distribution of retinal equivalent in selected legumes, tubers and starchy roots, vegetables and fruits.

Table 4. Retinol Equivalent (RE) of Selected Legumes, Tubers and Starchy Roots.

	Retinol Equivalent (RE)[a]
Legumes and legume products	
Kidney bean	2
Black eye bean	1
Green gram bean	21
Red gram bean	1
Black gram bean	26
Black gram dhall	8
Chickpea (small, brown)	11
Chickpea (big, yellow)	7
Bengal gram dhall	6
Egyptian kidney bean	1
Mysore dhall (orange)	3
Australian dhall	8
Soya bean	2
Soya bean curd	1
Soya bean curd, sheets	2
Soya bean, fermented	1
Appalam	0
Starchy roots and tubers	
Breadfruit	0
Potato (yellow)	1
Saga (large)	0
Saga (small)	0
Sweet potato (yellow)	3
Sweet potato (orange)	192
Tapioca	4
Tannia	1
Taro	3

[a]Mean of duplicate analysis; expressed as ug per 100 edible portion of sample

CONCLUSION

The HPLC procedure previously developed for the determination of carotenoid composition and content in vegetables and fruits, foods of animal origin, as well as blood samples has been shown to be applicable also for the analysis of legumes, starchy roots and tubers. Although all the legumes. tubers and roots studied are considered to be a poor sources of vitamin A activity, they are still of nutritional importance. If these foods are consumed in significant amounts, they can contribute significantly to total vitamin A intake. Furthermore, the foods studied can also provide other essential nutrients, especially as a source of complex carbohydrates and energy.

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