Tropical Agricultural Research Vol. 5 1993

Separation and Estimation of Pro-vitamin A Carotenoids from Common Green Leafy Vegetables in Sri Lanka

R.M.S. Ratnayake, W.R. Wimalasiri[1], P.A.J. Perera[2] and K.N.H. Weligama[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya

ABSTRACT. Vitamin A deficiency is observed mainly among the population in rural, dry and arid zones of Sri Lanka. It is probably due to the non availability and poor knowledge of vitamin A rich foods. Although vitamin A is most bio-available in foods of animal origin, the high cost of these foods make them less accessible to most people in Sri Lanka.

This project tries to identify cheap sources rich in pro-vitamin A and to evaluate the concentrations of different carotenoids in them. Further, the bio-availability of carotenoids will be calculated in terms of retinol equivalents.

The edible portion of fresh food articles was extracted with a mixture of petroleum and acetone. The extract after concentration was chromatographed on calcium hydroxide using a gradient of petroleum and acetone mixture. The carotenoids eluted from the column were identified by UV spectroscopy and thin layer chromatography. The concentration of each carotenoid was determined by colorimetry. The concentrations of pro-vitamins are expressed as μg/g of edible portion.

Of the green leafy vegetables, retinol equivalent was highest in Kura-thampala (18.65 μg/g) closely followed by Passion fruit leaves (17.84 μg/g). Kalukamberiya had the lowest (1.59 μg/g) with moderate values ranging from 11.85 μg/g to 6.08 μg/g in Beet leaves, Minchi, Koppa leaves, Vel kohila and Gotukola.

---

1 Division of Oral Biochemistry, Faculty of Dental Science, University of Peradeniya.

2 Department of Biochemistry, Faculty of Medicine, University of Peradeniya.

INTRODUCTION

Vitamin A deficiency is a condition which mostly affects the poorer population of the developing countries. Vitamin A deficiency is observed among the population mainly in the rural, dry and arid zones, probably due to inadequate intake, poor understanding of dietary sources rich in pro-vitamin A or vitamin A, in addition to other factors.

Bitot's spots and xerosis of the conjunctivae are a few of the common clinical signs seen among those affected. Vitamin A is also known to be involved in growth, reproduction and maintenance of the integrity of epithelial tissues (Harper, 1988).

Although preformed vitamin A is present only in animal foods and is more bio-available than the carotenoids, the high cost of animal foods make them less accessible to most people in Sri Lanka. Of the cheap pro-vitamin A rich foods, green leafy vegetables are considered to be rich in carotenoids. Therefore, it is important to identify rich sources of carotenoids, among the green leafy vegetables, that could contribute towards the dietary vitamin A requirements.

MATERIALS AND METHODS

Materials

Vegetables, in their raw, uncooked state, were purchased in duplicate from the open market in Kandy. Edible portions of each were analyzed in duplicate, for their carotene content and their mean and SEM were calculated. The food samples were washed thoroughly with distilled water and dried between filter paper before analysis. Analytical grade chemicals were used in all experiments.

Methods

Estimation of pro-vitamin A carotenoids in leafy vegetables

Twenty five grams of each food sample were ground with fine silica powder of chromatography grade and allowed to stand overnight in the dark, at room temperature, with 100 ml of petroleum ether-acetone (1:1). The

extract was then filtered and the residue was washed at the filter with 200 ml of the same solvent. This was continued until all the green colour was extracted. The filtrate was shaken with 50 ml of distilled water in a separating funnel and the water layer was discarded. This was repeated once and the organic layer obtained was dried over anhydrous sodium sulphate and concentrated under a vacuum. The residue obtained was subjected to column chromatography to isolate the different carotenoids. A glass chromatographic column of diameter 1 cm was packed with 10 g of anhydrous calcium hydroxide powder in petroleum ether. Pure alpha, beta and gamma carotenes were eluted separately from the column with 1%, 5% and 20% acetone in petroleum ether, respectively (Ratnayake *et al.*, 1993). The optical densities of alpha, beta and gamma carotenoids were measured at 400-450 nm using a Klett Summerson Colorimeter.

Verification of carotenoids

The alpha and beta carotenoids were identified by the Rf values they produced on thin layer chromatography. They were further confirmed by the spectra they produced in the UV region, which were identical to those produced by authentic standards of alpha and beta carotenoids. Identification of gamma carotene was made only by comparison of its UV spectrum with those reported in the literature (Karrer and Jucker, 1950).

Estimation of concentration of carotenoids

Carotenes in solution when exposed to air gets readily oxidised. Hence to standardise unknown carotene solutions it was necessary to prepare a stable coloured solution, equivalent in concentration to carotene, for comparison. This was done with the help of a stock standard solution of Potassium dichromate, containing 10 mg of potassium dichromate in 10 ml of absolute ethanol. A standard curve was drawn using dilutions of the stock standard. The concentration of carotenoids was determined by reading the concentration from the graph and multiplying by a factor of 0.006 (Seaber, 1940).

Bio-availability of carotenes

1 μg retinol activity	=	6 μg beta carotene activity
	=	12 μg of alpha and gamma carotene activity (Wikramanayake, 1987)

Recovery of Carotenoids

Pure beta carotene (NACALAI TESQUE, Ing; KYOTO, Japan) was used to prepare the standard solution. The absorbance of the diluted standard was checked prior to use. The standard solutions were processed in the same manner as the food samples. The mean percentage recovery was 97.3.

RESULTS AND DISCUSSION

The carotenoids from leafy vegetables were expressed as μg of alpha, beta and gamma carotenoids per gram of the edible portion (Table 1). These were then collectively expressed in terms of μg of retinol equivalents per gram of the edible portion (Table 2). In doing so, their mean values and standard error of mean (SEM) were calculated.

The method of choice for separation of carotenoids is the High Performance Liquid Chromatography (HPLC). However, the method described here is simple and inexpensive. It is based on different affinities of carotenoids towards calcium hydroxide. Calcium hydroxide had been used as an absorbent for the separation of carotenoids, by Karrer and Jucker, as early as 1950. The new method proposed is a modification of this method, where a discontinuous solvent gradient consisting of a mixture of petroleum ether-acetone was used in conjunction with a column packed with calcium hydroxide. This enabled the separation of carotenoids into alpha, beta and gamma fractions (Ratnayake *et al.*, 1993). The previously published results of carotenoids in local foods were those for beta carotene content only and hence could not be expressed method available retinol equivalent of the food (Atukorala, 1985). Table 2 also describes the amount of green leafy vegetables that should be eaten to provide the daily requirement of vitamin A for an adult (Harper, 1988).

Table 1. Carotenoids in leafy vegetables.

Food Article	Pro-vitamin content (μg/g)					
	Alpha Carotene		Beta Carotene		Gamma Carotene	
	Mean	SEM	Mean	SEM	Mean	SEM
1. Beet leaves (*Beta vulgaris*)	13.55	0.55	64.50	0.90	nil	nil
2. Gotukola (*Centella asiatica*)	2.40	0.10	32.80	0.20	5.15	0.02
3. Kalukamberiya (*Solanum nigrum*)	7.75	1.00	5.70	0.30	nil	nil
4. Kohila leaves (*Lasia spinosa*)	1.65	0.05	19.60	0.60	3.20	0.10
5. Koppa leaves (*Panex fruiticosum*)	6.50	0.43	34.80	0.89	5.40	1.56
6. Kura-thampala (*Amaranthus spp.*)	19.35	0.35	93.60	2.00	16.65	0.35
7. Minchi (*Mentha viridis*)	3.45	0.14	64.05	3.15	6.00	0.20
8. Passion fruit (*Passiflra edulis*)	11.00	0.80	97.50	0.60	8.20	0.10
9. Raddish leaves (*Raphanus sativas*)	4.30	0.30	19.55	0.55	1.75	0.15
10. Vel kohila (*Lasia sp.*)	5.60	0.09	34.25	0.35	5.25	0.05

Table 2. Content of retinol equivalents and amounts of green leafy vegetables that should be eaten.

Food article	Retinol equivalents (μg/g)	RDA* (g)
1. Beet leaves (*Beta vulgaris*)	11.85	63.3
2. Gotukola (*Centella asiatica*)	6.08	123.4
3. Kalukamberiya (*Solanum nigrum*)	1.59	471.6
4. Kohila leaves (*Lasia spinosa*)	3.65	205.4
5. Koppa leaves (*Panex fruiticosum*)	6.79	110.4
6. Kura-thampala (*Amaranthus spp.*)	18.65	40.2
7. Minchi (*Mentha viridis*)	11.45	65.5
8. Passion fruit (*Passiflra edulis*)	17.84	42.0
9. Raddish leaves (*Raphanus sativas*)	3.74	200.5
10. Vel kohila (*Lasia sp.*)	6.59	113.8

* RDA - Recommended Daily Allowances

Of the green leafy vegetables, retinol equivalent was highest in *Kura-thampala* (18.65 μg/g) closely followed by the Passion fruit leaves (17.84 μg/g). *Kalukambariya* had the lowest (1.59 μg/g), with moderate values ranging from 11.85 μg/g to 6.08 μg/g in Beet leaves, *Minchi*, *Coppa* leaves, *Val kohila* and *Gotukola*.

CONCLUSIONS

It is believed that carotenoids are rich in dark green leafy vegetables. This hypothesis was tested by dividing the leafy vegetables according to the darkness of the green colour and matching against the total retinol equivalent. The leaves of Passion fruit, *Minchi*, *Koppa*, Beet and *Kura-thampala* were the darkest in green colour and these had the highest level of retinol equivalents, except for *Koppa* leaves, which was considered moderate. All other leaves were moderate to light green in colour and had low retinol equivalent levels ranging from 6.59-1.59 μg/g.

The recommended dietary allowance of retinol for an adult male is 750 μg (Harper, 1988). This could be obtained by eating daily; 50 g of *Kura-thampala* or Passion fruit leaves, or 75 g of *Minchi* or Beet leaves. The others such as *Gotukola*, *Koppa* leaves, *Kohila* leaves, *Kalukambariya*, Raddish leaves, and *Vel kohila* leaves contained less retinol but could add variety to the total diet and some contribution to the daily vitamin A requirement.

ACKNOWLEDGEMENTS

The authors wish to thank the Postgraduate Institute of Agriculture, Peradeniya, Sri Lanka for providing a research grant, Mr. D.V. Chaminda Niroshana for technical assistance and Miss H.M.L.A. Kolongahapitiya for typing the manuscript.

REFERENCES

Atukorala, T.M.S. (1985). Vitamin A and Beta carotene content of some common foods. J. Natn. Sci. Coun. Sri Lanka. 13(2): 187-196.

Harper's Biochemistry (1988). Appleton and Lang Norwalk, Connecticut/San Mateo, California.

Karrer, P. and Jucker, E. (1950). Carotenoids, Elsevier Publishing Company, INC.

Ratnayake, R.M.S., Wimalasiri, W.R., Perera, P.A.J. (1933). A simple method for separation of pro-vitamin A carotenoids. Proc. I. Chem. (Sri Lanka), Vol. 10(1): 6.

Seaber, W.M. (1940). Analyst. 65: 266-281.

Wikramanayake, T.W. (1987). Food and Nutrition, Trumpet Publishers, Colombo, Sri Lanka.