Food Chemistry 27 (1988) 245-257

Total Carotenoid and *B***-Carotene Contents of Thai Vegetables and the Effect of Processing**

Andries J. Speek," Supinun Speek-Saichua^b & Wil H. P. Schreurs^a

aTNO-CIVO Toxicology and Nutrition Institute, Department of Clinical Biochemistry, PO Box 360, 3700 AJ Zeist, The **Netherlands**

^bKhon Kaen University, Faculty of Associated Medical Sciences, Khon Kaen 40002, Thailand

> (Received 4 December 1986; revised version received 10 June 1987; accepted 30 June 1987)

ABSTRACT

Total carotenoid and fl-carotene contents of 55 vegetable products and fruits commonly consumed in the northeastern part of Thailand have been determined by spectrophotometry and high-performance liquid chromatography. The vitamin A activities, as retinol equivalents, are calculated using the in vivo *conversion factors given by the World Health Organization (WHO, 1982).*

The data obtained in the present study are, in general, markedly lower than those stated in the Thai Food Composition Table of 1978.

Leaf vegetables contain considerably more carotenoids than tuberous vegetables and fruits. The distribution of carotenoids over leaves and stalks has been determined. The carotenoids of a plant are mainly deposited in the leaves which, in general, have a higher relative β *-carotene content than the stalks.*

The average losses of vitamin A activity as a result of local processing, i.e. cooking, frying, fermenting, sun-drying and sun-drying followed by cooking, were found to be 14, 24, 29, 44 and 60%, respectively.

INTRODUCTION

Carotenoids represent a group of aliphatic or aliphatic-alicyclic fat-soluble yellow to red coloured compounds which are synthesized *de novo* in plants

245

Food Chemistry 0308-8146/88/\$03'50 © Elsevier Applied Science Publishers Ltd, England, **1988. Printed in Great Britain**

and in some microorganisms. They occur in the green chloroplasts present in the parenchyma of the leaves and hence contribute considerably to the pigmentation in the plant kingdom. It has been estimated that nature produces about 100 million tons of this pigment per year (Isler *et al.,* 1965).

Man absorbs and stores carotenoids rather unselectively and hence they are found in liver, blood, fat tissue and in breast milk (Goodman, 1969). However, the human body is also able to alter certain carotenoids in the digestive tract or during absorption, prior to tissue storage or *in vivo* function.

The relation between dietary carotenoid intake and health has been studied by many researchers. Ong & Chytil (1983) showed the existence of an inverse relationship between cancer and dietary intake of β -carotene, while antiulcer properties of β -carotene and β -cryptoxanthin were reported by Moszik *et al.* (1983) and antiageing effects of carotenoids were demonstrated by Cutler (1984).

A well-known aspect of dietary carotenoid intake is conversion of certain carotenoids into retinol. The number of carotenoids known to occur naturally now exceeds 500, whereas the number of compounds with vitamin A activity is only about 50.

This *in vivo* conversion into retinol, which is part of the active light receptor in the rods of the retina (Marks, 1975), plays an important role in developing countries since retinol-containing dairy and other animal products are not available or are too expensive (Simpson, 1983). The main source of vitamin A activity for large groups of the population is the green • leaf vegetable. In many developing countries, prolonged vitamin A deficiency occurs among young children, especially during the weaning period (Mrisho, 1981). This constitutes a public health problem due to the possibly resulting xerophthalmia, which may cause irreversible destruction of the cornea and subsequent blindness. An extensive compilation concerning the importance of vegetables in human nutrition is given by Oomen & Grubben (1978).

The vitamin A activity of vegetables cannot be established unequivocally since many factors affect the concentration and the composition of the group ofcarotenoids in the plant or their *in vivo* conversion into retinol. The genetics and the environment of the plant influence the occurrence of carotenoids. Interplant variations can be considerable (Blessin *et al.,* 1963). A diurnal carotenoid pattern for leaves has already been shown by Thompson (1949). Furthermore, the age of the plant, the position of the leaf on the stalk and enzymatic and photochemical processes during and after harvesting influence the carotenoids pattern. Processing also affects vitamin A activity. Losses of activity as a result of processing have been compiled by Kläui & Bauernfeind (1981).

The conversion of certain carotenoids into retinol is influenced by the

animal species, the structure of the compound, its solubility, its state of isomerization and the dietary levels of carotenoids, lipids, proteins, antioxidants and absorption inhibitors. Hence no defined conversion factor per carotenoid can be given. The conversion factor in humans of β -carotene, the most active compound mentioned in the literature, varies from the theoretically highest of $\frac{1}{2}$ to $\frac{1}{8}$ (weight units of retinol proceeding from one unit of carotenoid). For dietary carotenoid mixtures it is generally acknowledged that, in terms of vitamin A activity, 6μ g of β -carotene or 12 μ g of mixed dietary carotenoids are converted into 1 μ g of retinol (WHO, 1982). The vitamin A activity, as a percentage of the activity of β -carotene of a large number of carotenoids obtained from bioassays, has been tabulated by Bauernfeind (1972).

As part of a nutritional intervention study (Egger *et al.,* 1985), a food consumption inquiry has been conducted among 150 families living in villages in northeast Thailand. Green leaf vegetables are their main source of vitamin A activity. Since there is evidence that methodology, to determine vitamin A activity mentioned in the food composition Tables currently in use, has led to overestimations in some cases (Zakaria & Simpson, 1979; WHO, 1982; Simpson & Tsou, 1986), the contents of total carotenoids and specific β -carotene have been determined in 55 commonly eaten vegetable products. The vitamin A activity as μ g retinol equivalents per g (μ g RE/g) was Calculated using the above-mentioned conversion factors. Because exposure of carotenoids to heat, oxygen and light may lead to decomposition and/or structural changes which decrease activity, the effects of local processing, i.e. cooking, frying, fermenting and sun-drying, were determined. The data presented in this study have been compared with literature data.

MATERIALS AND METHODS

Sampling of fresh vegetables

The vegetables examined were bought in June-July, 1986 at the local market of Khon Kaen, a provincial capital in northeast Thailand, and analysed on the same day. Per specimen, three bunches were bought at different market stalls. In the laboratory two intact plants were selected from every bunch and dried by pressing gently between towels. Subsequently, the plants were weighed, cut in small pieces and analysed. Carrots were finely grated.

Sampling of vegetables for processing

In order to obtain two samples with equal carotenoid contents of the same specimen the vegetables were sampled as follows.

Of vegetables consisting of leaves and stalk the leaves were systematically picked off from the top to the roots and were alternately divided into two portions. After drying by gently pressing the leaves between towels, one portion was analysed directly and one after processing.

Tuberous plants, like egg plants and sugar peas, were dried with a towel and cut in symmetrical halves. Two weighed portions, each consisting of a number of halves of different plants, were analysed, one directly, the other after processing. Onions of approximately the same size were selected from the same crop and divided in two portions. Onions were fermented as intact plants.

Processing of vegetables

Processing was carried out according to the traditional local habits:

Analysis of total carotenoids and *B***-carotene**

Total carotenoids and β -carotene were determined using the method described by Speek *et al.* (1986). The method is based on saponification of the sample, followed by organic extraction whereafter total carotenoids and β carotene in the extract are determined using spectrophotometry and reversed-phase high-performance liquid chromatography, respectively.

RESULTS AND DISCUSSION

Fresh and non-processed vegetable products

The data for individual products, arranged alphabetically according to genus and species, are given in Table 1. The results for leaf and tuberous vegetables and fruits have been grouped in Table 2. In both Tables the vitamin A activity as stated in the Thai Food Composition Table (TFT) of 1978 has been included.

Striking differences are observed when comparing the measured vitamin A activity data for vegetable products with those given in the Thai Food Composition Table (Table 1). With a few exceptions the Thai Table gives higher activities for all products, probably because the relevant data were collected from rather old investigations in which less specific analytical methods were applied (WHO, 1982; Simpson & Tsou, 1986). This means that making use of the Thai Table in food consumption inquiries will lead to overestimations of the provitamin A carotenoid intake.

The percentage of β -carotene of the total content varies from 2.1 to 55%. Thus, in spite of its higher vitamin A activity, β -carotene, with exceptions for carrots and durian, contributes less to the plant's total activity than the other mixed carotenoids.

Table 2 shows that leaf vegetables contain considerably more carotenoids than tuberous vegetables and fruits. Leaf vegetables $(1.2-18.8 \,\mu g$ RE/g, Table 2) have a higher vitamin A activity than milk (up to 1 μ g of retinol/g), contain approximately the same quantity of activity as eggs $(3-6 \mu g)$ of retinol/g), but considerably less than cattle liver $(150-1500 \,\mu g)$ of retinol/g, Marks, 1975). The average per group of the ratios of the vitamin A activities of vegetable products as stated in the Thai Food Composition Table and as measured shows that the Thai Table generally gives remarkably higher values.

Inhomogeneity of the plant

In order to investigate the distribution of the carotenoids over leaves and stalks, the leaves of fresh and non-processed vegetables and the whole plants of the same bunch were analysed. The results are shown in Table 3.

It appears from Table 3 that carotenoids mainly occur in the leaves. In general, the leaves contain relatively more β -carotene than the other parts of the plant.

Interplant variations

In order to determine interplant variations, amaranth, celery, vine spinach and lettuce were analysed for vitamin A activity on each of four consecutive

TABLE 1

Vitamin A Activity of Commonly Eaten Fresh and Non-processed Thai Vegetable Products and Fruits

	Total carotenoid $(\mu g/g)$	β- carotene $(\mu g/g)$	$% B -$ of total	Calculated activity (RE/g)	Thai Food Table		
					Activity Ref. (RE/g)		No.
Tuberous vegetables and beans							
Parkia speciosa Hassk. sato (local name)	8.2	0.66	$8-0$	0.74	2.2	5	256
Pisum sativum Linn. sugar peas or garden peas	8.9	1.7	$19-0$	0.88	$1-2$	1	85
Solanum melongena Linn. egg plant, long shape	7.9	0.87	$11-0$	0.73	1.06	2	234
Solanum melongena sw. egg plant, round shape	5.3	0.41	$7 - 7$	$0-48$	1.94	$\overline{2}$	230
Solanum torvum sw. egg plant, small and round	$8-7$	0.35	$4 - 0$	0.75	5.7	$\overline{2}$	231
Solanum xanthocarpum sw. egg plant, oval shape	3.9	0.10	$2-6$	0.33			236
Vigna sinensis Savi ex Hassk. yard-long beans,							
green	6.8	0.44	6.6	0.60	$1-7$	3	73
Zea mays. corn, yellow	$12-6$	0.74	5.9	$1 - 11$	1.31	3	18
Fruits							
Artocarpus heterophyllus Lamk. jackfruit, mature	4.5	0.23	51	0.39	$1-18$	1	9
Carica papaya Linn. papaya, ripe	$6-4$	0.38	5.9	0.57	3.5	1	94
Cucurbita maxima Duch. winter squash or							
pumpkin	$23 - 0$	0.49	2.1	20 ₀	$7-4$		223
Durio zibethinus Linn. durian	$3 - 8$	1.9	50.0	0.48	$3-1$	2	35
Mangifera sp. mango, ripe	$3 - 33$	0.63	$18-9$	0.33	$9-4$	1	76
mango, unripe	0.62	0.10	$16-1$	0.06	0.55	1	74
Lagenaria siceraria Standl. bottle gourd, fruit	0.46	0.04	$8-7$	0.04	$0 - 05$	1	90

TABLE *l--cont.*

TFT: Thai Food Composition Table of 1978 composed of data collected from:

[1]: Food Composition Table for use in East Asia, by FAO and US Department of Health, Education and Welfare.
[2]: Division of Food Analysis, *The Bulletin of Department of Medical Science, Thailand*, Vol. 1–2, 1973, 47–67. [2]: Division of Food Analysis, *The Bulletin of Department of Medical Science, Thailand,* Vol. 1-2, 1973, 47~57. (Thai).

[3]: *Food Composition Table by Food and Nutrition Research Center in Manila, Philippines Handbook* l(3rd revision), 1964.

[4] *Composition of Foods, Raw, Processed, Prepared,* USDA Agriculture Handbook No. 8, Revised, 1963.

[5]: Analytical results from The Analytical Research Subcommittee of the National Nutrition Committee, Thailand (Thai).

TABLE 2

Vitamin A Activity of Groups of Fresh and Non-processed Leaf Vegetables, Tuberous Vegetables and Beans, and Fruits

Vegetable	Number TFT	Vitamin A activity $(\mu$ g RE/g)		$% \beta$ -carotene of total	
		Whole plant	Leaf	Whole plant	Leaf
Coriander	181	14.2	23.2	$13-3$	19.6
Neem tree	202	$10-6$	$17-4$	7.8	12.5
Chinese swamp cabbage	188	$10-5$	$9 - 1$	$24 - 7$	$26 - 7$
Amaranth	173	$9-4$	$28 - 7$	$21 - 1$	$20-0$
Sweet basil	139	$8-1$	21.5	5.5	12.3
Celery	19	6.9	$16-0$	$10-3$	15.5
Chinese cabbage	163	6.8	13.3	6.7	$15-3$
Vine spinach	196	6.3	14.2	$8-0$	6.4
Hairy basil	$130 \cdot$	5.5	16.5	5.6	7.8
Ivy gourd	52	4.9	$14-4$	$11-5$	$16-4$
Chinese kale Swamp cabbage, white	177	3.8	4.9	5.3	$21-4$
stalk and small leaves	189	1.3	19.8	2.8	50.0

TABLE 3 Vitamin A Activity of Whole Plants and Their Leaves

days. Every day a bunch of vegetables was bought at a different market stall and analysed. From the results: amaranth, $9.3 \pm 17\%$; celery, $6.8 \pm 22\%$; vine spinach, $6.6 \pm 17\%$ and lettuce, $5.7 \pm 9.0\%$ (mean \pm coefficient of variation, μ g RE/g, n = 4), it can be concluded that, irrespective of seasonal variations, interplant variations are rather large. Therefore, fresh vegetables were sampled by selecting a few intact plants from three bunches bought at different stalls (Table 1).

Reproducibility of sampling vegetables

In order to establish processing effects which are expected to be of the same extent as the above-mentioned interplant variations, a method had to be found to obtain two samples with equal carotenoid contents of the relevant vegetable. Three different sampling methods were tried out. Analysis of vitamin A activity was done in duplicate on amaranth, sweet basil, Chinese chive and swamp cabbage.

- A: The plants, in the form in which they are consumed or processed, were cut in pieces of 3-4 cm which were mixed. Two portions were weighed and analysed.
- **B:** The plants were cut lengthwise through the stalk in more or less symmetrical parts. Two portions, each consisting of different halves of plants, were weighed and analysed.

C: The leaves were systematically picked off from the top to the roots and alternately divided into two portions which were weighed and analysed.

The average of the four duplicate differences was, for method A, $17\% \pm 7.5$; for method B, 24% \pm 13.5, and for method C, 1.9% \pm 1.0 (percentage of the average value $+$ SD, $n = 4$). Based on these results it was decided to apply method C to obtain samples for the determination of the effects of processing. The high duplicate differences obtained with methods A and B can be explained by the finding that stalks contain a considerably lower amount of carotenoids than the leaves while they contribute rather a lot to the weight of the plant.

Effects of processing

A number of vegetables were sampled as described above and analysed fresh and after processing according to the local habits. The results are given in Table 4.

After cooking the water was somewhat green. Probably, this resulted from migration of green chloroplasts from the leaves into the water. Loss of carotenoids is obvious as both chloroplasts and carotenoids occur in the parenchyma of the leaves. Our data concerning the losses of vitamin A activity are, in general, comparable with literature data as compiled by Kläui & Bauernfeind (1981).

The effect of cooking on the vitamin A activity (average loss of 14%) agrees very well with the finding of Sood & Bhat (1974) who reported an average loss of $13\% + 2.6$ (mean + SD) for eight leaf vegetables cooked according to three comparable methods. Sweeney & Marsh (1971) observed losses of activity of 15 to 20% in a similar experiment with green vegetables.

Ogunlesi & Lee (1979) reported an average loss of vitamin A activity of 15 % for carrots after cooking for 30 min at 116°C. They took into account losses as a result of decomposition as well as of the conversion *ofall-trans-* to less active *cis-isomers.* Although their three-step separation method probably results in a more accurate assay of the vitamin A activity, it is not suitable for large-scale determinations as the method is very complex, and thus time-consuming.

All five types of processing mentioned in Table 4 cause relatively larger losses of β -carotene, probably because this compound is less heat and UVlight stable than the other carotenoids.

The highest average effect is caused by sun-drying (44% loss) and sundrying followed by cooking (60% loss). Sun-dried vegetables are always cooked before consumption. Cooking fresh vegetables causes vitamin A

TABLE 4 Influence of Processing on Total Carotenoids and β -Carotene Contents and Vitamin A Activity of Vegetable Products

Vegetable	Number Min TFT	Total carotenoid	<i>B</i> -carotene	Vitamin A activity		
		Carotenoids as percentage of fresh after sun drying (2 days) followed by cooking (4 min)				
Amaranth	173	43		40		
Amaranth duplicate			38	39		

TABLE *4---contd.*

^aIn a blank experiment the soya-bean oil used for frying was checked for the absence of vitamin A activity $(0.2μ g RE/g).$

^b These vegetables are usually not sun-dried.

activity losses of $14\% \pm 8$ (av. \pm SD) while higher losses are involved in cooking of sun-dried vegetables, i.e. 25 %, probably on account of the lower integrity of the cell walls.

CONCLUSION

As many food composition Tables in the region are composed using the same sources, it may be expected that generally too high values for the vitamin A activity of vegetable products are reported. The Tables should be revised using more specific analytical methods. Furthermore, processing of vegetable products causes a considerable loss of vitamin A activity, which should be taken into account in using food composition Tables for the calculation of vitamin A intake.

ACKNOWLEDGEMENTS

The current investigation was carried out at the Central Research Division of the Faculty of Medicine of the Khon Kaen University, Khon Kaen, Thailand, as part of the bilateral Thailand-Netherlands Nutrition/Health Development programme in northeast Thailand (under the name 'Nutritional Supplement Cooperation Project') with financial support of the Netherlands Government. The authors are grateful to Ms Sam-ang Homchuen, Faculty of Science, Khon Kaen University and Mrs Kalaya Pharadai, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, for their assistance in identifying the vegetables.

REFERENCES

- Bauernfeind, J. C. (1972). Carotenoid vitamin A precursors and analogs in foods and feeds. J. *Agric. Food Chem.,* 20, 456-73.
- Blessin, C. W., Brecher, J. D., Dimler, R. J., Grogan, C. O. & Campbell, C. M. (1963). Carotenoids of corn and sorghum. III. Variation in xanthophylls and carotenes in hybrid, inbred and exotic corn lines. *Cereal Chem.,* 40, 463-72.
- Cutler, R. G. (1984). Carotenoids and retinal: Their possible importance in determining longevity of prunate species. *Proc. Nat. Acad. Sci.,* 81, 7627-31.
- Egger, R. J., Wedel, M. & Schreurs, W. H. P. (1985). In: *Health problems in Asia and the Federal Republic of Germany. How to solve them?* (Schelp, F. P. (Ed)), *Medezin in Entwicklungsliindern,* Vol. 21, Verlag Peter Lang, Frankfurt am Main, 307-14.
- Goodman, D. S. (1969). Biosynthesis of vitamin A from *ß*-carotene. *Amer. J. Clin. Nutr.,* 22, 963-5.
- Isler, O., Ruegg, R. & Schwieter, U. (1965). Carotenoids as food colorants. *Pure Appl. Chem.,* 14, 245-63.
- Kläui, H. & Bauernfeind, J. C. (1981). Carotenoids as food colors. In: *Carotenoids as colorants and vitamin A precursors. Technological and nutritional applications* (Bauernfeind, J. C. (Ed)), Academic Press, New York, London, 156.
- Marks, J. (1975). *A guide to the vitamins. Their role in health and disease.* MTP Co. Ltd, Lancaster, UK, 39-49.
- Moszik, G., Monika, B., Tibor, J., Fancisco, M., Joszef, S. & Toth, G. (1983). *Taplakozastud. Helyzete Feladatia Magyarorszagon,* 8, 781.
- Mrisho, F. H. (1981). The importance of vitamin A in weaning food. In: *Practical considerations for childfeeding in East, Central and Southern African countries,* Workshop Arusha (Tanzania), 152.
- Ogunlesi, A. T. & Lee, C. Y. (1979). Effect of thermal processing on the stereoisomerisation of major carotenoids and vitamin A value of carrots. *Food Chem.,* 4, 311-18.
- Ong, D. E. & Chytil, F. (1983). In: *Vitamins and hormones* (Aurbach, G. D. (Ed)), Academic Press, New York, 105.
- Oomen, H. A. P. C. & Grubben, G. J. H. (1978). *Tropical leaf vegetables in human nutrition.* Royal Tropical Institute, Amsterdam, Tesink, Zutphen, The Netherlands.
- Simpson, K. L. (1983). Relative value of carotenoids as precursor for vitamin A. *Proc. Nutr. Soc.,* 42, 7-17.
- Simpson, K. L. & Tsou, S. C. S. (1986). Vitamin A and Provitamin A Composition of Foods. In: *Vitamin A deficiency and its control.* (Bauernfeind, J. C. (Ed)), Orlando, Academic Press (In press).
- Sood, R. & Bhat, C. M. (1974). Changes in ascorbic acid and carotene content of green leafy vegetables on cooking. J. *Food Sci. Tech.,* 11, 131-3.
- Speek, A. J., Temalilwa, C. R. & Schrijver, J. (1986). Determination of β -carotene content and vitamin A activity of vegetables by high-performance liquid chromatography and spectrophotometry. *Food Chem.,* 19, 65-74.
- Sweeney, J. P. & Marsh, A. C. (1971). Effect of processing on provitamin A in vegetables. J. *Am. Diet. Ass.,* 59, 238-43.
- Thompson, C. R. (1949). The carotene content of several varieties and strains of alfalfa. *Agron. J.,* 41, 294-7.
- WHO (1982). *Control of vitamin A deficiency and xerophthalmia.* World Health Organization, Technical Report Series No. 672: Report of a joint WHO/UNICEF/Helen Keller International/IVACG meeting.
- Zakaria, M. & Simpson, K. (1979). Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes. *J. Chromatogr.,* 176, 109-17.

J.