ELSEVIER

Food Chemistry 64 (1999) 163–167

Food Chemistry

Retinol and beta carotene content of indigenous raw and home-prepared foods in Northeast Thailand

Pongtorn Sungpuag*, Sommai Tangchitpianvit, Uraiporn Chittchang, Emorn Wasantwisut

Institute of Nutrition, Mahidol University at Salaya, Nakhon Pathom 73170, Thailand

Received 21 April 1998; received in revised form and accepted 30 June 1998

Abstract

Retinol and β-carotene contents of raw and home-prepared indigenous foods in Northeast Thailand were studied. The criteria used to select the vitamin A-rich foods were high retinol or β-carotene contents of the individual food items, the amount and frequency of consumption, preference of consumption and food availability. Items selected were chicken liver, chicken egg, ivy gourd, amaranth, swamp cabbage (Chinese), Chinese cabbage, pumpkin, yellow sweet potato and two traditional menu items, kang-nho-mai and om-kruang-nai-kai. Raw food items were purchased from the local market; the traditional cooking procedures of the community were duplicated in the laboratory. Retinol and β-carotene contents were determined prior to and following cooking using HPLC methodology. The results indicated that boiling intact chicken liver resulted in 5% loss of retinol; boiling with cutting into small pieces and grilling resulted in losses of 8 and 16%, respectively. Greater losses (43%) were observed for egg omelet compared to hard-boiled egg (11%). For vegetables, blanching resulted in 7–11% loss of β-carotene, while steaming, frying and boiling showed losses of 15, 18 and 43%, respectively. Traditional foods such as bambooshoot soup (Kang-nho-mai) and chicken organ soup (Om-kruang-nai-kai) exhibited β-carotene losses ranging from 6–21%. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Retinol; β-Carotene; Cooking

1. Introduction

Thailand is classified by WHO as a country with moderate degree of subclinical vitamin A deficiency among preschool children (WHO, 1995). The most appropriate and long term approach to its prevention and control is to ensure that diets provide adequate amounts of the vitamin. In order that locally available retinol and carotene rich foods be effectively used for combating vitamin A deficiency, it is important to obtain accurate analytical data concerning the content and bioavailability of vitamin A, in both raw and ready-to-eat form.

Both preformed vitamin A (retinol) and provitamin A carotenoids are susceptible to destruction by heat, light and oxygen (Olson, 1991). Certain local food preparation techniques in Northeast Thailand can result in considerable losses of vitamin A activity (Speek, Speek-Saichua, & Schreurs, 1988; Wasantwisut, Sungpuag, Chavisit, Chitang, Jittnandana, & Viriyapanich, 1995). Thus, there is a need to evaluate the effects of home-processing and cooking procedures on the preformed and provitamin A contents of traditional Thai foods.

The objective of this study was to determine the effects of local preparation and cooking procedures on vitamin A content of traditional Thai food and to identify and recommend preferable local cooking methods which minimize destruction of vitamin A activity.

2. Materials and methods

2.1. Selection, collection and preparation of food samples

2.1.1. Selection

The preparation and procedures of vitamin A-rich foods in selected rural households of Nong Wang Noi and Chai Mongkhol villages in Mukdaharn province of Northeast Thailand were observed and recorded by trained nutritionists. The selection of vitamin A-rich foods of both animal and plant origin was based on

* Corresponding author.

0308-8146/98/$—see front matter
PII: S0308-8146(98)00154-X

high concentrations of retinol or β-carotene and the amount, the availability, frequency, and preference of consumption. The sequential preparation and cooking processes, temperature and duration were observed and recorded in the households and were duplicated in the laboratory of the Institute of Nutrition, Mahidol University (INMU). The retinol and β-carotene content, before and after cooking, were determined by HPLC, using the modified method of Speek, Temaliwa, & Schrijver (1986).

2.1.2. Collection
Chicken livers were obtained from Bangkok Livestock Processing Co. Ltd. and a chicken farm in Minburi, in order to minimize dietary variations and breed. Vegetables were purchased from the markets of Bangkok on the morning of each day of preparation and cooking.

2.1.3. Preparation
For the vegetable-based foods, control and cooked samples were prepared in triplicate while single control and cooked samples were prepared for the animal-based foods, on the day the raw materials were purchased. Ascorbic acid (1% of sample weight) was immediately added as an antioxidant to each of the composite raw and cooked samples; they were blended under a flow of oxygen-free nitrogen (OFN) and light-protected conditions. The homogenized samples were stored at approximately −20°C and analyses were carried out within 3 days, to minimize losses due to storage.

2.2. Pre-cooking and sample procedure

Duplicate portions of each food sample were purchased and prepared for retinol and/or β-carotene analysis. One portion was processed raw while the other was prepared by grilling, boiling or simmering using local cooking practices; they were then aliquoted prior to analysis. The cooking conditions for each of the foods are shown in Table 1.

2.3. Analytical procedure

Retinol and β-carotene contents were determined in duplicate. Food specimens were digested with 50 ml 2 N potassium hydroxide (KOH) for 30 min. The β-carotene or retinol fraction was extracted with diisopropyl ether according to the method (Speek et al., 1986), then resaponified with 5% KOH and washed with 10% sodium chloride. The final wash was with distilled water until it was alkaline free. The sample was then dried and dissolved with mobile phase for determination of β-carotene and retinol concentration by HPLC.

The HPLC instrumentation included a 5 μm Micro Pak MCH, 15×0.4 cm (Varian Associates, Sunnyvale, USA) and a 5 μm ZorbaxODS, 25×0.46 cm (Jones Chromatography Ltd., CO, USA) for β-carotene and retinol analyses, respectively. A mixture of acetonitrile (CH_3CN), methanol (CH_3OH) and acetone $(CH_3)_2CO$ in the ratio 60:30:10 served as the mobile phase solvent for β-carotene analysis at a flow rate of 1.2 ml min^{-1}; methanol (CH_3OH) was the mobile phase for retinol determination at a flow rate of 1.5 ml min^{-1}. Peak responses of β-carotene and retinol were measured at wavelengths of 450 and 325 nm, respectively. Pure standards of retinol were obtained from Sigma Chemical Co., St. Louis, USA, and all-*trans*-β-carotene were obtained from Fluka Chemie AG, Buchs, Switzerland. Purity of the standards was checked by HPLC analysis. Stock and standard solutions were prepared in ethanol and concentrations were calculated using extinction

Table 1
Conditions of cooking of food items

Food	Method	Temperature, duration
Chicken liver	Grilling/Boiling	175–180°C, 20 min
Chicken liver	Boiling	77–100°C, 7 min
Chicken egg	Boiling	100°C, 10 min
Yellow sweet potato (*Ipomoea batatas* L.)	Boiling	27–100°C, 15 min
Ivy gourd (*Coccinia grandis* L. Voigt) in soup with ground pork	Boiling	100°C, 2 min
Pumpkin (*Cucurbita maxima*)	Steaming	27–100°C, 40 min
Amaranth (spineless) (*Amaranthus viridis*)	Steaming	100°C, 8 min
Ivy gourd, Chinese cabbage (*Brassica chinensis*), Swamp cabbage (Chinese) (*Ipomoea reptans*)	Blanching	100°C, 5 min
Chicken egg (omelet)	Frying	200°C, 2 min
Swamp cabbage (Chinese)	Frying	190°C, 4 min
Chicken liver/Ivy gourd/Coriander (*Anethum graveolens*)/ Pumpkin young leaves/Holy leaves (*Ocimum canum*) and Onion leaves (*Allium ascalonicum* Linn.)	Simmering soup Traditional I san soup (Om-kruang-nai-kai)	99°C, 10 min
Bamboo shoot (*Bambusa arundinacea*)/Pumpkin young leaves/Holy leaves/Cha-om (*Acacia insuavis*)/ Pag-ka-yang/ and Bai-yah-nang (*Tiliacora triandra* Diels) juice	Traditional I san bambooshoot vegetarian soup (Kang-nho-mai)	45–100°C, 10 min

coefficients (1850 for Retinol at 325 nm in ethanol and 2592 for all-*trans*-β-carotene at 450 nm). Working solutions of retinol and β-carotene were analysed with each batch of samples on the day of analysis.

The percentage loss of retinol and β-carotene was calculated per 100 g wet weight of raw and cooked samples. The changes of retinol and/or β-carotene content in each food due to various cooking methods were analysed by Mann–Whitney U-test (Saunders & Trapp, 1994).

3. Results and discussion

3.1. Effect of cooking on retinol and β-carotene content

In general, the retinol content of cooked foods was less than for uncooked (Table 2). The highest concentration of retinol was found in uncooked chicken liver (average 10514 μg/100 g) compared to chicken egg (average 166 μg/100 g). Although the retinol content of cooked chicken egg is much less than cooked chicken liver (mean 120 μg vs 9402 μg/100 g), it is a potentially rich source of vitamin A, due to its reasonable cost, ready availability in the community and ease of preparation. Moreover, chicken eggs are popular for regular consumption at all ages, especially among young children.

β-Carotene content in raw vegetables varied widely as indicated by the large standard deviations (Table 3). β-Carotene content is affected by variety, maturity, growing conditions, season and which part of the vegetable is consumed (Hart & Scott, 1995). Leafy vegetables contain considerably more β-carotene than other vegetables (Speek et al., 1988). Raw ivy gourd leaves (Table 5), contained more β-carotene (2474 μg/100 g) than swamp cabbage leaf and stems, 1541 μg/100 g (Table 3). In contrast, the amount of β-carotene in other leafy vegetables like Chinese cabbage (Table 3) and amaranth showed lower values.

Yellow sweet potato in light syrup (Table 3), Kang-nho-mai and Om-kruang-nai-kai (Table 4) and ivy gourd soup with ground pork (Table 5) showed β-carotene contents ranging from 4.51 to 514 μg/100 g.

3.1.1. Boiling, frying and grilling

Hard boilling of chicken egg resulted in 11% loss of retinol, while losses in omelette were up to 43% (Table 2). The protection from retinol loss in hard-boiled egg is attributed to the shell, while exposure to direct heat and air, as in omelette preparation, resulted in greater loss. Boiling chicken liver with the lid in place resulted in greater retinol retention compared to grilling the liver. Since fresh liver was grilled using direct heat with a higher temperature for longer time, this method resulted in a greater loss of retinol. Dripping loss of solids from fresh liver during grilling was another reason for the vitamin loss. Losses in retinol were thus affected by higher temperature, longer cooking time and exposure to air and heat during cooking. The cutting size also affected losses during cooking (Table 2 and Table 4). Cutting results in cell damage and increase of surface area exposure to direct heat and air, consequently leading to oxidative degradation and destruction of retinol.

Table 2
Loss of retinol in chicken liver and egg during various cooking methods

Food	Methods	Retinol content (μg/100 g)[a]		% Retinol loss
		Raw	Cooked	
Chicken liver	Boiling 77–100°C, 7 min	10586 ± 408	10032 ± 164	5 ± 0.8[b]
	Grilling 175–180°C, 2 min	10442 ± 203	8770 ± 446	16 ± 3.6[b]
Chicken egg	Boiling 100°C, 10 min	162 ± 6.4	144 ± 8.7	11 ± 5.8[b]
	Frying 200°C, 2 min	169 ± 5.8	96.5 ± 17.0	43 ± 11.0[b]

[a] Mean ± SD.
[b] Significant difference ($p < 0.05$).

Table 3
Loss of β-carotene by cooking raw vegetables using various methods

Food	Method	β-carotene (μg/100 g)[a]		% β-Carotene loss
		Raw	Cooked	
Swamp cabbage	Blanching 77–100°C, 5 min	1541 ± 80	1379 ± 60	11 ± 1.4[b]
	Stir-frying 175–180°C, 2 min	1541 ± 63	1262 ± 90	18 ± 2.4[b]
Chinese cabbage	Blanching 100°C, 5 min	1488 ± 84	1387 ± 77	7 ± 0.5
Hand-peeled pumpkin	Steaming 27–100°C, 40 min	176 ± 7.5	150 ± 4.3	15 ± 1.9
Yellow sweet potato	Boiling 27–100°C, 15 min	7.86 ± 0.2	4.51 ± 0.3	43 ± 4.4

[a] Mean ± SD.
[b] Significant difference ($p < 0.05$).

Table 4
Loss of retinol and β-carotene in Kang-nho-mai and Om-kruang-nai-kai

Food	Retinol (μg/100 g)[a]		β-Carotene (μg/100 g)[a]		% Retinol loss	% β-Carotene loss
	Raw	Cooked	Raw	Cooked		
Kang-nho-mai	ND[b]	ND[b]	31.3 ± 0.5	24.7 ± 0.4	ND[b]	21 ± 1.7
Om-kruang-nai-kai	2189 ± 124	2006 ± 118	335 ± 26	315 ± 24.5	8 ± 1.5	6 ± 0.8

[a] Mean ± SD.
[b] ND = not determined.

Table 5
Increase in β-carotene after cooking green leafy vegetables using different methods

Food	Method	β-Carotene (μg/100 g)[a]		% increase of β-Carotene[b]
		Raw	Cooked	
Amaranth (spineless)	Steaming 100°C, 8 min	1438 ± 763	1656 ± 79	15 ± 0.9[c]
Ivy gourd	Blanching 100°C, 5 min	2474 ± 185	2882 ± 255	16 ± 1.7[c]
Ivy gourd soup with ground pork	Boiling 100°C, 2 min	453 ± 21	514 ± 23	13 ± 2.1[c]

[a] Mean ± SD.
[b] % increase of β-carotene = $\frac{\beta\text{-carotene}_{cooked} - \beta\text{-carotene}_{raw}}{\beta\text{-carotene}_{raw}} \times 100$
[c] Not significantly different.

3.1.2. Blanching and frying

Blanching of swamp cabbage and Chinese cabbage led to minimal loss of 7–11% β-carotene compared to 18% loss by stir-frying (Table 3). High-temperature blanching for short periods has been reported to result in greater vitamin retention than low-temperature blanching for longer periods (Reddy, Vijayaraghawan, Bhaskarachary, & Rani, 1995). No loss of β-carotene occurred when vegetables were water-blanched (Dietz, Kantha, & Erdman, 1988). In contrast, employing higher temperatures, even for a short time during frying, caused significant reduction in the content of the heat-labile vitamin (Adams & Erdwan, 1988). Previously, Sood and Bhat (1974) reported that losses of retinol may occur at high temperatures, when butter or palm oil was used in open-pot stir-frying of vegetables. Similar findings have been noted with stir-fried spinach and cabbage (Speek et al., 1988; Rahman, Wahed, & Akbar, 1990).

3.1.3. Steaming and boiling

Hand-peeled pumpkin, when steamed, resulted in 15% loss of β-carotene, while boiling yellow sweet potato showed up to 43% destruction (Table 3). Steaming or pressure-cooking of vegetables have been recommended to minimize nutrient loss (Adams & Erdwan, 1988; Dietz et al., 1988). However, Reddy et al. (1995) have demonstrated varying losses of β-carotene from 0 to 83% in steaming of vegetables. Minimal losses of carotene and ascorbic acid are obtained when vegetables are cooked without water, while maximum loss is associated with cooking in large volumes of water. The time–temperature relationship is important for all types of food preparation employing heat, but the impact varies with different cooking methods and products. Some vegetables require longer heat processing to inactivate enzymes or to render the product tender (Adams & Erdwan, 1988). Boiling vegetables (2–15 min) under home conditions in Indonesia, resulted in β-carotene loss of 39 to 86% while shorter cooking time reduced the loss to 6–75% (Hermana & Muhilal, 1995). Home preparation in the Brazilian tradition indicated that β-carotene in pumpkin and sweet potato decreased (12–33%) after boiling for 10 minutes (Rodriguez-Amaya, 1996). In the present study, boiling yellow sweet potato, cut into small pieces in light syrup, showed losses of up to 43% (Table 3).

3.1.4. Simmering

The loss of β-carotene in bai-yah-nang juice simmered in soup as Kang-nho-mai averaged 21% (Table 4). However, vegetables and chicken liver combined in Om-kruang-nai-kai was found to be a potentially good source of vitamin A, with low losses of β-carotene and retinol, amounting to only 6 and 8%, respectively.

3.2. Apparent increase in vitamin A activity due to cooking

Apart from the loss of β-carotene, on increment in β-carotene was observed. Table 5 shows changes in β-carotene after cooking 100 g of raw green leafy vegetables using different cooking methods. When compared on a wet weight basis, green leafy vegetables such as ivy gourd and amaranth showed an average increase of 15% β-carotene. This varied according to the type of vegetables and cooking methods. Similar findings have been reported earlier, wherein lower values observed in raw vegetables are due to incomplete extraction of carotenes from the stable lipoprotein complexes (Park, 1987). Chandler and Schawartz (1988) found a significant increase in β-carotene after blanching for 2 min and attributed this to an increase in extractability of carotene in the blanched products. Changes in tissue morphology, which occur as a result, allow greater penetration of organic solvents into the cells and enhance release of β-carotene. Khachick, Goli, Beecher, Holden, Lusby, Tenorio and Barrera (1992), observed increase in β-carotene in cooked broccoli, spinach, green beans and tomato compared to the raw food. Hart and Scott (1995) demonstrated that boiled spinach, broccoli and green beans had higher β-carotene contents than the raw form. In the present study, ivy gourd and amaranth are thinner and softer than swamp cabbage and Chinese cabbage. Thus by cooking at appropriate temperature and time, the cell wall might have been disrupted more readily and yielded more extractable β-carotene.

In conclusion, a loss of retinol or β-carotene was mainly due to destruction during cooking. In contrast, some vegetables such as ivy gourd and amaranth which were blanched, boiled or steamed showed an increase in β-carotene content. Likewise, use of moist heat in cooking animal-derived food resulted in higher retention of retinol compared to frying or grilling. There is a need to promote the use of locally available sources of carotene and retinol, and to encourage beneficial cooking practices, through nutrition communication/education programmes in the community.

Acknowledgements

This work was partially supported from the Vitamin A Field Support Project of the United States Agency for International Development (USAID). The authors also would like to thank Dr Lalita Bhattacharjee, International Affairs Program, INMU, for her editorial assistance.

References

Adams, C. E., & Erdwan Jr, J. W. (1988). Effect of home food preparation practices on nutrient content of foods. In E. Karmas & R. S. Harris (Eds.), *Nutrition evaluation of food processing* (3rd ed., pp. 557–605). New York: Van Nostrand Reinhold.

Chandler, L. A., & Schawartz, S. J. (1988). Isomerization and losses of *trans*-β-carotene in sweet potatoes as affected by processing treatments. *Journal of Agricultural and Food Chemistry, 36*, 129–133.

Dietz, J. M., Kantha, S. S., & Erdman Jr, J. W. (1988). Reversed phase HPLC analysis of alpha and beta carotene from selected raw and cooked vegetables. *Plant Foods for Human Nutrition, 38*, 333–341.

Hart, D. J., & Scott, K. J. (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods and measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chemistry, 54*, 101–111.

Hermana, H., & Muhilal, M. K. (1995). Identifying seasonal vitamin A rich foods and recommended preparation and preservation methods in Indonesia. In E. Wasantwisut & G. A. Attig (Eds.), *Empowering vitamin A foods* (pp. 53–59). Salaya, Thailand: Institute of Nutrition, Mahidol University.

Khachick, F., Goli, M. B., Beecher, G. R., Holden, J., Lusby, W. R., Tenorio, M. D., & Barrera, M. R. (1992). Effect of food preparation on quantitative and qualitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *Journal of Agricultural and Food Chemistry, 40*, 390–398.

Olson, J. A. (1991). Vitamin A. In L. Lawrence (Ed.) *Handbook of vitamins* (2nd ed, pp. 2–27). New York: Marcel Dekker.

Park, Y. W. (1987). Effect of freezing, thawing, drying and cooking on carotene retention in carrots, broccoli and spinach. *Journal of Food Science, 52*, 1022–1025.

Rahman, M. M., Wahed, M. A., & Akbar, A. M. (1990). Beta carotene losses during different methods of cooking green vegetables in Bangladesh. *J. Food Comp Anal., 3*, 47–53.

Reddy, V., Vijayaraghavan, K., Bhaskarachary, K., & Rani, M. (1995). Carotene rich foods: the Indian experience. In E. Wasantwisut & G. A. Attig, (Eds.), *Empowering vitamin A foods* (pp. 15–27). Salaya, Thailand: Institute of Nutrition, Mahidol University.

Rodriguez-Amaya, D. B. (1996). Assessment of the provitamin A contents of foods—the Brazilian experience. *J. Food. Comp. Anal., 9*, 196–230.

Sood, R., & Bhat, C. M. (1974). Changes in ascorbic acid and carotene content of green vegetables leafy on cooking. *J. Food. Sci. Tech., 11*, 131–133.

Saunders, B. D. & Trapp, R. G. (1994). *Basic and clinical biostatistics* (2nd ed., pp. 117–119). New Jersey: Appleton and Lange.

Speek, A. J., Temaliwa, C. R., & Schrijver, J. (1986). Determination of beta-carotene content and vitamin A activity of vegetables by high performance liquid chromatography and spectrophotometry. *Food Chemistry, 19*, 65–74.

Speek, A. J., Speek-Saichua, S., & Schreurs, W. H. P. (1988). Total carotenoid and beta carotene contents of Thai vegetables and the effect of processing. *Food Chemistry, 27*, 245–257.

Wasantwisut, E., Sungpuag, P., Chavasit, V., Chitchang, U., Jittnandana, S. & Viriyapanich, T. (1995). Identifying and recommending vitamin A rich foods in Northeast Thailand. In E. Wasantwisut & G. A. Attig (Eds.), *Empowering vitamin A foods* (pp. 69–90). Salaya, Thailand: Institute of Nutrition, Mahidol University.

WHO (1995). Global prevalence of vitamin A deficiency. MDIS Working Paper no. 2. WHO, Geneva.