J. Natn. Sci. Foundation Sri Lanka 2003 31(3&4): 437-444

BIOCONVERSION OF PRO-VITAMIN A CAROTENOIDS AND ANTIOXIDANT ACTIVITY OF *CARICA PAPAYA* FRUITS

U. G. CHANDRIKA, E. R. JANSZ*, S. M. D. N. WICKRAMASINGHE and N. D. WARNASURIYA

[1] *Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda.*

(Received: 06 August 2002; accepted: 24 January 2003)

Abstract: Previous studies have shown that the red-fleshed *Carica papaya* has significantly higher β-carotene and lycopene than the yellow-fleshed variety. This study with Wistar rats has shown that irrespective of the colour of the flesh, a significant increase of serum vitamin A is shown only if papaya is fed along with a standard diet ($p<0.001$).This is not so if the papaya is fed separately. The red-fleshed variety shows higher (66.7± 4.2μg/dL) β-carotene levels in the liver compared to the yellow fleshed variety (5.4 ± 1.0μg/dL, $p<0.001$). This is probably due to both (a) higher β-carotene levels of the red-fleshed variety and (b) inhibition of 15-15[1] dioxygenase activity by lycopene which is also higher in the red-fleshed variety. *In vivo* antioxidant activity as judged by thiobarbituric acid reactive substances (TBARS) in the heart muscles showed that papaya fed rats had significantly higher antioxidant activity than the control. This is probably due to the known high antioxidant activity of both lycopene and β-carotene. Both vitamin A and β-carotene were determined using RP-HPLC.

Keywords: β-carotene, bioavailability, *Carica papaya*, lycopene, RP-HPLC, TBARS, vitamin A, Wistar rats

INTRODUCTION

Vitamin A deficiency is a national deficiency disorder of public health importance in Sri Lanka. A recent national survey revealed that 36% of pre-school children in Sri Lanka have vitamin A deficiency (serum retinol < 20 μg/dl). In view of its well-established association with child morbidity and mortality,[1,2] it is a cause for concern.

Vitamin A is available from animal sources in the form of retinol, retinal, retinoic acid or esters, and from plant sources, particularly fruits and vegetables, in the form of pro-vitamin A carotenoids. There are approximately 50 known active pro-vitamin A carotenoids, of which β-carotene makes the largest contribution to vitamin A activity in plant foods.[3] Recent findings suggest that the bioavailability of carotenoids in fruits and vegetables may be much lower than previously estimated.[4,5] Research is currently under way to revise these previously established conversion factors.

* Corresponding author

In addition to their well-known vitamin A activity, carotenoids with or without vitamin A activity are known to be involved in immuno-enhancement[6] and are useful in the treatment and prevention of cancer owing to their antioxidant capacity.[7] The main strategy for prevention of vitamin A deficiency in Sri Lanka has been the promotion of the general consumption of pro-vitamin A, especially as carotenoids from plant sources.[8] Papaya is one of the main fruits recommended for vitamin A deficiency in Sri Lanka. There are different types (red-fleshed and yellow-fleshed) of *Carica papaya*. In a previous study, identification and quantification of pro-vitamin A and non-vitamin A carotenoids of the two main types (red and yellow-fleshed) of *Carica papaya* grown in Sri Lanka using MPLC (Medium Pressure Liquid Chromatography) and UV/visible spectrophotometry was reported.[9,10] The study indicated that the major carotenoids of yellow-fleshed papaya had β-carotene and β-cryptoxanthin, which correspond to the 106.3 ± 2.8 μg of mean retinol equivalent (RE). Red-fleshed papaya contained pro-vitamin A carotenoids β-carotene, β-cryptoxanthin and β-carotene epoxide,[5,6] corresponding to 211.8 ± 3.8 μg of mean RE. As far as we are aware, there has not been any *in vivo* bioavailability studies on the carotenoids of *Carica papaya* found in Sri Lanka. Therefore, the objective of this study was to investigate *in vivo* bioavailability of carotenoids from major types of red-and yellow-fleshed papaya when consumed by Wistar rats, either incorporated or not incorporated into standard rat chow. Studies carried out recently also showed the red-fleshed papaya to be rich in lycopene,[9,10] a potent antioxidant. Lycopene is reported to be beneficial in cardiovascular ailments and cancer.[11,12] Since many of the protective effects and health benefits of lycopene and other carotenoids have been hypothesized to occur via protection against oxidative damage[13,14,15] the antioxidant activity of *Carica papaya* has been studied by determining the levels of heart thiobarbituric acid- reactive substances (TBARS).

METHODS AND MATERIALS

Plant materials: Yellow- and red-fleshed papaya (*Carica papaya* L.) were purchased from the market. The yellow fruits selected were of the same skin colour, shape (globular), and ripening level (pH =4.5-5.0). Similarly, the red-fleshed fruits selected for this study were of the same skin colour, shape (elongated), and ripening level (pH=4.5-5.0).

Chemicals: β-carotene and retinol were obtained from Sigma Chemical Co (St Louis, Mo., USA). All the other chemicals used were of HPLC or analytical grade and were purchased from LABO-TECH, Uppsala, Sweden.

Biological assay: Healthy, 2 months old male Wistar rats (weight 205 ± 10.8 g) from the Medical Research Institute (MRI) were used. They were housed in stainless steel cages under standard conditions. The rats were randomly divided into three groups (6 rats each). Two experiments were carried out. In the first study, papaya (red-fleshed and yellow-fleshed) was incorporated (10% dry weight) into the standard

WHO rat feed.[16] In the second study, while being maintained on the standard rat feed, papaya was given orally (10 g/day) to rats in the morning. All rats given papaya consumed the full amount.

Each day for 4 weeks the two groups received test diets and a control group was given only the standard diet. At the end of four weeks, the rats were anaesthetized and blood was drawn for estimation of vitamin A. In the first study, hearts and livers were excised and stored at -20 ^{0}C for estimation of vitamin A and β-carotene content in the liver and *in vivo* lipid peroxidation assay in the case of the heart.

Extraction of serum vitamin A: Serum (100 µl) and ethanol (100 µl) were mixed vigorously in a vortex mixer.[17] HPLC grade hexane was added and the contents mixed a gain in a vortex mixer, until the bottom layer was thoroughly extracted. The contents were centrifuged at 2000 rpm for 5 minutes. The upper hexane layer was transferred to a small test tube and evaporated under nitrogen. The residue was dissolved in 50 µl of 95% methanol and analysed using RP-HPLC.

Extraction of liver vitamin A: The liver tissues were first chopped and minced. Liver (1 g) was placed in a mortar.[18] The tissue was ground to a powder with anhydrous sodium sulfate. After the addition of 2 volumes (v/w) of 2-propanol/ dichloromethane (1:1), the mixture was ground further with a pestle and then allowed to stand for 2 to 3 minutes. The extract was filtered, and the residue was extracted with dichloromethane 3 to 4 times, as described. The pooled extract, after being filtered, was divided into two portions and evaporated to dryness in a rotary evaporator. One portion was dissolved in 95% methanol for analysis of vitamin A and the other dissolved in methanol: acetonitrile: trifluroacetic acid (58: 35: 7) for analysis of β-carotene. An aliquot (50 µL) was analysed by RP-HPLC.

RP-HPLC for vitamin A analysis: For reverse-phase HPLC, Waters Associates (Milford, MA) pumps (model 515), Shimpak Column, CLC-ODS (M) C_{18}, 25 cm x 4.6 mm, rheodyne injection valve, SCL-6A system controller and CR-6A recorder were used. Serum extracted solution of 50 µl was injected onto the RP-HPLC column and vitamin A levels were analysed using 95% methanol as mobile phase and detected at 325nm.[17] Plasma retinol concentration was calculated using a standard curve. For quantification, a standard curve was utilized (r^2=0. 992). In order to correct for recovery a parallel HPLC run was conducted using a known amount of vitamin A mixed into each test.

RP-HPLC for β-carotene analysis: β-carotene was analysed using the above HPLC system but the mobile phase was acetonitrile: methanol: triflouroacetic acid (58: 35: 7).[19] Detection was at 450nm. Plasma β-carotene concentrations were calculated using a standard curve. For quantification, a standard curve was available (r^2= 0.989). In order to correct for recovery, a parallel HPLC run was conducted using a known amount of β-carotene mixed into each sample.

In vivo lipid peroxidation: Measurement of lipid peroxidation was performed according to the methods by Munasinghe *et. al.*,[20] with slight modifications. Each heart was homogenized in ice with 20% (w/v) of MDA buffer (KCl 0.9%w/v, Na_2HPO_4 0.033M) followed by incubation for 1 hour at 37 °C. These were centrifuged at 2500 rpm in a Kubota 5100 for 15 minutes. Aliquots (1 ml) were withdrawn from the incubation mixture and 0.5 ml each of 40% (w/v) trichloroacetic acid (TCA) and 0.2% (w/v) thiobarbituric acid (TBA) with 0.02% (w/v) butylated hydroxytoluene (BHT) added. The samples were vortexed and boiled for 15 minutes followed by a 5 minutes cooling period on ice. One ml of 70% TCA was added to each tube, vortexed, and allowed to stand for 20 min at room temperature. Then the samples were centrifuged at 3500 rpm for 20 min (Kubota 5100) and the colour developed was measured at 532 nm in a Shimadzu UV-1201 V spectrophotometer.

Statistical analysis: Statistical analysis was carried out in Microsoft Excel. All the results are presented as mean ± SEM. The significance of the differences between the means of the tests and controls were established by student t-test. p-value less than 0.001 was considered to be significant.

RESULTS

Bioconversion of pro-vitamin A carotenoids to vitamin A

Table 1 shows serum vitamin A concentrations of rats given papaya, incorporated into standard diets and separately, whereas Table 2 shows liver vitamin A and β-carotene concentrations of rats given papaya incorporated into the standard diet. Results of Table 1 show that in order to increase serum vitamin A, papaya must be incorporated into the standard diet. Table 2 shows that red-fleshed papaya markedly increases liver β-carotene content.

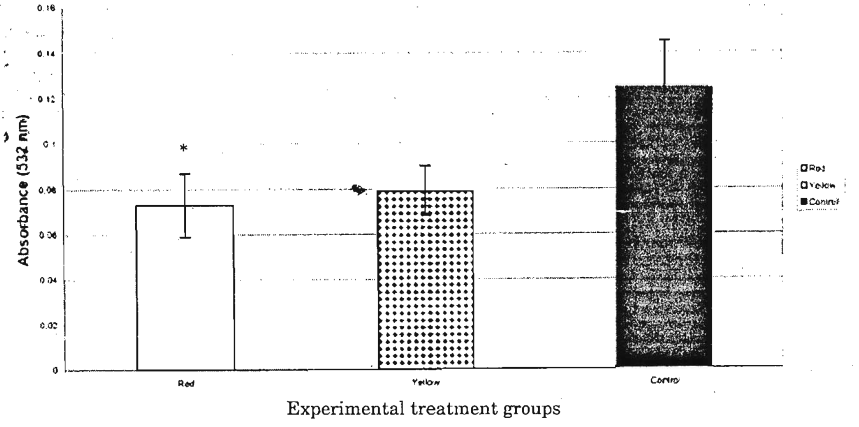

Figure 1: Effects of *Carica papaya* (yellow and red-fleshed) incorporated into standard diet on cardiac lipid peroxidation *p=0.0022 compared with control

In vivo lipid peroxidation

Figure 1 shows the effects of *Carica papaya* (yellow and red-fleshed) incorporated into the standard diet on cardiac lipid peroxidation. Both papaya groups significantly reduced TBARS in the heart (36.3% and 41.12% respectively) compared to the control. There were no significant differences between the groups fed on yellow -fleshed and red-fleshed papaya.

Table 1: Serum vitamin A in Wistar rats

	Papaya incorporated into standard diet (μg/dL)	Papaya given separately (μg/dL)
Red-fleshed Papaya	14.3±2.8*	6.2±1.6
Yellow-fleshed Papaya	12.0±1.8*	5.8±2.0
Control	4.8±1.4	6.4±1.2

* Significantly different from control at $p<0.001$
Values shown are the mean ± SEM of six determinations.

Table 2: Liver vitamin A and β-carotene in rats given papaya incorporated into standard diet

	Vitamin A (μg/g)	β-carotene (μg/g)
Red-fleshed Papaya	1.6±0.1	66.7±4.2*
Yellow-fleshed Papaya	1.7±0.7	5.4±1.0
Control	0.8±0.1	5.3±0.4

* Significantly different from control at $p<0.001$
Values shown are the mean ± SEM of six determinations.

DISCUSSION

Among the three groups of rats, the serum vitamin A levels were significantly ($p<0.001$) higher for groups given yellow-fleshed and red-fleshed papaya incorporated into the standard diet (12.0 ± 1.8 μg/dL and 14.3 ± 2.8 μg/dL respectively), compared to the control (4.8 ± 1.4 μg/dL). There was no significant

difference between yellow-fleshed (5.8 ± 2.0 μg/dL) and red-fleshed papaya (6.2 ± 1.6 μg/dL) given separately and the control fed on only standard diet (6. 4 ± 1.2 μg/dL). These results show that bioavailability of pro-vitamin A is greater when papaya is incorporated into the standard diet, than when the two are given separately. This may be due to fats and oils in the diet, which enhance bioavailability of pro-vitamin A carotenoids. This would tend to suggest that in humans, papaya taken as a dessert soon after a main meal is more likely to increase vitamin A bioavailability than when taken separately (e.g. as breakfast). Previous carotenoid analysis studies[9,10] showed that red-fleshed papaya had a higher retinol equivalent compared to the yellow-fleshed papaya. However, the *in vivo* bioavailability studies show that there is no significant difference in the vitamin A content of rats fed on red-fleshed papaya and yellow-fleshed papaya. It is likely that lycopene, a known inhibitor of 15-15' dioxygenase enzyme,[21] which is present in high levels in the red-fleshed variety[9,10] affects the bioconversion of pro-vitamin A carotenoids to vitamin A. This is supported by significantly higher levels of β-carotene in the liver of rats fed on red-fleshed type. This is evidence of the inhibitory effect of lycopene present in red-fleshed papaya.[5,6] Further studies are needed to confirm the molecular mechanism of the action of lycopene.

Papaya significantly reduced TBARS in the heart of the rats fed on red-and yellow-fleshed papaya compared to the control ($p=0.0022$ for red-fleshed and $p= 0.0129$ for yellow fleshed). The differences in TBARS on feeding of rats with the two types of papaya were not statistically significant. This can be interpreted as being due to carotenoids such as β-carotene and lycopene of papaya[9, 10] and their known antioxidant effects. Therefore, rats fed papaya incorporated into a standard diet had significantly higher levels of heart antioxidant activity compared to the control.

Acknowledgement

The authors acknowledge financial assistance by IPICS from research grant Sri: 07 and the University of Sri Jayewardenepura from research grant No: ASP/6/PR/2000/13.

References

1 Milton R. C., Reddy V. & Naidu A. N. (1987). Mild vitamin A deficiency and childhood morbidity-an Indian experience. *American Journal of Clinical Nutrition* **46**: 827-829.

2 Sommer A, Hussaini G. & Tarwotjo I. (1983). Increased mortality in children with mild vitamin A deficiency. *Lancet* **2**: 585-588.

3 Bauernfeind J. C. (1981). *Carotenoids as colorant and vitamin A precursors*. Academic Press, New York.

4 de Pee S. & West C. E. (1996). Dietary carotenoids and their role in combating vitamin A deficiency: Review of the literature. *European Journal of Clinical Nutrition* **50**: 85-126.

5 de Pee S., West C. E., Muhilal K. D. & Hautvast J. G. A. J. (1995). Lack of improvement in vitamin A status with increased consumption of dark green leafy vegetables. *Lancet* **346**: 75-81.

6 Bendich A. (1991). Non-provitamin A activity of Carotenoids: Immunoenhancement. *Trends in Food Science and Technology* **2**: 127-130.

7 Matthews-Roth M. M. (1991). Recent progress in the medical applications of carotenoids. *Pure and Applied Chemistry* **63**: 147-156.

8 Solomons N. W. & Bulux J. (1993). Plant sources of vitamin A and human nutrition. *Nutrition Review* **51**: 199-204.

9 Chandrika U. G., Jansz E. R., Wickramasinghe S. M. D. N. & Warnasuriya N. D. (2001). Separation, identification, and quantification of carotenoids of two major varieties of *Carica papaya* grown in Sri Lanka using MPLC. *Proceedings of the Sri Lanka Association for the Advancement of Science* **57**(1): 254.

10 Chandrika U. G., Jansz E. R., Wickramasinghe S. M. D. N. & Warnasuriya N. D. (2002). Carotenoids in yellow-fleshed and red-fleshed papaya, *Journal of the Science of Food and Agriculture* **83**:1279-1282.

11 Kohimeier L., Kark J. D. & Gomez-Gracia E. (1997). Lycopene and myocardial infarction in the EURAMIC study. *American Journal of Epidemiology* **146**: 618-628.

12 Rao A. V. & Agarwal S. (2000). Role of antioxidant lycopene in cancer and heart disease. *Journal of the American College of Nutrition* **19**(5): 563-9.

13 Gerster H. (1997). The potential role of lycopene for human health. *Journal of the American College of Nutrition* **16**: 109-126.

14 Dimascio P., Kaiser S. & Sies H. (1998). Lycopene has the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics* **274**: 1-7.

15 Halliwell B. (1994). Free radicals and antioxidants: a personal view. *Nutrition Research* **52**: 253-65.

16 Sabourdy M. A. (1988). Breeding and care of laboratory animals. WHO/Lab/ **88**. 1 I: 45.

17 Barua A. B., Duitsman P. K. & Olson J. A. (1998). The role of vitamin A status in the conversion of all-*trans* retinoyl ß-glucuronide to retinoic acid in male Sprague-Dawley rats. *Journal of Nutritional Biochemistry* **9**: 8-16.

18 Mahapatra S. and Monorama R. (1997). The protective effect of red palm oil in comparison with massive vitamin A dose in combating vitamin A deficiency. *Asia Pacific Journal of Clinical Nutrition* **6** (4): 246-250.

19 Waters Corporation (1996). HPLC products for sample preparation, analysis and purification. pp 52.

20 Munasinghe T. C. J., Seneviratne C. K. Thabrew M. I. & Abeysekara A. M. (2001). Antiradical and Antilipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection. *Phytotherapy Research* **15**: 519-523.

21 Ershov I. V., Dmitrovski A. A. & Bykhovskii V. I. (1993). The character of the interaction of beta-carotene-15,15'-dioxygenase from rabbit small intestine with lycopene, 15,15'-dehydro-beta-carotene, lutein, and astaxanthine. *Biokhimiia* **58**(5): 733-739.