Vidyodaya J. of Sci, (2006) Vol. 13, pp 95-98

An investigation of the carotenoids of *carica papaya*

A.M. Buddhika Priyadarshani, E.R Jansz and H. Peiris
Department of Biochemistry, University of Sri Jayewardenepura, Nugegoda.

Corresponding author : ***E.R. Jansz***
Department of Biochemistry, University of Sri Jayewardenepura, Nugegoda.

Received on : 12/09/06
Accepted on : 04/26/07

Abstract

The carotenoids of yellow-fleshed papaw *(Carica papaya),* were collected at random from different areas of Kurunegala, a major papaw producing District in Sri Lanka. Results showed that the carotenoids present were phytofluene, ζ-carotene, β-carotene, β-cryptoxanthin, β-cryptoxanthin 5,6-epoxide and an unknown mono epoxy-carotenoid. Carotenoid content varied markedly from sample to sample and the retinol activity equivalent (RAE) and retinol equivalent (RE) ranged from 25 - 156.7 $100g^{-1}$ fresh weight (FW) and 50 - 313.3 $100^{-1}g$ FW respectively. This is 2 to 16 fold, values reported for papaw previously. *In-vitro* bio-accessibility studies showed values 5.2±0.9 $\mu g.g^{-1}$ FW for β-carotene and 6.9±1.1 $\mu g.g^{-1}$ FW for β-cryptoxanthin (50.5% and 45.7% extraction respectively).

Keywords: *Carica Papaya,* Carotenoids, Retinol Equivalent, Retinol Activity Equivalent, *In-vitro* bio-accessibility

1. Introduction

The carotenoids of yellow-fleshed papaw have been described previously[1,2] and the retinol equivalent (RE) of 1516. Kg^{-1} on the basis of dry weight was reported. Papaw is widely recommended for vitamin A deficiency in Sri Lanka[3] and also that is one of the main causes for hypercarotenemia in Sri Lanka. Therefore, the value reported above seems low even in relation to *Lasia spinosa* stem[4]. Further the standard deviation of carotenoids reported[1,2] appeared very low for a random selection having only a coefficient of variation of 20-30%. The objectives of the present study were to re-investigate (i) the carotenoid profile (ii) the carotenoid content (iii) RE and in addition (a) to calculate retinol activity equivalent (RAE) values and (b) to investigate the *in-vitro* bio-accessibe carotenoids of yellow-fleshed papaw.

2. Materials And Methods

Yellow fleshed-papaws (n=6), a day or two from full ripening, were collected from Pothuhera, Alauwa and Polgahawela, Thulhiriya and Boyagane of the Kurunegala District. On ripening they were extracted for carotenoids, saponification was done with 10% methanolic KOH, the components were separated by open column chromatography[5].Identification was carried out by UV spectrophotometrically and by the following chemical tests (i) fuming HC1 test on the thin layer chromatography (Tlc) (ii) test for epoxides with 0.1N HC1 (iii) for *cis-trans* configuration by iodine catalysis[5] . The last test had to be modified by adding exactly the same quantity of iodine into the blank for the spectral reading after isomerisation. The extracts were analyzed by HPLC at 450 nm as described previously[4] using β-apo-8' carotenal *(trans)* as internal standard after correcting for difference of absorbances of ζ-carotene and phytofluene which had markedly different λ_{max} values. Retinol equivalent (RE) was calculated by dividing μg β-carotene by 6 and other pro-vitamin A carotenoids by 12μg[6] and retinol activity equivalent (RAE) was calculated by dividing μg β-carotene by 12 and other pro-vitamin A carotenoids by 24[7]. Results were expressed both on fresh weight and dry weight. Moisture content was determined by freeze drying to constant weight. *In-vitro* bio-accessibility was determined by the method reported previously[8] using the specimen No: 4 (See Table I).

3 Results And Discussion

Table I: Carotenoid profile, RE and RAE of random specimens of yellow-fleshed papaw

Specimen No:	1	2	3	4	5	6
Carotenoids (μg.100g^{-1} FW)						
*Phytofluene	235	241	163	214	135	193
ζ-Carotene	120	29	46	75	220	238
β-Carotene	520	480	160	1030	140	900
β-Cryptoxanthin	610	600	230	1510	330	1180
* β-Cryptoxanthin-5,6-epoxide	130	100	50	190	20	110
** Unidentified *trans* mono epoxide	119	178	131	210	62	223
RE 100g^{-1} fresh weight	148.3	138.3	50	313.3	52.5	257.5
RAE 100g^{-1} fresh weight	74.2	69.2	25	156.7	26.3	128.8
RE 100g^{-1} dry weight	1158.6	1055.7	344.8	2410	372.3	1788.2
RAE 100g^{-1} dry weight	579.7	528.2	172.4	1205.4	186.5	894.4

*Not identified previously **Not separated previously
Each sample was analysed in duplicate

Table I shows the carotenoid profile of randomly selected individual samples and the RE and RAE. The levels of all carotenoids varied markedly from sample to sample. According to the previous studies, carotenoid concentrations of four varieties of papaw from different cities of Brazil: (μg/g edible portion, raw) were as follows: β- carotene ranged from 1.2 to 6.1; β-cryptoxanthin from 5.3 to 9.7. β-cryptoxanthin-5,6-epoxide from 1.8 to 3.8 and ζ-carotene from 1.4 to 2.0 showing the variations among and within the varieties of *Carica papaya*[5]. According to the study carried out in Indonesia, β-carotene ranged from 322 to 664 and β-cryptoxanthin ranged from non detectable amount to 425 μg/100 g wet weight edible portion and United States database in 1993 reported, β-carotene ranged from 62 to 910 and β-cryptoxanthin ranged from 519 to 1264 μg/100 g wet weight edible portion for papaw[9]. In present study the content of carotenoids ranged from 1.4 to 10.3 μg/g FW for β-carotene, from 2.3 to 15.1 μg/g FW for β-cryptoxanthin, from 0.5 to 1.9 μg/g FW for β-cryptoxanthin-5,6-epoxide and 0.29 to 2.38 μg/g FW for ζ-carotene and percentage contribution to the recommended daily allowance of vitamin A of the pre-school children that is set to 400 RE[7] ranged from 12.5% to 78.3% from a 100 g portion of papaw. The lowest and the highest values for β-carotene were approximately 7 fold to 57 fold higher than that of the previously reported for papaw in Sri Lanka [1,2] on the basis of dry weight. Our data compares well with the data of Brazil[5] where β-carotene is 6-30 fold higher than the previous Sri Lankan study[1,2]. It is felt that the underestimation of the previous studies[1,2] was due to using medium pressure liquid chromatography (MPLC) with silica gel. It is reported that silica gel causes degradation of carotenoids[5]. The low coefficient of variation in that study[1,2] is difficult to explain especially as yellow fleshed papaw has no agricultural selection and is highly heterogeneous. In this study as expected, random sampling is not amenable to calculation of standard deviation. These results will provide a major input to nutritionists and others concerned with vitamin A deficiency who may have been misled previously[1,2].

Special mention needs to be made on the following observations (i) extraction of carotenoids from papaw was harder than that experienced from other carotenoid bearing materials (ii) in all samples some of the colour in papaw was contributed to non-carotenoids (iii) among the petroleum ether extract was a non carotenoid which reacted with iodine (iv) the test for *cis-trans*[5] had to be modified as in the test because iodine is a catalyst (v) correction had to be made to HPLC values for those carotenoids having a λ_{max} deviating from 450 nm.

In-vitro bio-accessibility of papaw was done for the first time. Study revealed that in the *in-vitro* procedure reagents are not limiting. Percentage bio-accessibility for β-carotene and β-cryptoxanthin (the main pro-vitamin A carotenoids) were

5.2 ± 0.9 $\mu g.g^{-1}$ FW and 6.9 ± 1.1 $\mu g.g^{-1}$ FW respectively. This is 0.51 and 0.47 the fraction of total available β-carotene and β-cryptoxanthin respectively, and this reduction is due to the "matrix effect". If there is no absorption effect then RAE = RE multiplied by matrix effect. This is closely reflected in the results from papaw.

4. Acknowledgment

A grant for IPICS (SRI:07) is gratefully acknowledged.

5. References

1. Chandrika UG (2004) Content, bioavailability, bioconversion and antioxidant activity of carotenoids of some Sri Lankan fruits and green leafy vegetables. Ph.D. thesis, University of Sri Jayewardenepura.

2. Chandrika UG, Jansz ER, Wickramasinghe SMDN, Warnasuriya ND (2003) Carotenoids in yellow and red-fleshed papaya *(Carica papaya* L). *Journal of the Science of Food and Agriculture* **83**: 1279-1282.

3. Medical Research Institute Sri Lanka (1998). Report of the survey on vitamin A deficiency status of children in Sri Lanka 1995/96 Publ. UNICEF.

4. Priyadarshani AMB, Jansz ER (2006). The effect of maturity on carotenoids of *Lasia Spinosa* stem and the effects of cooking on *in-vitro* bioaccessibility of carotenoids. *Journal of National Science Foundation Sri Lanka.* **34** (3) : 131-136

5. Rodriguez-Amaya DB (1999). A Guide to carotenoid analysis in foods. ILSI press. Washington.

6. FAO (1988). Requirements of vitamin A, iron, folate, and vitamin B_{12}: Report of a joint FAO/WHO Expert Consultation, Rome, Italy.

7. Hedren E (2004). *In-viro* accessibility of provitamin A carotenoids in vegetables and fruits. Ph.D. thesis. University of Chalmers, Sweden.

8. Hedren E, Diaz V, Svanberg U, (2002). Estimation of carotenoid accessibility from carrots determined by an *in-vitro* digestion method. *European Journal of Clinical Nutrition* **56:** 425-430.

9. Setiawan B, Sulaeman A, Giraud DW and Driskell, JA (2001). Carotenoid content of selected Indonesian fruits. *Journal of Food Composition and Analysis* **14**: 169-176.