Vidyodaya J. of Sci. (2006) Vol. 13, pp 33-39

Variations in carotenoid profiles of pulp morphological fruit types of palmyrah from Kalpitiya

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Received on : 10-03-05
Accepted on : 07-11-06

Abstract

Depending on the morphology of fruit, palmyrah (Borassus flabellifer) had been classified in 5 types. On extraction, total carotenoid of the types from Kalpitiya was found to vary from 3.3-9.3 mg.100g^{-1} fresh weight. On separation by open column chromatography using a solvent gradient, carotenoids of the 5 types of fruit were found to vary markedly. Type I was dominated by violaxanthin and neoxanthin, type IIA by neoxanthin, β-zeacarotene, violaxanthin and phytofiuene, type IIB by phytoene, phytofluene, violaxanthin and neoxanthin, Type III by phytoene and violaxanthin and type IV by phytofluene and zeta-carotene. Of the nine carotenoids isolated only β-carotene and β-zeacarotene are provitamin A. The 5 types gave varying retinol equivalent 18, 155, 32, 21 and 33 per 100g on fresh weight basis for types I to IV respectively. The carotenoid profile differed from those of the same types previously collected from Mannar with the carotenoid content five to twelve fold more in the latter. However a common feature was that lycopene and compounds of the right fork of the carotenoid biosynthesis pathway were not detected from all types except the uncommon type IV (found only in Kalpitiya), indicating that the main types had a similar genetic overall biosynthetic motif. The variation of contents of the individual components of the left fork from location to location could be influenced by climatic and edaphic factors too. Type IV which had considerable morphological differences appears much less related to the other types.

Keywords Palmyrah, *Borassus flabellifer L*, morphological types, fruit pulp, carotenoids, Kalpitiya.

1. Introduction

Ariyasena (2002) described the variation of the total carotenoids of palmyrah *(Borassus flabellier L)* morphological fruit types. Carotenoid profiles for fruit types from Mannar were reported by Pathberiya & Jansz (2005) who showed that carotenoid content and retinol equivalent was low and was dominated by the non-provitamin A carotenoid neurosporene. That study showed that components of right

fork of the carotenoid biosynthesis pathway and lycopene was absent in all types. However studies on bulk samples by Samarasinghe & Jansz (2001), Chandrika (2004) and Uluwaduge (2005) indicated the presence of lycopene and moderate retinol equivalent.

This work of Pathberiya and Jansz (2005) also for the first time quantified the colourless carotenoids phytoene and phytofluene which showed variations with type. This is of significance as these two acylic carotenoids bind flabelliferins (steroidal saponins of palmyrah) and alter their antimicrobial and ATP^{ase} inhibitory activities (Uluwaduge *et.al,* 2005).

The objectives of this study were (a) to quantity the carotenoids of the pulp of the 5 types of fruit laying emphasis on the effect of this location especially on retinol equivalent and phytoene : phytofluene ratios (b) to attempt to gain some insight into the carotenoid biosynthesis motifs of palmyrah.

2. Materials and methods

General Precautions

Since carotenoids are unstable to light, elevated temperature and oxygen, all operations were carried out in dim light, at 25°C and once pulp was extracted, storage was at -20°C under N_2. Analytical grade solvents were used.

Raw materials

Palmyrah fruit (n=40), selected according to 5 morphological fruit types was collected one day after fall (optimum ripening) from Kalpitiya in the North-West of Sri Lanka in August 2005. Six (6) fruits of each of the 5 types described by Ariyasena (2002) and Pathberiya (2005) were separated. Two types were pulped immediately and the other three stored in a freezer, without opening the pericarp, until needed (1-3 weeks). In each case the pulp was extracted manually with a spoon the same day or the next day and stored as described above.

Extraction oficarotenoids

Palmyrah fruit pulp (PFP) (50g) was extracted using acetone (AC). The AC was extracted into petroleum ether 40-60°C (PE) until colourless and concentration achieved by N_2 flushing to give an extract of 1ml.

Separation of carotenoids

Open column chromatography as described by Rodriguez-Amaya (1999) and modified by Pathberiya and Jansz (2005) was carried using celite:MgO (1:1) as the matrix. Elution was by a solvent gradient of PE, PE and diethyl ether and PE and AC in the ratios specified by Rodriguesz-Amaya (1999). Eluents were monitored by a scanning uv-vis spectrophotometry using a double beam Shimadzu (model 1070) spectrophotometer. Identical fractions were pooled.

Identification

The procedure of Rodriguesz Amaya (1999) was followed using the following criteria: eluent:solvent ratio, λ of peaks, ratio of λ max, should be the shape of spectrum on comparison to data published (Rodriguesz Amaya, 1999). In addition, mono and diepoxides were distinguished by the fuming HCI test in a TLC tank (spots of carotenoids on plastic backed SiO_2 gel G_{60} plates), the hypsochromic shift with 0.1N HCI to distinguish 5,6 and 5,8 epoxides and the I_2 in PE test for a shift in λ max to detect *cis* and *trans* isomers (Rodriguesz Amaya, 1999).

Quantification

The uv-vis spectrophotometric method and extinction coefficient values of Rodriguesz Amaya (1999) were used to calculate quantity of individual carotenoids.

Retinol equivalent (RE)

RE was calculated on the basis that for one and two β- ionone moieties in a carotenoid, the R.E. $100g^{-1}$ equated to 12 and 6μg carotenoid respectively this was expressed on both fresh and dry weight basis.

Moisture content

The method of Dean and Stark (1984) was used to determine moisture.

3. Results

Total carotenoid content

Total carotenoids of the 5 types were 3.27, 9.26, 7.80, 5.34 and 5.9mg. 100^{-1}g PFP on wet basis for types I, IIA, IIB, III and IV respectively. This was 5-12 fold that reported from PFP from Mannar.

Carotenoid profile

Table I shows the carotenoid profiles. Of special note are (i) the variation in content among type of the colourless carotenoids (this is significant in that they are hydrophobic binders of flabelliferins, Uluwaduge *et al.,* 2005) (ii) the presence of the epoxides, violaxanthin and neoxanthin, reported for the first time in PFP. (iii) crocetin also reported for the first time in PFP, (iv) the absence of the neurosporene, this is the carotenoid at the junction of the biosynthesis pathway and is common in Mannar PFP (Pathberiya, 2005) (v) the paucity of provitamin A carotenoids, with only relatively small amounts of β-carotene in all types and β-zeacarotene in type IIa and III and (vi) the presence of δ-carotene which is the only member of the right fork of the carotenoid biosynthesis pathway present and that too in the very uncommon type IV found only in Kalpitiya.

Table 1 Carotenoid profiles of the 5 types of fruit pulp of palmyrah from Kalpitiya.

Type	I	IIA	IIIB	III	IV
Carotenoid	Content (mg.100g^{-1} fresh weight basis)				
Phytoene	-	0.76	2.49	2.19	-
Phytofluene	0.15	1.47	1.58	0.94	1.88
β - carotene	0.11	0.07	0.19	0.09	0.20
Zeta- carotene	0.31	0.85	0.43	0.40	1.15
Crocetin	0.38	0.23	0.44	0.33	0.98
β-zeacarotene	-	1.73	-	0.07	-
Violaxanthin	1.60	1.67	1.39	1.19	0.59
Neoxanthin	0.72	2.49	1.28	0.21	0.92
β-carotene	-	-	-	-	0.26

Total carotenoid, fold increase over Mannar 7.1, 11.8, 5.4, 9.5 for type I, IIA, IIB and III

Retinolequivalent (RE)

Despite the low levels of β-carotene in relation to the other carotenoids retinol equivalent of type I, IIb, III and IV was in the range 18-33. 100g^{-1} fresh weight PFP which are comparable to that reported by Chandrika (2004). The very much higher retinol equivalent of type IIa (table 2) is due to high levels of β-zeacarotene which brings RE levels to values in to a favourable comparison with other Sri Lankan fruits (Chandrika, 2004)

Table 2 - Retinol equivalent (100g^{-1})

Type	Moisture content	Fresh wt.	Dry wt.
I	76	18	75
IIA	84	155	969
IIB	74	32	1-23
III	76	21	81
IV	76	33	-

4. Discussion

Apart from the study of Pathberiya and Jansz (2005) this is the only report that quantifies isolated carotenoids in the different morphological types of fruit of palmyrah separately. In this study fruits were collected from Kalpitiya and the earlier study selected those from Mannar. There were major differences in this study (i) total carotenoid content (5-12 higher) (ii) retinol equivalent (several fold higher) (iii) the presence of neoxanthin, violaxanthin, crocetin and δ-carotene (the last in type IV only) and (iv) the absence of neurosporene in Kalpitiya.

There were two major factors in common in both studies (i) the absence of lycopene (ii) the absence of the carotenoids of the right fork of its biosynthesis pathway (fig 1) for all types except type IV. Type IV is found only in Kalpitiya and is uncommon, it had δ-carotene in small quantities. This suggests that the main types of palmyrah in Sri Lanka may not have the right fork in the carotenoid biosynthesis pathway.

The wide variations in total carotenoid content in Mannar and Kalpitiya is consistent with the findings of Ariyasena (2002) who reported wide disparity in carotenoid content and suggests that carotenoids of palmyrah are affected by climatic, edaphic and genetic factors.

Studies on bulk samples (mixed type PFP) which had been stored for long periods of time, used by Samarasinghe & Jansz (2001), Chandrika (2004) and Uluwaduge (2005) reported the presence of lycopene. Scrutiny of the spectral data indicates that the first two reports above were misinterpretations for the precursor of lycopene, namely neurosporene. The third had no backup spectral data. The only other possible explanation is that lycopene is formed as a post pulping - storage phenomenon in bulk samples from the Palmyrah Development Board which contained sodium metabisulphite, where while attaining the temperature of the freezer at which enzymes are inactive takes time in bulk samples. The enzyme activities of such samples are supported by the finding of Rajapakse (2005) who found pectin hydrolyzing activity under these conditions.

The marked variation of phytoene and phytofluene among types in both Mannar and Kalpitiya gave credence to the finding of Uluwaduge (2005) who could not obtain correlation of content of phytofluene on one hand and flabelliferin-II or flabelliferin B on the other. This can be explained by the different content and ratios of phytoene and phytofluene in different samples noting that these two colourless carotenoids compete with each other for the hydrophobic binding site of the β-sitosterol moiety of flabelliferins (Uluwaduge et.al., 2005).

5. Conclusion

The carotenoids of PFP are mainly non provitamin A although due to high overall carotenoid content some may contribute towards a significant RE. The variations in types and locations appear to be genetic and environmental respectively.

6. Acknowledgements

The authors thank IPICS Sri:07 for a grant.

7. References

AOAC (1984). Official methods of analysis of the-Association of Official Analytical Chemists - 14th edition, AOAC, Washington, DC 7:004

Ariyasena DD (2002). The diversity, bioactivity and structural studies of flabelliferins from palmyrah (*Borassus flabellifer* L) fruit pulp. M.Phil, thesis, University of Sri Jayewardenepura.

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

Pathberiya LG (2005). Studies on the hypocholesterolaemic effect, in-vitro antioxidant activity and carotenoids of palmyrah fruit pulp. M.Phil, thesis, University of Sri Jayewardenepura.

Pathberiya LG and Jansz ER (2005). Studies on the carotenoids and in-vitro antioxidant capacity of palmyrah fruit pulp. Accepted. *Journal of National Science Foundation,* Sri Lanka.

Rajapakse RANK (2005) Studies on endogenous pectin hydrolysis and carotenoid profile of two palmyrah fruit pulps. Final year, B.Sc. Human Biology project dissertation.

Rodriguesz -Amaya DB (1999). In a guide to carotenoid analysis in foods. ISLI press Washington pp 1-30.

Uluwaduge DI (2005). Investigation of palmyrah (*Borassus flabellifer* L) fruit pulp mediated inhibition of intestine glucose uptake and its toxicity. Ph.D. thesis, University of Sri Jayewardenepura.

Uluwaduge DI, Keerthi AAP, Senadheera SN and Jansz ER (2005). Studies on the natural hydrophobic binder of flabelliferins and their effect on some bioactivities. *Journal of the National Science Foundation.* **33(3),** In press.

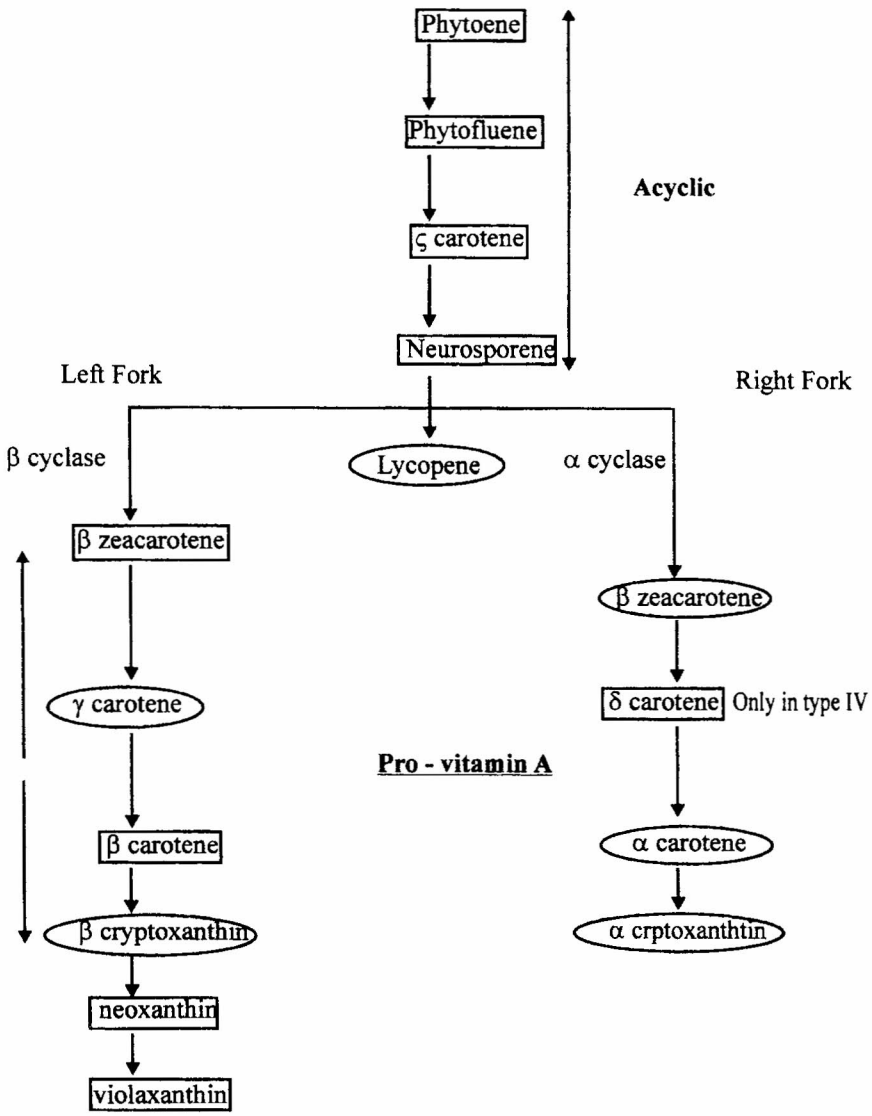

Fig. 1 : Carotenoid biosynthetic pathway adapted from (Rodriguez - Amaya, 1999)

Identified in Kalpitiya, Mannar PEP type studies

Not detected