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The carotenoids of the fruit pulp of palmyrah (*Borassus flabellifer*) from Hambantota

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Abstract

The Hambantota district has relatively new plantations of palmyrah (*Borassus flabellifer*) and appear to be the future for industrial products from this tree. The tree with morphological type IIB fruit dominates plantations (>90%). Specimens of this type were selected for carotenoid analysis. Total carotenoid content was relatively low; 15.5 to 35.4 mg.100g^{-1} on the basis of dry weight (DW). Carotenoids were dominated by those of the hydrocarbon type. No oxygenated carotenoids were detected in the petroleum ether and diethyl ether extracts. Highest contents of carotenoids were phytofluene; from 2.8 to 4.8 mg.100g^{-1} DW, phytoene from 5.7 to 10.3 mg.100g^{-1}DW and unidentified carotenoids I, II, III and IV from 0.7 to 1.9 mg.100g^{-1} DW, 0.7 to 3.4 mg.100g^{-1} DW, 4.7 to 16.4 mg.100g^{-1} DW and 0.6 to 1.1 mg.100g^{-1} DW, respectively. β-Carotene and ζ-carotene were present in traces. Retinol equivalent (RE) was negligible. As is usual for palmyrah, lycopene and the right fork of carotenoid biosynthesis pathway were absent.

Key words : Palmyrah, Fruit Pulp, *Borassus flabellifer*, Carotenoids

Introduction

The traditional cultivation of palmyrah (*Borassus flabellifer*) has been in the North, East and coastal North West of Sri Lanka. About three decades ago under United Nations Development Programme (UNDP) and later Palmyrah Development Board (PDB) sponsored projects, seeds were planted in the Hambantota district. This is now the most accessible source of this palm as the traditional districts of Jaffna, Mannar, Kalpitiya and the East have been debilitated due to war, substitute plantations and the use of this palm as a building material is not only for houses but also for bunkers. Further the Hambantota stand is more homogenous with morphological type of palmyrah fruit IIB dominating (>90%).

Past studies on carotenoids of palmyrah were carried out in Mannar where content and retinol equivalent (RE) was low (Pathberiya and Jansz, 2005) and in Kalpitiya where carotenoid content was 5-12 fold higher and RE 100^{-1}g fresh weight were 18, 155, 32, 21 and 33 for type I, IIA, IIB, III and IV respectively (Wijemanne *et al.*, 2006). In both cases lycopene and the right fork of carotenoid biosynthesis pathway were absent. A report on carotenoids from a bulk sample said to be obtained from the PDB originating from Hambantota was reported (Chandrika, 2004), but was not convincing as PDB did not at that time did not have bulk samples from Hambantota. The objective of this study was to assess for the first time the carotenoid content of palmyrah fruit pulp (PFP) from Hambantota to predict its potential use.

Materials and Methods

Samples:

Representative specimens (n=6) were collected from Hambantota district; Palatupana, Baragama, Mirijjawila, Magama and two locations of Hambantota town area. They represented trees from the sea shore, near a paddy field, in arid low country and hillocks. The fruits were collected on the day of fall on 3rd September 2006 and transported to the laboratory, if necessary, storing some fruits for less than 5 days at -20 °C. All small amounts of type I, IIA, III and IV fruits were available but all fruits collected were type IIB which was black with a large orange area at the distal end (Pathberiya and Jansz, 2005).

Extraction of fruit pulp:
This was done manually as described previously (Wijemanne *et al.*, 2006).

Carotenoid extraction:
The procedure of Rodriguez-Amaya (1999) was followed. β-Apo-8' carotenal (*trans*) was employed as internal standard.

Open column chromatography (OCC):
Separation of carotenoids was done by OCC using celite:MgO (1:1) as described previously (Rodriguez-Amaya, 1999).

Identification:
The procedure of Rodriguez-Amaya (1999) was used. That is λ_{max} of the three peaks, spectral fine structure, order/position in OCC, chemical tests, Tlc data, HPLC peak enrichment was used for identification.

High performances liquid chromatography (HPLC):
The method based on reverse phased chromatography as described previously was followed for the quantification (Priyadarshani and Jansz, 2006).

Chemical tests:
The epoxy-furanoid rearrangement, fuming HCl test and iodine catalysed *cis-trans* isomerisation test were conducted as described previously (Rodriguez-Amaya, 1999).

Thin layer chromatography (Tlc):
Tlc was done in 5% methanol in toluene on activated Tlc plate (Rodriguez-Amaya, 1999).

Care was taken to carry out all experimental procedures to away from light, heat and oxygen.

Moisture determination:
Triplicate samples were freeze dried to a constant weight.

Results

Moisture content of the six specimens was 71.2, 74.9, 73.4, 78.1, 79.5 and 76.6%. Table 1 shows the results of Tlc, OCC, HPLC and chemical tests. The data base did not contain data to identify four carotenoids. It was suspected that some were 5,8 epoxide but comparing the fuming HCl test with a 5,8 epoxide showed that this was not so.

Unidentified II and IV showed anomalous behavior on the iodine catalised *cis-trans* isomerisation test where bathochromic shift of 33.5 and 24.5 nm, respectively was observed. According to the shape and retention times of peaks on HPLC and Tlc R_f values all compounds were hydrocarbons.

Table 2 shows the profile of carotenoids with standard deviation (SD). SD was large, but ranges were much lower than what was reported previously (Pathberiya and Jansz, 2005; Wijemanne *et al.,* 2006). β-Carotene was found only in traces and no other pro-vitamin A carotenoids were present. RE was therefore negligible.

Table 1. Chemical characteristics of the carotenoids from palmyrah

OCC Fraction	R_f (Tlc)	HCl vapour test for 5,6 and 5,8 epoxides	Epoxy-furanoid rearrangement for5,6 epxide	Iodine catalysed *cis/trans* isomerisation	HPLC retention time (min)	Carotenoid
1	0.98	ND	ND	ND	42	Phytoene
2	0.98	ND	ND	ND	43	Phytofluene
3	0.98	Absent	Absent	A mixture of *cis/trans* isomers	40	β-Carotene
4	0.98	Absent	Absent	A mixture of *cis/trans* isomers	37	ζ-Carotene
5	0.98	Absent	Absent	A mixture of *cis/trans* isomers	32	Unidentified I
6	0.98	Absent	Absent	Anomalous behavior	29	Unidentified II
7	0.98	Absent	Absent	A mixture of *cis/trans* isomers	26	Unidentified III
8	0.98	Absent	Absent	Anomalous behavior	23	Unidentified IV

- ND-Not done

Table 2. Carotenoid profile and content of palmyrah from Hambantota district

Carotenoid	Concentration ($mg.100^{-1}g$, dry weight)
Phytoene	7.7±1.5
Phytofluene	3.8±0.9
Unidentified I	1.2±0.4
Unidentified II	1.8±1.0
Unidentified III	10.0±4.0
Unidentified IV	0.9±0.3
β-Carotene	Trace amounts
ζ-Carotene	Trace amounts

- Phytoene and phytofluene were detected at 286 and 348 nm, respectively
- Each sample was analysed in duplicate

Discussion

Total carotenoid content was low and RE negligible. Therefore these carotenoids have no pro-vitamin A value. Since carotenoids are hydrocarbons they are likely to be absorbable and therefore have potential for antioxidant capacity. They can also been used as a fat based food colour.

The anomalous behavior of unidentified II and IV on *cis-trans* is puzzling as such a shift can be explained only by the exposure of a hydroxy group. However the moiety linked must be hydrophorbic (HPLC and Tlc data) and become detached on treatment with iodine. This remains a mystery. The fact that hydroxy groups may be present as derivatised moieties appears possible as a water soluble carotenoid has been isolated from this source, on which work in proceeding.

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