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RESEARCH ARTICLE

The effect of maturity on carotenoids of *Lasia spinosa* stem and the effects of cooking on *in-vitro* bioaccessibility of carotenoids

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Abstract: In a continuing search for pro-vitamin A carotenoids, *Lasia spinosa* (Linn.) stem (Sinhala: *kohila ala*) was examined for the first time. Specimens purchased from the market showed a wide variation in carotenioid content (0.4 -1.8 μg.g^{-1} fresh weight (FW) and 0.9-7.2 μg.g^{-1} FW for α-carotene and β-carotene respectively) and retinol activity equivalent (RAE, 9.2-67.5 100g^{-1} FW). Percentage contribution to recommended daily allowance (RDA) of pre-school children by a 25 g portion of *Kohila* stem ranged from 1.1 to 8.4. Sampling from a home garden showed that there were two distinct leaf types {sagittate (A) and pinnatifid (B)} and a third plant a hybrid type (C). Sampling from the foliage end to terminal root end gave an index of maturity. Carotenoids were separated by Open Column Chromatography (OCC) and identified by chromatographic behavior, UV/Vis spectrophotometrically and chemical tests. The following were identified; α-carotene, β-carotene, β-carotene-5,6,5',6'diepoxide, 5,6,5',6'-diepoxy-5,8,5',8'-tetrahydro-β,β-carotene-3,3'-diol, *cis*-neoxanthin and unidentified carotenoids I, II, III and IV. There was a 14 and 2.3 fold higher content of pro-vitamin A carotenoids in the edible part of the most mature stems of type A and B respectively compared to the most immature stems. Total pro-vitamin A carotenoid content of the most mature part of the stems were 261.4 and 234.6 μg.100g^{-1} FW (RAE was 19.5 and 15.7 100g^{-1} FW) for type A and B respectively. *In-vitro* bioaccessibility showed that preparation of curry with coconut milk releases α-carotene and β-carotene in the amounts of 1.1±0.41 μg.g^{-1} (15.1%) {(Dry weight (DW)} and 5.8±2.1 μg.g^{-1} (18.3%) DW respectively. On frying, the values were 0.4±0.11 μg.g^{-1} (10.8%) DW and 1.6±0.48 μg.g^{-1} (12.1%) DW for α-carotene and β-carotene respectively as determined by high performance liquid chromatography (HPLC). Frying and making curry are the two traditional methods of cooking this vegetable in Sri Lanka.

Key words: Carotenoids, effect of maturity, *in-vitro* bioaccessibility, *Lasia spinosa*, retinol activity equivalent.

INTRODUCTION

In the course of a search for unconventional sources of pro-vitamin A carotenoids, it was decided to analyse the edible part of the trailing stem of *Lasia spinosa* (Linn.) (Sinhala: *kohila ala*) which is consumed for its reputation as a source of dietary fibre. Its carotenoid content has not been studied earlier. It is a cheap vegetable and therefore is within the budget of low-income groups, in which vitamin A deficiency is most prevalent.[1] Vitamin A deficiency together with iron and iodine deficiency are the three most prevalent deficiencies in Sri Lanka.[1] Considerable studies have been carried out on carotenoids of fruits and vegetables in Asia. Some of the pro-vitamin A carotenoids are as follows. In Sri Lanka, yellow fleshed papaw [(β-carotene, 1.4±0.4 μg.g^{-1} dry weight (DW)][2], jackfruit (β-carotene, 5.6±0.3 μg.g^{-1} DW and α-carotene, 1.7±0.1 μg.g^{-1} DW)[3], fresh blanched green leafy vegetables (β-carotene ranged from 149 to 565 μg.g^{-1} DW)[4] have been studied. In Thailand ripe mango [β-carotene, 0.785 mg.100 g^{-1} fresh weight(FW)], pumpkin (β-carotene, 0.780 mg.100 g^{-1} FW)[5] and leafy vegetables (β-carotene ranged from 12 to 50 μg.g^{-1} edible portion)[6], have been studied. Indian data include leafy vegetables where β-carotene ranged from 30 to 197 μg.g^{-1} edible portion[6] and carrot (β-carotene, 65±15 μg.g^{-1} edible portion).[6] A Bangladesh report on leafy vegetables gives values of 54-100 μg.g^{-1} edible portion)[6]. Considerable work has also been reported in Malaysia[6] and Indonesia[7] too. The objectives of the study were: (a) determination of Retinol Activity Equivalent (RAE) of the plant material purchased from the market (b) determination of the carotenoid profile and RAE of material collected from a home garden (c) attempt to determine why the profiles in above (b) vary markedly (d) determination of the *in-vitro* bioaccessibility after traditional cooking and (e) indicate to consumer how to select the plant material for cooking if RAE is the major concern.

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METHODS AND MATERIALS

Raw material: Plant material (trailing stem) (n=6) was purchased at random from vendor stalls (250 g x 6) in and around the Colombo suburbs. These samples were not chosen according to the variety, maturity, climatic conditions, soil, etc., in order to determine variations in carotenoid content in different samples. Samples were analysed in duplicate. Plant material was also harvested from a home garden for the maturity study where it had been grown for 12 years for leaf shoots and not harvested for the trailing stem (Sinhala: *kohila ala*). It was noticed that there were three types of plants depending on leaf morphology. Type A – sagittate. Type B – pinnatifid (6-8) up to about halfway from edge to center.[8] Type C had both types of leaves. Type A and B were selected for the maturity study. Type A and B had been grown in plots about 5 m apart. Soil and climatic conditions were therefore similar. The plot sizes were approximately 2 x 4 m each.

The trailing stem had adventitious roots and the mode of growth gave a gradation in maturity of the stem; the end closest to the point of growth from the soil being most mature and the end with leaves attached, least mature. For the maturity study, the stem was removed in its entirety and both types A and B were subdivided into pieces of 15-17 cm in length. Type A had a mean diameter of about 4 cm while type B was more thorny with a mean diameter of about 2.5 cm. The stems were shaved with a knife to remove the thorny exterior and the material was transported to the laboratory and stored at -20 °C. Analysis for the effect of maturity was completed within 11 days. Just before sampling for analysis, the edible inner core (edible portion 2-3 cm) was separated and cut into pieces. Depending on maturity the edible part (core) was light yellow to pale orange in both types A and B.

Sample preparation: The stems were peeled and cut into pieces. They were then grated with a simple grater and made homogenous manually with a spoon.

Extraction of carotenoids: The homogenized sub-samples of 50 g was used for Open Column Chromatography (OCC) for the maturity study. For vendor samples 10g was used for High Performance Liquid Chromatoghaphy (HPLC)). Samples were ground with celite. These were extracted immediately. Carotenoids were extracted with cold acetone followed by petroleum ether (boiling point 40-60 °C)[9], evaporated by nitrogen flushing and stored at -20 °C under nitrogen. Separation was carried out the next day.

Separation of carotenoids by OCC: This was performed according to the procedure of Rodriguez-Amaya[9] using a celite: MgO (1:1) column activated at 110 °C for 2 h and a solvent gradient of petroleum ether, diethyl ether and acetone was used as described previously.[9]

Identification of carotenoids: The following tests were performed as described previously[9].

- Scanning UV/Vis spectrophotometry. λ_{max} For three peaks and spectral fine structure using a Shimadzu model UV-1601 double beam spectrophotometer.
- Exposure of a thin layer plate to HCl vapour in the Thin Layer Chromatography (TLC) tank (carotenoid spots on SiO_2 gel G_{60} plates, Merck) to identify epoxy carotenoids.
- Epoxy-furanoid rearrangement in ethanol was monitored spectrophotometrically. A hypsochromic shift of 10-12 and 20-25 nm was observed with 0.1 HCl for mono and di 5,6 epoxide respectively.
- Iodine-catalyzed *cis-trans* isomerization was monitored spectrophotometrically. Hypsochromic and bathochromic shifts of 3-5 nm was observed for *trans* and *cis* respectively with the addition of iodine in petroleum ether, using an iodine containing blank, after isomerisation.
- Chromatographic behavior TLC (plastic backed SiO_2 gel G_{60} plates, Merck).
- HPLC retention time.

Cooking procedures: Sample No:3 (given in Table 1) was selected for cooking purposes.

Preparation of "kohila curry": Stems (250 g) were peeled, washed and the edible part cut into small pieces (4 x 2 x 25 mm). This was cooked for 20 min with 400 mL of thick and thin coconut milk.

Preparation of "fried kohila": Stems (250 g) were peeled, washed and the edible part cut into pieces of 4 x 2 x 25 mm size. This was fried for 30 s in coconut oil.

Determination of in-vitro accessible a-carotene and b-carotene: This was performed according to Hedren *et al.*[10] Samples were prepared as pieces similar in sizes to chewed items. The digestion was initiated with the addition of porcine pepsin solution at pH 2. The sample was incubated at 37 °C in a shaking water bath for 1 h. The sample was then treated with

pancreatin and bile salt solutions at pH 7.5 and incubated for a further 30 min. After centrifugation the aqueous fraction was transferred to petroleum ether and quantified by HPLC. b-apo-8'-carotenal (*trans*) was used as an internal standard.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) conditions: Waters separation model 515 with Waters UV/Visible detector 2487. Waters spherisorb ODS_2 5 µm, monomeric C_{18} column was used. The column was made of stainless steel 4.6 x 250 mm in size. The mobile phase was acetonitrile: methanol : tetrahydrofuran 58 : 35 : 7. The injections were done with a 20 µl loop and the flow rate was 1 mL/min.

Precautions were taken to minimize the loss of carotenoids during the analysis. All experiments were conducted under dim light, samples were stored under nitrogen gas at -20 °C in amber bottles and experiments were completed within the shortest possible time. All analyses were carried out on the day following carotenoid extraction.

Quantification: Carotenoid content of the randomly collected samples from vendor stalls were quantified by HPLC in duplicate and quantification for the carotenoid content in maturity study was performed by UV/Vis spectrophotometry using absorption coefficients and the absorbance at the λ_{max}.[9,11]

Retinol Activity Equivalent (RAE) and Retinol Equivalent (RE): RAE was calculated according to the conversion factors, 12 µg of β-carotene and 24 µg of α-carotene giving rise to 1 µg of retinol (1RAE)[12] and RE calculated according to the conversion factors, 6 µg of β-carotene and 12 µg of α-carotene giving rise to 1 µg of retinol (1 RE).[13]

Determination of moisture content: This was determined by freeze drying to constant weight.

Determination of fat content in cooked samples: This was done by Soxhlet extraction[14] using petroleum ether (boiling point 60-80 °C) as the solvent.

RESULTS

Random sampling

Table 1 shows the carotenoid content of randomly selected samples from vendor stalls. Since carotenoid content is subject to many variables such as genetic composition, environmental conditions and maturity etc., as expected, a standard deviation could not be calculated for the six samples. a-Carotene and b-carotene content as determined by HPLC varied markedly between 0.4 -1.8 $mg.g^{-1}$ FW and 0.9-7.2 $mg.g^{-1}$ FW respectively.

Carotenoid profile

The effects of maturity on type A and B determined by OCC are shown in Table 2. The carotenoids identified in both type A and B were α-carotene, β-carotene,β-carotene-5,6,5',6'diepoxide, 5,6,5',6'-diepoxy-5,8,5',8'-tetrahydro-β,β-carotene-3,3'-diol, *cis*-neoxanthin and unidentified I. Unidentified II and III were found only in type A and unidentified IV was found only in type B. All isomers were *trans* except β-carotene-5,6,5',6'diepoxide and neoxanthin. Unidentified carotenoids were as follows. Unidentified I and IV were diepoxy, *trans* carotenoids, unidentified II was a 5,8 epoxy, *trans* carotenoid and unidentified III was a mono epoxy, *trans* carotenoid for which no spectral data were available in databases.

Table 1: Carotenoid content in randomly selected samples of *Lasia spinosa* stem (edible part) from the vendor stalls

Sample No:	α-carotene $\mu g.g^{-1}$ FW	β-carotene $\mu g.g^{-1}$ FW	Retinol Activity Equivalent(RAE)$100g^{-1}$ FW
1	1.5	4.1	40.4
2	1.2	4.1	39.2
3	1.8	7.2	67.5
4	0.7	1.8	17.9
5	0.4	0.9	9.2
6	0.9	2.1	21.3 ·

· Quantification by HPLC

· Each sample was analysed in duplicate

· RAE was calculated as 12 µg of β-carotene =1 µg retinol and 24 µg of α-carotene =1 µg retinol

Table 2: Effect of maturity on carotenoids of edible part of stem

Carotenoid	Maturity stages									
	1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B
α-carotene	8.4	44.8	16.0	46.2	45.8	51.8	48.4	52.4	53.8	92.4
β-carotene	10.4	56.2	17.0	59.3	181.2	83.2	191.4	132.4	207.6	142.2
β-carotene 5,6,5',6' di-epoxide	122.0	34.4	10.0	60.2	24.8	15.6	53.8	-	58.0	-
5,6,5',6' di-epoxy-5,8,5',8' tetrahydro – β–β-carotene 3-3'-diol	-	-	-	132.6	31.6	66.4	19.0	65.0	35.2	45.2
Cis-neoxanthin	-	-	-	-	3.2	-	-	37.6	-	34.6
Unidentified I	-	75.2	-	-	7.2	-	-	-	-	-
Unidentified II	-	-	-	-	25.6	-	-	-	-	-
Unidentified III	-	-	-	-	11.6	-	-	-	-	-
Unidentified IV	-	-	-	-	-	12.4	-	-	-	-
Retinol activity equivalent $100g^{-1}$ FW	1.2	6.6	2 .1	6.9	17.0	9.1	18.0	13.2	19.5	15.7
Retinol activity equivalent $100g^{-1}$ DW	14.1	77.6	19.8	109.5	144.1	101.1	150.0	100.8	158.5	109.8

* A-Type A plant
* B-Type B plant

- Quantification by OCC and UV/Vis spectrophotometry
- Carotenoid content is given as $\mu g.100g^{-1}$ FW
- 1 refers to stem at leaf tip end and 5 refers to stem at main root end
- 1 ⟶ 5 Increase in maturity
- RAE was calculated as 12 μg of β-carotene =1 μg retinol and 24 μg of α-carotene =1 μg retinol

Effect of maturity

Table 2 shows the increase in pro-vitamin A carotenoid content with maturity. Type A and type B increased about 14 fold and 2.3 fold respectively with maturity. There was also a change in profiles. RE also increased with maturity (Figure I).

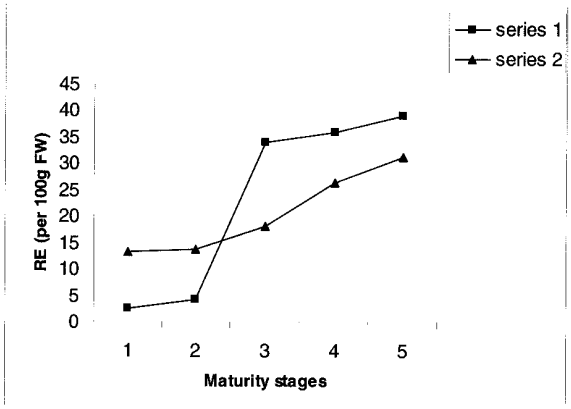

Figure 1: Change in retinol equivalent with maturity

- Series 1– type A
- Series 2 – type B

In-vitro bioaccessibility

In-vitro bioaccessibility of both α-carotene and β-carotene was low with both cooking procedures. Currying with coconut milk gave a better bioaccessibility (Table 3).

DISCUSSION

Studies on carotenoids in *Lasia spinosa* (Linn.) stem (Sinhala: *kohila ala*) has not been reported before. Random samples collected from the market (Table 1) showed major variations. Further studies showed that this could be due to variety and maturity variations. Since the plant can grow both on sandy soil and can tolerate water logging, climatic and soil factors can also play a part in the variation.

Stem of type A and type B showed that pro-vitamin A carotenoid content increases from the stem tip to the terminal root attachment (termed maturity) by 14 and 2.3 fold in type A and B respectively. Epoxides were present in each type but β-carotene was dominant at maturity.

RE was contributed to only by the pro-vitamin A carotenoids α-carotene and β-carotene. RE in the most mature stem was higher than in fruits such as yellow-fleshed papaw (1516 kg^{-1} DW)[2], palmyrah (159.1 100 g^{-1} DW)[11], jackfruit (141.6 100 g^{-1} DW)[3], beli (trace amount)[11] and lavalu (68 100 g^{-1} DW)[15] and lower than carrot, pumpkin, squash, sweet potato, based on fresh weight Priyadarshani, (2005) unpublished results. Relavant data of foreign studies are given in the introduction. The carotenoid composition also indicated that anti-oxidant potential would be high as most of the carotenoids have conjugated double bonds. The percentage contribution to recommended daily allowance of pre-school children is set to 400RE[12] by a 25 g portion is from 1.1 to 8.4.

The consumer, if interested in RE, could select the vegetable depending on the intensity of colour in the inner core (edible part of the stem), which is easily visible at the cut end. *In-vitro* digestibility studies indicated that currying with coconut milk gave a better release of carotenoid, probably due to the softer matrix. Percentage *in-vitro* accessibility values were comparable with that of some green leafy vegetables.[4,11]

CONCLUSION

Maturity and variety can affect the composition and the content of carotenoids of *Lasia spinosa* (Linn.) stem (Sinhala: *kohila ala*). The mature stem is a good source of pro-vitamin

Table 3: *In-vitro* bioaccessible pro-vitamin A carotenoids in kohila preparations

Kohila preparation	Fat%	Moisture%	*In-vitro* bioaccessibility $\mu g.g^{-1}$ DW Mean±SD[a]	
			α-carotene	β-carotene
Currying	14.1	73.6	1.1±0.41 (15.1%)	5.8±2.1 (18.3%)
Frying	48.3	6.2	0.4±0.11 (10.8%)	1.6±0.48 (12.1%)

[a]Mean±SD in 6 samples in duplicate

A carotenoids. Preparation of a curry with coconut milk is a better method of cooking the stems compared to deep frying in respect of *in-vitro* bioaccessibility.

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