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SHORT COMMUNICATION

Studies on the carotenoids of jakfruit *(Artocarpus heterophyllus* Lam.) from Matale and Kurunegala Districts

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Abstract: The kernels of ripe jakfruit (*Artocarpus heterophyllus* Lam.) (Sinhala: *waraka*) is widely consumed in Sri Lanka. Specimens were collected from Kurunegala and Matale Districts. The yellow fruit kernels were found to contain carotenoids. The variations from specimen to specimen were manifest not only in total content but also in components. β-Carotene varied from traces to 50, α-carotene from traces to 20.5, lutein from 2.7 to 221.5 µg. 100^{-1}g of dry weight (DW). A range of contents was also shown in unidentified fractions. Retinol equivalent (RE) and retinol activity equivalent (RAE) varied from traces to 10 and 5/100g DW, respectively. The major compounds common to all samples in the saponified petroleum ether extract were lutein and unidentified fraction II as determined by Open Column Chromatography (OCC), Reverse Phase High Performance Liquid Chromatography (RP-HPLC), Thin Layer Chromatography (TLC) and chemical tests. A carboxylic acid carotenoid was extracted into the alkaline water layer after saponification and tentatively identified by its chemical characteristics and spectrum as crocetin. *In vitro* bioaccessibility was low (~ 8%) probably due to the rubbery texture of the kernel.

Key words: *Artocarpus heterophyllus*, carotenoids, *in vitro* bioaccessibility, jakfruit, retinol equivalent

INTRODUCTION

Different pro-vitamin A sources have the potential to alleviate to varying extents, the deficiency of vitamin A in Sri Lanka[1]. Carotenoids of ripe jakfruit (*Artocarpus heterophyllus*) kernel have been studied before and reportedly had a retinol equivalent (RE) of 141.6/100g dry weight (DW)[2]. This study also showed that the coefficients of variation of the components ranged from 4.8 to 9.7%[2], which appears to be low. Crocetin has been identified in the saponified petroleum ether extract. This is not possible as it is a dicarboxylic acid. The R_f value of crocetin in two reports of the same analysis has been given as 0.33[2] and 0[3]. There was therefore ample justification for ripe jakfruit kernel to be studied in more detail.

The main objectives of the study are to determine carotenoid profile of ripe jakfruit kernel collected from the Kurunegala and Matale Districts and estimating the *in vitro* bioaccesibility of ripe jakfruit kernel taking cognizance of the fact that its inherent texture could be an impediment to disintegration in the gastrointestinal tract. Minor objectives included: a study of crocetin, and determining the effect of heat (121 °C) on carotenoids of the kernel.

METHODS AND MATERIALS

Raw material: Mature jakfruits (*A. heterophyllus* Lam) (variety:*waraka*) were collected from 1.Dambagahamula, 2.Dembawa, 3.Wegodapola, 4.Kanandana, 5.Maussawa and 6.Omaragolla of Kurunegala and Matale Districts. The fruits showed the normal variations in shape, size and texture of pericarp. On ripening (1-5 d), as judged by the softening of the pericarp and characteristic odour and colour, the fruit was cut open and the bulbs were removed. The number of bulbs varied from 55 to 162. Specimen No: 5 had thin, long, light coloured bulbs. 20 bulbs were randomly selected, de-seeded and cut into pieces of about 5×5 mm and mixed thoroughly. From this homogeneous mixture 10-20 g (depending on the colour intensity) were used for the extraction of carotenoids. In this study one fruit was analysed from each location.

Extraction of carotenoids was done as described previously[5]. β-Apo-8'-carotenal (*trans*) was employed as an internal standard. As the water layer showed a yellow colour on partition into petroleum ether (BP, 40-60 °C),

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this step was repeated with increased concentration of diethyl ether (10%). The extract was saponified using 10% methanolic KOH[4] because fatty acid esters of carotenoids were present as indicated by Open Column Chromatography (OCC) and High Performance Liquid Chromatography (HPLC) of the unsaponified sample. The sample was concentrated by nitrogen flushing and subject to OCC. The alkaline water extract of the saponification washing was used for isolation of crocetin. OCC, identification and HPLC were carried out as previously described[5].

In-vitro bioaccessibility: This was carried out as described previously[6] with specimen No:1 (given in Table 1). Jakfruit kernel was cut up into pieces of 9×9 mm (as determined by a panel masticating jakfruit) and pounded to mimic action by teeth. In a further procedure each member of the panel masticated and re-gurgitated a jakfruit kernel for *in vitro* bioaccessibility studies.

Isolation and methylation of crocetin: The water extract from saponification was adjusted to pH 2 and extracted with petroleum ether. The spectrum of the extract was scanned with a double beam spectrophotometer. As the extract appeared to be impure with ultraviolet absorbing impurities, it was subjected to OCC and TLC as in the case of petroleum ether extract. The crocetin in methanol was dried with anhydrous Na_2SO_4 for 24 h and two drops of concentrated H_2SO_4 was added and kept at room temperature (27 ^{0}C) for 24 h. TLC was carried out using the solvent system mentioned above. Procedures for carotenoid extraction and identification were carried out in dim light and away from oxygen (except TLC). Storage was done under nitrogen at -20 °C. Solvent evaporation was done by nitrogen flushing.

Effect of autoclaving: Jakfruit kernel (specimen No:1 which contained the highest pro-vitamin A carotenoid content) was autoclaved (STURDY SA – 232) in a sealed container for 20min at 1.3 kg/cm^2 (121°C). Extraction and HPLC analysis were carried out as described previously.

Retinol equivalent (RE) and retinol activity equivalent (RAE): RE was calculated according to the conversion factors; 6 µg of β-carotene and 12 µg of α-carotene to give 1µg of retinol (1 RE)[7] and RAE was calculated according to the conversion factors; 12 µg of β-carotene and 24 µg of α-carotene to give 1µg of retinol (1 RAE)[8].

Determination of moisture content: Moisture determination was done by freeze-drying (1g) in triplicate to constant weight.

RESULTS

Carotenoid composition and retinol equivalent

The carotenoids identified from ripe jakfruit kernels were β-carotene, α-carotene, lutein, α-zeacarotene, unidentified I (*trans* form), II (*cis* and *trans* forms), III (*cis* form) and IV (*trans* form). In most specimens lutein was the main contributor to the total carotenoid content. Among the unidentified carotenoids, all were epoxy carotenoids except for unidentified fraction I and according to chromatographic properties they were polar carotenoids. α-Zeacarotene was detected from OCC but as its concentration was very low it was not detected by HPLC at 450 nm (λ_{max} of α-zeacarotene is 421 nm). All the carotenoids showed a marked variation in their content from specimen to specimen. Unidentified fractions III and IV were found only in specimen No: 5 and 6 relatively in large amounts. Table 1 shows the vast variation in content, profile, RE and RAE.

In vitro bioaccessibility

In vitro bioaccesssibility is very low (8%). On inclusion of *in vivo* mastication prior to the *in vitro* digestion, bioaccessibility ranged from 12% to 18%. This may be due to differences in the mastication process among individuals and therefore has to be repeated using a large number of individuals.

Effect of heat

On autoclaving, the carotenoid content decreased to trace amounts with some changes to the carotenoid profile. Extensive splitting of the β-carotene peak indicated considerable isomerisation. After autoclaving a new unidentified carotenoid was released into the water medium. Characteristics of this carotenoid were as follows; λ_{max} = 420, 440 and 468, di-epoxy, *trans* with the R_f values of 0.21 on an activated TLC plate.

Crocetin

As crocetin is a dicarboxylic acid it is expected in the water layer after saponification with methanolic KOH. After acidification to pH 2, the yellow coloured extact gave a spectrum with petroleum ether at 398, 420 and 442 nm (λ_{max} for all *trans* form 400, 422, 450 nm in petroleum ether)[4] with spectral fine structure of 50 and R_f value of 0.09 on an activated TLC plate. On attempted methylation with H_2SO_4 the yellow colour was lost as H_2SO_4 reacts with double bonds conjugated to (COOH) carbonyl groups and the solution was colourless and no carotenoid peaks were observed in the *uv/vis* spectrum.

Table 1: Carotenoid content RE and RAE of random samples of jackfruit

Carotenoid	Specimen number					
(μg.100g^{-1} Dry weight)	1	2	3	4	5	6
β-Carotene	50.0	43.6	48.0	22.9	Tr**	12.8
α-Carotene	20.5	15.8	18.6	4.3	Tr**	Tr**
Lutein	51.2	131.2	221.5	62.1	2.7	7.2
Unidentified I	20.9	33.7	23.2	Tr**	Tr**	Tr**
Unidentified II – *trans*	8.3	45.5	36.2	23.6	13.7	27.8
Unidentified II – *cis*	15.0	51.0	59.9	26.4	15.8	11.1
Unidentified III	ND*	ND*	Tr**	ND*	32.2	15.6
Unidentified IV	Tr**	Tr**	Tr**	Tr**	32.8	26.7
α-Zeacarotene	ND*	ND*	ND*	ND*	ND*	ND*
RE 100^{-1} g Dry weight	10.0	8.6	9.6	4.2	Tr**	2.1
RAE 100^{-1} g Dry weight	5.0	4.3	4.8	2.1	Tr**	1.1

* ND, Not detected (α-zeacarotene was not detected at 450 nm)

** Tr, Trace amount

Locations of the specimens: Specimen No: 1.Dambagahamula, 2.Dembawa, 3.Wegodapola, 4.Kanandana, 5.Maussawa, 6.Omaragolla; Quantification was done by HPLC; Each sample was analysed in duplicate

RE was calculated from 6 μg of β-carotene = 1 μg retinol and 12 μg of α-carotene = 1 μg retinol and RAE was calculated from 12 μg of β-carotene = 1 μg retinol and 24 μg of α-carotene = 1 μg retinal

DISCUSSION

The results concerning the carotenoid profiles obtained in this study are different from those obtained in the previous study [2,3]. The carotenoid profile given in the previous study includes β-carotene, α-carotene, β-carotene-5, 6-epoxide, α-zeacarotene, β-zeacarotene and crocetin. The identification of β-carotene-5, 6-epoxide is doubtful because R_f given by that carotenoid was 0 in 6% methanol in toluene solvent system[2]. Variations observed in the current study are very large and it is concluded that previous sampling [2,3] may be biased as there were very low coefficients of variation (4.8 to 9.7%). In this study RE is seen to vary markedly from trace amounts to 10/100 g DW making it impossible to predict the percentage contribution to recommended daily allowance (RDA) of vitamin A per portion of jakfruit. On comparison with other fruits and vegetables of Sri Lanka eg. RE values for different preparation methods of carrot, pumpkin, squash and sweet potato based on *in-vitro* accessible pro-vitamin A carotenoids were 31.3 to186.6, 52.7 to 87.3, 4.6 and 20.9 to 43.1 from a 10 g portion vegetable dish[9] and for papaw from 344.8 to 2410/100g DW[10]. Therefore jakfriut does not appear to be a consistent contributor to RDA of vitamin A. This contribution would be even less as the matrix effect of jakfruit kernel and tendency for some individuals not to masticate this fruit kernel completely in the mouth gives very low bioaccessibility.

The presence of crocetin in the water layer of saponification is expected, as it will be soluble in alkaline water and not in petroleum ether. The identification of crocetin is interesting however a spectrum is not available for comparison. It is felt that this method of isolation used can be utilized for other fruits. The crocetin structure due to its conjugated double bonds system is favorable for high antioxidant capacity, but no data is available to determine if it is absorbed. The slight difference in spectrum from that of the database is probably due to extensive isomerisation in alkaline medium.

Autoclaving changed the carotenoid profile and reduced the pro-vitamin A carotenoid contents to only trace quantities. As carotenoids are highly sensitive to high temperatures, canned products will also not be a significant contributor to the RDA of vitamin A. This study shows that it is not possible to predict contribution of pro-vitamin A to recommended daily allowance. Further, due to the rubbery matrix *in vitro* bioaccessibility is very low.

Acknowledgement

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