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STUDIES ON CAROTENOID CONTENT OF SELECTED FRUITS AND NON-LEAFY VEGETABLES OF SRI LANKA AND THEIR BIOAVAILABILITY AND METABOLITES

BY

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DECLARATION BY CANDIDATE

The work described in this thesis was carried out by me under the supervision of Prof. E.R. Jansz and Prof. H. Peiris (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and a report on this has not been submitted in whole or in part to any University or any other Institution for another Degree/Diploma.

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DECLARATION BY SUPERVISORS

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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ABBREVIATIONS

A	Absorbance
$A^{1\%}_{1cm}$	Absorption coefficient for 1% solution
AC	Acetone
BHA	Butylated hydroxyanisole
BHT	2,6-Di-tert-butyl-4 methylphenol
CV	Coefficient of variation
CRBP	Cellular retinoid-binding protein
DEE	Di-ethyl ether
DMAPP	Dimethylallyl diphosphate
DW	Dry weight
e^-	Electron
FAO	Food and Agriculture Organisation
FW	Fresh weight
GGPP	Geranylgeranyl diphosphate
GGPS	Geranylgeranyl diphosphate synthase
HDL	High density lipoproteins
Holo-RBP	Holo-retinol binding protein
HPLC	High performance liquid chromatography
IPI	Isopentenyl diphosphate isomerase
IPP	Isopentenyl diphosphate
IVACG	International Vitamin A Consultative Group
λ_{max}	Maximum wavelength of absorption
LDL	Low-density lipoproteins

MPLC	Medium pressure liquid chromatography
MRI	Medical Research Institute
$^3O_2$	Triplet state of oxygen
$^1O_2$	Singlet oxygen
OCC	Open column chromatography
PDS	Phytoene desaturase
PE	Petroleum ether
PFP	Palmyrah fruit pulp
PSY	Phytoene synthase
$R^{\bullet}$	Free radical
RAE	Retinol activity equivalent
RDA	Recommended daily allowance
RE	Retinol equivalent
R_f	Retardation factor
RPE	Retinal pigment epithelium
SD	Standard deviation
TBARS	Thiobarbituric acid reactive substances
TEAC	Trolox equivalent antioxidant capacity
Tlc	Thin layer chromatography
TSH	Thyroid stimulating hormone
VLDL	Very low-density lipoproteins
WHO	World Health Organization

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Athapaththu Mudiyanselage Buddhika Priyadarshani

ABSTRACT

Carotenoids are important as precursors of vitamin A as well as for prevention of cancers, coronary heart diseases, age-related macular degeneration, cataract etc. The objective of this study was to determine the carotenoids of some non-leafy vegetables and fruits of Sri Lanka and the effect of over-feeding of carotenoid rich diets. Carotenoid analysis procedure included specimen collection, extraction, identification and quantification. The estimation of carotenoids in selected non-leafy vegetables and fruits was carried out having made some improvements to the existing procedure with regard to sampling, identification and quantification. The mean contents of β- and α-carotenes in *Daucus carota* (carrot) were 43.8±5.6 and 20.5±1.7 $\mu g.g^{-1}$ fresh weight (FW), respectively whereas in *Cucurbita moschata* (squash) it was 6.0±0.8 and 5.1±1.1 $\mu g.g^{-1}$ FW, respectively. β-Carotene and α-carotene contents in the most common *Cucurbita maxima* (pumpkin) variety 'Arjuna' was 50.9±5.7 and 27.3±3.1 $\mu g.g^{-1}$ FW, respectively. The highest β-carotene content reported from *Ipomoea batatas* (sweet potato) was 59.0±6.2 $\mu g.g^{-1}$ FW. Stems of *Lasia spinosa* (kohila ala) purchased from the markets showed a wide variation in carotenoid content (0.9-7.2 and 0.4-1.8 $\mu g.g^{-1}$ FW for β- and α-carotenes, respectively). The fruits *Carica papaya* (papaw) and *Artocarpus heterophyllus* (jakfruit) which have no agricultural varieties showed high heterogeneity. Therefore carotenoid content varied markedly from specimen to specimen and standard

deviation (SD) could not be calculated. In *Carica papaya* retinol activity equivalent (RAE) ranged from 25.0 to 156.7 .100g^{-1} FW. In *Artocarpus heterophyllus* RAE was only in traces on the basis of fresh weight. *Borassus flabellifer* (palmyrah) fruit type IIB from Hambantota gave large SDs for carotenoids. Its RAE was negligible. *In-vitro* bioaccessibility of β-carotene was high in *Daucus carota* curry (74.7%), boiled and homogenized *Daucus carota* (73.9%) and *Carica papaya* (50.5%) but low in all other types of cooked foods due to matrix effect.

Stems of *Lasia spinosa* showed 14 and 2.3 fold increase in total pro-vitamin A carotenoid content with maturity for type A (sagittate) and B (pinnatifid) plants respectively. Carotenogenesis was found to occur in *Ipomoea batatas* under the open and gunny bag storage conditions at ambient temperature. β-Carotene content increased 2.2 and 2.3 fold after twelve days storage for open and bagged samples, respectively.

A water-soluble carotenoid was isolated from the fruit pulp of *Borassus flabellifer* and the solubility was due to glycosylation mainly by glucose and some rhamnose. The sugars were released by action of naringinase. This finding provides a basis for making a yellow natural food colour.

Hypercarotenemia was studied in eight patients with a history of frequent intake of excess of *Daucus carota*, *Carica papaya* and *Cucurbita maxima* over a period of time. This study was important as high carotenoid food intake appears to be sometimes high in Sri Lanka giving rise to this problem. Six of the patients had typical hypercarotenemia. Two atypical cases were detected. Control subjects showed no carotenoids and its metabolites in serum. The results indicate that boiled and homogenised *Daucus carota* with high β-carotene and high *in-vitro* bioaccessibility was the main cause for hypercarotenemia in Sri Lanka.