DOI: 10.31357/fmesphd.2005.00116

CONTENT, BIOAVAILABILITY, BIOCONVERSION AND ANTIOXIDANT ACTIVITIES OF CAROTENOIDS IN SOME SRI LANKAN FRUITS AND GREEN LEAFY VEGETABLES

By

UDUWALAGALA GAMAGE CHANDRIKA

Ph. D 2004

CONTENT, BIOAVAILABILITY, BIOCONVERSION AND ANTIOXIDANT ACTIVITIES OF CAROTENOIDS IN SOME SRI LANKAN FRUITS AND GREEN LEAFY VEGETABLES

BY

UDUMALAGALA GAMAGE CHANDRIKA

B.Sc. Hons., M.Phil., M.IChem., C.Chem.

Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Doctor of Philosophy in Biochemistry on 30th June 2004.

DECLARATION BY THE CANDIDATE

The work described in this thesis was carried out by me under the supervision of Professor E. R. Jansz (Senior Professor, Head of the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Professor N. D. Warnasuriya (Dean of the 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.

Chandrika Gamage ------------------------------------

Udumalagala Gamage Chandrika

07/02/05 ----------------

Date

DECLARATION OF THE 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.

Professor E. R. Jansz (Supervisor)	Professor N. D. Warnasuriya (Supervisor)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

AC	Acetone
Acetyl-CoA	Acetyl coenzyme A
AUC	Area under the curve
BHT	Butylated hydroxytoluene
CAR	Carotenoids
CRBP	Cellular Retinal-Binding Protein
DMAD	Dimethylallyl diphosphate
DW	Dry weight
EBP	Epididymal-Binding Protein
EE	Diethyl ether
FAO	Food and Agriculture Organisation
FFQ	Food Frequency Questionnaire
GDP	Geranyl diphosphate
GGDP	Geranylgenanyl diphosphate
GLV	Green leafy vegetables
HIV	Human Immuno-Deficiency Virus
HKI	Helen Keller International
HPLC	High Performances Liquid Chromatography
ICR	Institute of Cancer Research
IDP	Isopentenyl diphosphate
IOM	Institute of Medicine
IPP	Isopentenyl pyro-phosphate
IRBP	Interphotoreceptor Retinal-Binding Protein

LDL	Low-density lipoproteins
LRAT	Retinol acyltransferase
LRAT	Lecithin Retinal Acyltransferase.
MDA	Malonaldehyde
MPLC	Medium Pressure Liquid Chromatography
MRI	Medical Research Institute
ND	Not detected
OCC	Open Column Chromatography
OMNI	Opportunities for Micronutrient Intervention
PDA	Photodiode Array
PE	Petroleum Ether
PFP	Palmyrah Fruit Pulp
RA	All *trans* retinoic acid
RBP	Retinol Binding Protein
RDA	Recommended daily allowance
RDR	Relative Dose-Response Tests
RE	Retinol equivalent
RGR	Retinol G protein coupled receptor
RPE	Retinol Pigment Epithelium
RP-HPLC	Reverse Phase High Performances Liquid Chromatography
SD	Standard deviation
SEM	Standard Error of Mean
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances

TCA	Trichloroacetic acid
TEA	Triethylamine
TLC	Thin Layer Chromatography
UNICEF	United Nations Children's Fund
UV	Ultra Violet
VAD	Vitamin A deficiency
VLDL	Very low-density lipoproteins
WHO	World Health Organization

ACKNOWLEDGEMENTS

I wish to thank all those who contributed in different ways to the work involving my thesis:

In particular, I would like to express my sincere thanks and heartfelt gratitude to my supervisor Prof E. R. Jansz for his encouragement, invaluable guidance throughout the research project, suggestions on the manuscript preparation and providing opportunities to do part of this work in recognized carotenoid laboratories abroad.

I wish to thank my supervisor Prof N. D. Warnasuriya for his invaluable ideas, encouragement, constructive criticism on the manuscript preparation and continuous guidance on the clinical relevance of my work given to me during the past four years.

My thank also go to Prof. S. M. D. N. Wickramasinghe for her supervision and valuable advice during first two years of my project.

It is my obligation to extend my gratitude to Prof. Delia Rodriguez–Amaya, Department of Food Sciences, Faculty of Food Engineering, State University of Campinas, Brazil for giving me an opportunity to work in her world famous carotenoid laboratory and for her valuable training in carotenoid analysis and help during my stay in the Brazil.

I also wish to thank Prof. Ulf Svanberg, Department of Food Science, Chalmers University of Technology, Sweden for training me in *in vitro* bioavailability studies, guidance, valuable advice and help given to me during my stay at Gothenburg, Sweden

My sincere appreciation also extended to: -

International Programme for Chemical Sciences (IPICS) project SRI: 07 for equipments, chemicals, travel and training abroad and University of Sri Jayewardenepura (Research Grant No: ASP/6/PR/2000/13) for purchase of raw materials, rats, rat feed and costs for local travels.

Professor Malin Akerblom, the Director IPICS and her entire staff for all the support including helping me with the IPICS fellowships that enabled me to work abroad (Brazil for four months, Sweden for six months) with well respected international carotenoid researchers (Prof. Rodriguez-Amaya and Prof. Svanberg) and to present papers at International vitamin A Consultative Group meetings in Morocco and Peru.

Staff and graduate students at Department of Food Sciences, Chalmers University of Technology, Sweden for their hospitability given to me during my stay, especially Ellen and Erika.

Staff and graduate students at Department of Food Sciences, State University of Campinas, Brazil for their friendliness, especially Patricia, for acting as my interpreter.

All members of academic and non academic staff and research students at the Department of Biochemistry, University of Sri Jayewardenepura for their support in many ways, especially Mr. Nalaka Ileperuma for helping me with HPLC equipment and Mr. Jayaweera Banda for providing me with tea and lunch when I was working after hours and week ends.

Mrs P. S. Perera for helping me with typing the thesis and Mrs. I Dayaratne for editing.

My former teachers in the Department of Chemistry, University of Sri Jayewardenepura, especially, Prof. A. M. Abeysekera for his encouragement, kind advice throughout my study and for explanations of chemical reactions and Prof. W.S. Fernando for providing transport and an assistant to transport animals from MRI to the animal house.

I am forever grateful to my parents and sisters who helped me in many ways.

I DEDICATE THIS THESIS TO MY PARENTS

CONTENT, BIOAVAILABILITY AND BIOCONVERSION AND ANTIOXIDANT ACTIVITIES OF CAROTENOIDS IN SOME SRI LANKAN FRUITS AND GREEN LEAFY VEGETABLES

UDUMALAGALA GAMAGE CHANDRIKA

ABSTRACT

The aim of this study was to evaluate the content and bioavailability/bioconversion of pro- vitamin A carotenoids in selected fruits and green leafy vegetables (GLV) from Sri Lanka and to elucidate the effects of different processing and preparation methods. The fruits used in this study was papaya, palmyrah, jak, lavalu and beal. GLV used in this study were kathurumurunga (*Seshania grandiflora*), mukunuwanna (*Alternathera sessilis*), gotukola (*Centella asiatica*), manioc (*Manihot esculenta*), sarana (*Triathema monogyna*), nivithi (*Spinacea oleracea*) and thampala (*Amaranthus caudatus*). In the case of papaya, palmyrah and jakfruits content of carotenoids were determined using MPLC and visible spectrophotometry and in the case of lavalu and GLV by HPLC method. The vitamin A and β- carotene status in Wistar rats was assessed with and without out fat in the diet in the case of papaya and mukunuwanna leaves using HPLC method. An *in vitro* method that simulates human digestion was used to measure the fraction of pro-vitamin A carotenoids that is release for absorption (bioaccessible) from selected GLV using HPLC method.

Separation and quantification of the carotenoids in two major varieties of *Carica papaya* grown in Sri Lanka indicated that red- and yellow-fleshed varieties had different carotenoid profiles. Yellow–fleshed papaya contained three major

carotenoids, i.e. β-carotene, β-cryptoxanthin and ζ-carotene. In addition to these three carotenoids, red-fleshed papaya also contained lycopene and β-carotene-5,6 epoxide. It is interesting to note that red-fleshed papaya has significantly higher β-carotene and lycopene than yellow-fleshed variety. Hence the calculated mean retinol equivalent (RE) was 506.7 ± 54.9 $\mu g kg^{-1}$ fresh weight (FW) in yellow-fleshed papaya, whereas in red-fleshed papaya it was 202 ± 48 $\mu g kg^{-1}$FW. Study with Wistar rats has shown that notwithstanding the colour of the flesh a significant increase of serum vitamin A is shown only if papaya is fed incorporated to standard diet ($p<0.001$) rather than given separately (with out incorporated to standard diet). Red-fleshed variety shows higher (66.7± 4.2 μg/g) β-carotene levels in the liver compared to the yellow fleshed variety (5.4 ± 1.0 μg/g, $p<0.001$).

Six carotenoids were detected in jakfruit kernel. The carotenes are β-carotene, α-carotene, β-zeacarotene, α-zeacarotene, β-carotene 5, 6 epoxide and a dicarboxylic carotenoid crocetin were identified. This corresponds theoretically 25.5 RE/100g FW. Our study indicated that jakfruit is a source of pro-vitamin A carotenoids, but not as good as papaya. Serum retinol concentrations of rats supplemented with jakfruit kernel shown to be significantly higher ($p=0.008$) compared with control group. The same is true for liver retinol ($p=0.006$). Quantification was carried out by RP-HPLC. This results shows biological conversion of pro-vitamin A in jakfruit kernel appears satisfactory. Thus increased consumption of ripe jakfruits could be advocated as a part of a strategy to prevent and control of vitamin A deficiency in Sri Lanka.

Four major carotenoids were detected in palmyrah fruit pulp (PFP). The carotenoids are α-carotene, ζ-carotene, lycopene and β-zeacarotene and this corresponds to 22.3 RE/100g FW. The results of *in vivo* bioavailability and bioconversion studied showed that pro-vitamin A carotenoids from PFP were also bioavailable and bioconvertible.

The major carotenoid in lavalu (*Chrysophyllum roxhburgii*) was *trans*-violaxanthin (113 mg.kg-1, 63%). Also present was *cis*-violaxanthin (19%), neoxanthin (3%), β-cryptoxanthin monoepoxide (11%), lutein, β-cryptoxanthin, ζ-carotene and β-carotene. The retinol equivalent of the pulp was only 13.8 RE/100g FW. The study shows that lavalu is not a good source of pro vitamin A. Further the structural properties of the carotenoids make bioconversion studies with animals or humans futile. So that the best use of the lavalu carotenoids is as a food colourent for oil based foods.

In the case of beli (*Aegle marmelos*) there were only trace amount of caroenoids and canot be used as a pro vitamin A supplement.

The all-*trans*-β-carotene content in fresh GLV ranged from 63.1 μg/g FW in leaves of mukunuwanna to 133.8 μg/g FW in the thampala. Cooking of fresh leaves resulted in some losses of all-*trans*-β-carotene and retention ranging from 56 to 76% in the malluma and 27 to 73% in the fried preparation. Cooking with water or coconut milk resulted in retentions ranging from 36 to 88%. The *in vitro* accessibility of all-*trans*-β-carotene in cooked vegetables ranged from 14 to 43% in malluma, and from 12 to 36% in the fried preparation. In the GLV cooked with coconut milk the *in vitro* accessibility ranged from 12 to 26% compared with 4 to 8% when cooked with water. The β-carotene content, retention and *in vitro* accessibility widely varied between different varieties of green leaves. The three types of different traditional cooking procedures show about the same retention. Preparations with oil, scraped coconut and coconut milk had improved *in vitro* β-carotene accessibility.

In conclusion, the green leafy vegetables and fruits studied have variable contents and *in vitro* accessibility of pro-vitamin A carotenoids and thus contribute in various degrees to recommended daily allowance (RDA) of vitamin A. For green leafy vegetables such as kathurumurunga, mukunuwanna, manioc, gotukola, sarana, thampala an adequate contribution to RDA of vitamin A can be obtained by consumption of green leafy vegetables (100g, FW) with high pro-vitamin A content judging from *in vitro* accessibility and traditional preparation methods of Sri Lanka with addition of coconut, coconut milk or coconut oil.

None of fruits studied would cover the total vitamin A requirement if consumed in normal amounts. Papaya, jak and palmyrah should be considered as good sources of pro-vitamin A carotenoids, as they would be a sound vitamin A contributing complement to the diet. This study also revealed that some fruits such as lavalu and beli are not good sources of pro-vitamin A as believed by the general population. These findings can be useful in dietary intervention programmes to alleviate vitamin A deficiency in Sri Lanka as well as other developing countries.