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A study of carotenoids of mango fruit and mango based products and their *in-vitro* bioavailability

by

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The work described in this thesis was carried out by me under the supervision of Prof. A Bamunuarahchi , Prof. U.G. Chandrika and Prof. K.K.D.S.Ranaweera 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.

Signature of the candidate

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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Affectionately
dedicated to
all Sri Lankans'
who spent money
time and tears for
my free education

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Study of carotenoids of mango (*Mangifera indica*) fruit and mango based products and their *in vitro* bioavilability

By Matara Arahchighe Jagath Wansapala

ABSTRACT

Vitamin A is an important micronutrient of animal origin responsible for structure and functioning of the skin, mucous membrane and maintaining of a good vision. Low-income, poor educational background and cultural and religious practices discourage the intake of recommended amount of vitamin A through animal sources. Therefore the attention should be given to the naturally available and affordable provitamin A sources such as green leafy vegetables, certain root crops and most of the fruits.

The major objectives of this study were to qualitatively analyze the carotenoid profile in three popular mango varieties namely "Karuthacolamban" , "Beti Amba" and "Gira Amba" grown in the three different agro climatic regions of the Sri Lanka, quantification and *in vitro* bioavailability of pro vitamin A content in all three varieties with a view to updating the database. The impact of different process conditions on the stability of provitamin A carotenoids in mango based products (ie. jam, cordial and osmotically dehydrated mango) were also studied. Sensory characteristics of the selected mango varieties were comparatively studied and the sensory web was developed for each variety.

A reversed phase HPLC method has been developed for the study for the separation and extraction of the carotenoids in crude and saponified extracts from the "Karuthacolamban" , "Beti Amba" , "Gira Amba" and mango based products. All fruits with the same maturity

indices were selected from local market and the cultivars were identified by Horticultural Crop Research and Development Institute Gannoruwa, Kandy, Sri Lanka.

Carotenoids from mango were extracted according to the method described by Rodriguez Amaya (1999), which involved extraction of carotenoids, partition to petroleum ether, separation of carotenoids by Open Column Chromatography (OCC), identification of carotenoids using Ultra violet Visible absorption spectra (maximum absorption and spectral fine structure), order of elution in OCC and chemical tests. Purity of the identified carotenoids were further confirmed by using the High Performance Liquid Chromatography with photo diode array detection (C_{18} column Spherisorb ODS2, 5µm, 4.6mm x 150mm; gradient elution of mobile phase of Methanol, Acetonitrile and 0.05% Tri ethyl amine in Ethyl acetate). The HPLC analysis of crude extract of variety Karuthacolamban highlighted the existence of four main peaks which had UV- Visible spectra similar to those reported for violaxanthin, neoxanthin, β-carotene and α-cryptoxanthin. The HPLC analysis of crude extract of "Beti Amba" and "Gira Amba" varieties highlighted the existence of three main peaks which had UV- Visible spectra similar to those reported for violaxanthin, neoxanthin, and β-carotene .A saponification step of crude extracts indicated the existence of esterified violaxanthin, neoxanthin, and α-cryptoxanthin. The Variety "Karuthacolomban" contains 2.7±0.3 µg/g (Fresh weight) , the variety "Beti Amba" contains 2.6±0.3 µg/ g (Fresh weight) and the variety "Gira Amba" (0.18 ± 0.6 µg/ g (Fresh weight) of β- carotene as the principal pro-vitamin A carotenoid .The amount of in vitro bioavailability β- carotene was highest in the "Beti Amba" variety (29.6%) than "Karuthacolomban" (24%) and "Gira Amba" (20.30%).

Thus, this study has indicated that varietal difference does not exist in the content but bioavailability of β-carotene in mango.

The percentages of destruction of β- carotene in jam, cordial and osmotically dehydrated mango during the process were recorded as 89%, 78% and 66% respectively. The in vitro bioaccessibility of β- carotene in all three products were recorded as 67 %, 25 % and 64% respectively. Higher amounts of provitamin A carotenoids (1.43±0.3 µg/ g Fresh weight) were obtained under Room temperature (28 °C) and the lowest amount of provitamin A carotenoids (0.20±0.3 µg/ g Fresh weight) were recoded at -2 °C. The provitamin A carotenoids content of artificially ripened mango was (0.30±0.3 µg/ g Fresh weight) lower than that of the natural. The highest sensory values were recorded "Gira amba" variety.