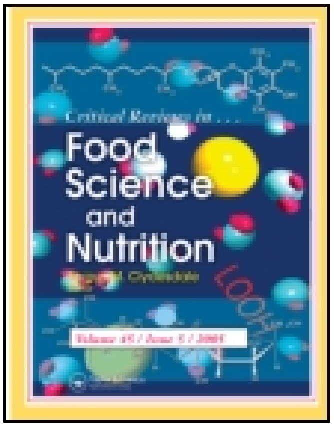

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

A Critical Review on Carotenoid Research in Sri Lankan Context and Its Outcomes

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Accepted author version posted online: 14 Jan 2013.Published online: 21 Nov 2013.

To cite this article: A. M. B. Priyadarshani & E. R. Jansz (2014) A Critical Review on Carotenoid Research in Sri Lankan Context and Its Outcomes, Critical Reviews in Food Science and Nutrition, 54:5, 561-571, DOI: 10.1080/10408398.2011.595019

To link to this article: http://dx.doi.org/10.1080/10408398.2011.595019

Critical Reviews in Food Science and Nutrition, 54:561–571 (2014)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2011.595019

A Critical Review on Carotenoid Research in Sri Lankan Context and Its Outcomes

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As determined by countrywide assessments, vitamin A deficiency is a public health problem in Sri Lanka. Study of carotenoid profile and content could be important to nutritionists as some carotenoids act as precursors of vitamin A. Sri Lanka has a remarkable diversity of carotenoid sources. A number of Sri Lankan sources of carotenoids have been studied by many authors. This study reviews carotenoid research done in Sri Lanka, comparing results which are generally in conflict with a few relevant studies abroad, while focusing on problems of carotenoid research and concluding that it is difficult for a dietician to predict carotenoid intake due to marked biological variation. Therefore, any database on carotenoid covering the entire country has its limitations. Further that even if carotenoid profiles are known using exhaustive sampling, there can be no single method of calculating retinol equivalent (RE) and retinol activity equivalent (RAE) especially as carotenoid uptake and bioconversion could be multifactorially affected and subject to control mechanisms. Therefore, RE and RAE should be calculated differently for different types of plant materials may even be expanded so that a unique calculation depending on plant material and method of cooking.

Keywords Carotenoid, fruits, nonleafy vegetables, green leafy vegetables, retinol equivalent, retinol activity equivalent

INTRODUCTION

Carotenoids are a well-known group of natural plant pigments responsible for most of the yellow, orange, and red colors throughout the natural world. They are found in the plant kingdom providing these brilliant colors to fruits, vegetables, flowers, and even birds. It is estimated that more than 600 different carotenoids excluding *cis* and *trans* isomers are broadly distributed in nature and many of them have been identified. Carotenoids are generally 40-carbon tetraterpenoids, formed by joining eight 5-carbon isoprene units ($-CH_2-C(CH_3) = CH-CH_2-$) in a head-to-tail manner except at the center where the linkage is in a tail-to-tail manner. This provides a symmetrical and linear basic molecule (Rodriguez-Amaya and Kimura, 2004).

Depending on structural uniqueness, carotenoids can be categorized as provitamin A and non-provitamin A carotenoids. The necessary structural requirement to possess provitamin A activity is that the molecule must have at least one unsubstituted β-ionone moiety with a polyene chain of at least 11 carbon atoms. Hence all-*trans*-β-carotene is essentially two molecules of vitamin A (retinol) (Figure 1). α-Carotene, γ-carotene, and β-cryptoxanthin are also provitamin A carotenoids and are assigned one half of the vitamin A activity of β-carotene on the molar basis. Lutein, zeaxanthin, astaxanthin, violaxanthin, phytofluene, ζ-carotene and lycopene are non-provitamin A carotenoids (Rodriguez-Amaya, 1999; Rodriguez-Amaya and Kimura, 2004).

During digestion, in the stomach caroteno-protein complexes are dissociated into carotenoids by the action of pepsin. Further digestion takes place in the small intestine by proteolytic enzymes. Once the carotenoids are released in the stomach, they associate with the dietary lipids. Bile salts emulsify the lipid containing carotenoids. Lipids are digested by pancreatic lipase in the upper part of the intestine. Fat digestion yields free fatty acids, monoacylglycerols, and diacylglycerols, which are incorporated into mixed micelles together with carotenoids and bile salts (Hedren, 2004). Absorption of carotenoids takes place in

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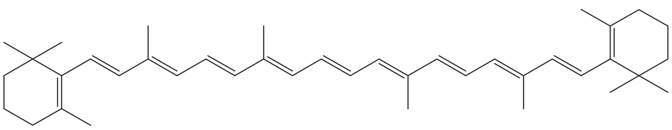

Figure 1 Structure of β-carotene.

the small intestinal mucosal cells. In general, carotenoids are deposited mainly in adipose tissue and liver. They have also been found in lung, kidney, and most of other tissues (Schmitz et al., 1991). The cleavage of provitamin A carotenoids into vitamin A is catalyzed by the cytosolic enzyme 15-15′-carotenoid dioxygenase and the key enzyme is β-carotene-15-15′-dioxygenase. The reaction initially takes place in the intestinal mucosa (Goodwin, 1986; Nagao et al., 1996) but it has been shown recently to be also present in approximately four times the quantity in liver (During et al., 2001).

Vitamin A plays a key role in vision, immunity, cell differentiation, maintenance of cell membrane integrity, embryonic development, and growth and reproduction (Garrow et al., 2000).

Hence, vitamin A deficiency results in clinical signs of conjunctiva and corneal aberrations including, xerophthalmia with all other ocular manifestations such as Bitot's spots, night blindness or inability to see under reduced level of illumination, keratomalacia, corneal ulceration, scarring, and resultant irreversible blindness. Other than the above, decreased immune responses and resultant infections of respiratory and gastrointestinal systems, decreased goblet cell frequency, abnormalities of epithelial cells in many tissues and greater incidence of mortality, therefore, have been reported (Underwood and Olson, 1993; IVACG, 2004). In embryogenesis, retinoic acid exhibits its function in sequential development of different parts of an organism. This occurs through its ability to involve in the expression of Hox genes (Garrow et al., 2000).

Apart from provitamin A activity, carotenoids have other well-defined health benefits. Carotenoids exert their antioxidant activity by singlet oxygen quenching and free radical scavenging effects; thereby they acquire a range of important protective mechanisms for human health. This involves protection against the pathogenesis of degenerative diseases especially coronary heart diseases, cancers, and an array of other free radical-mediated conditions (Krinsky, 1989; Halliwell, 1997; Kritchevsky, 1999). Neoxanthin appears to have anticancer activity being converted to neochrome in the gastrointestinal tract (Asai et al., 2004). Lutein and zeaxanthin usually referred to as "macula pigments" are selectively deposited in the retina. Macula pigments are known to provide protection against age-related macular degeneration mediated by their ability to scavenge harmful reactive oxygen species formed in the photoreceptors. They can act as blue-light filters as well and may protect the macula from blue-wavelength photons (Landrum and Bone, 2001). Moreover, lutein supplementation has been interconnected with the improved visual function of patients with cataract (Olmedilla et al., 2001).

Khachik et al. (1997) mentioned that metabolites of carotenoids exhibit more pronounced activity than carotenoids relevant to biological activities such as enhancement of the cellular communication, stimulation of the activity of the phase II enzymes (detoxification enzymes), and anti-inflammatory/ immune-related properties.

PITFALLS OF QUANTIFICATION OF CAROTENOIDS IN PLANT MATERIALS

A reliable carotenoid database is critical for use by nutritionists in recommending proper diets to the vitamin A deficient group. Unfortunately, carotenoid analysis is subject to many errors. As a result, there are many disagreements not only in Sri Lanka but also internationally on collection of data and their interpretation (Rodriguez-Amaya, 1999). Therefore, it appears that reliable techniques must be used in both sampling and analysis. The source of main error is sampling as carotenoids are affected by many factors, mainly biological variations due to genetics, maturity, environment, soil, climate, and also postharvest handling practices, etc. Table 1 shows some results on Sri Lankan fruits showing a vast variation in retinol equivalent (RE) that could reflect the effect of sampling, genetic, climatic, and edaphic factors on carotenoids. It is easy to observe why there are disagreements on the data on carotenoids internationally.

Table 1 Retinol equivalent (RE) of some fruits studied in Sri Lanka

Fruit	Reference	Area	RE /100 g DW
Papaw (yellow-fleshed)	Chandrika et al. (2003)	Not given	151.6 ± 34.2
Papaw (yellow-fleshed)	Priyadarshani et al. (2006)	Kurunegala district	344.8 to 2410
Jakfruit	Chandrika et al. (2005a)	Colombo suburbs	141.6
Jakfruit	Priyadarshani et al. (2007a)	Kurunegala, Matale districts	Traces to 10
Lavalu	Abeysekara, (1993)	Colombo district	1867
Lavalu	Chandrika et al. (2005b)	Kadawata, Piliyandala area of Colombo districts	68
Lavalu	de Lanerolle et al. (2008)	Kandy, Kurunegala, Gampaha, Colombo districts	Traces to 11,813
Palmyrah fruit	Chandrika (2004)	Hambantota district	159.1
Palmyrah fruit	Pathberiya (2005)	Mannar distric	0 to 44.5
Palmyrah fruit	Wijemanne et al. (2006)	Kalpitiya	75 to 969
Palmyrah fruit	Priyadarshani (2007)	Hambantota district	Negligible

DW, dry weight.

When superimposed, especially in the case of vegetables, with cooking practices, further variations arise.

In general, the techniques of Rodriguez-Amaya (1999) have been found to be acceptable by many researchers. In analyzing the carotenoid concentrations in plant materials, two extremes have been found. First, crops with bred agricultural cultivars (where there is no genetic effect), and second, crops with no agricultural selections in Sri Lanka. When species with specific agricultural varieties (cultivars) were analyzed, the diversity of carotenoid content is seen to be much less and, as a result, standard deviation (SD) could be calculated in a given locality. On the other hand, when there are no agricultural selections, SDs could not be calculated making it virtually impracticable for a nutritionist to predict RE per meal/portion. Additionally, the consumer rarely purchases agricultural varieties. Due to variations in carotenoid content, prediction of percent contribution of a portion to recommended daily allowance (RDA) is very difficult especially in the case of nonagricultural varieties (Priyadarshani, 2007).

Other errors identified include the problems in analysis techniques, the methods of extraction, separation, chromatographic techniques (open column chromatography (OCC), high-pressure liquid chromatography (HPLC), and thin layer chromatography (TLC). A limited database for identification leads to a number of unidentified carotenoids and problems of quantification. In the extraction of carotenoids, care must be taken to remove all color in the acetone extract. Acetone is used because the carotenoids in biological materials occur in an aqueous environment. Saponification should be carried out when it is absolutely necessary. In separation of carotenoids, medium-pressure liquid chromatography (MPLC) has been tried out instead of OCC (Chandrika, 2004). However, it was found to be unsuitable using celite:MgO (1:1) due to contraction of column in petroleum ether and breaks in the column under pressure which is applied from beneath. Silica gel has no such problems but carotenoids are reported to decompose on silica gel (Rodriguez-Amaya, 1999). Identification is also a problem as detailed spectroscopic data on carotenoids are available only for about 33 carotenoids. An internal standard must be used to ensure that injection errors are nullified (to correct for recovery) during the HPLC analysis and to limit other related errors. If not used, errors of calculation and questions of authenticity of results arise. Internal standards give credibility to the calculations.

PITFALLS OF QUANTIFICATION OF RETINOL EQUIVALENT (RE) AND RETINOL ACTIVITY EQUIVALENT (RAE)

Further calculation of potential bioavailability of carotenoids (RE and RAE) is a problem, as they are affected by many factors. RE has been defined as 6 μg of β-carotene and 12 μg of other provitamin A carotenoids, giving rise to 1 μg of retinol (1 RE). This ratio was established according to two assumptions (i) conversion of a maximum of half of absorbed purified β-carotene which was in oil into vitamin A in vivo and (ii) absorption of about one-third of the β-carotene from a mixed diet compared to the β-carotene in oil. More recently another term, RAE, has been proposed. Here double the amounts of β-carotene and other provitamin A carotenoids are needed for the formation of retinol, i.e., RAE is calculated according to the conversion factors, 12 μg of β-carotene and 24 μg of other provitamin A carotenoids giving rise to 1 μg of retinol (1 RAE). According to in vivo studies, these changes are due to the relative absorption of β-carotene being shown to be 1:7 and not 1:3. This ratio was adjusted to 1:6 due to low content of fruits in the tested mixed diet (Hedren, 2004). But some carotenoids are not bioavailable. Furthermore, there must be some control over bioconversion in order to prevent hypervitaminosis. In addition, the β-carotene molecule gives two molecules of retinol only if its cleavage is symmetrical. Among the dietary factors, fat is a key positive effector (Priyadarshani and Chandrika, 2007a). On the other hand, soluble dietary fiber has an adverse effect on absorption of carotenoids in humans (Yeum and Russell, 2002). Carotenoids can be affected by their different arrangements within the cell structure. When they are located in chromoplast as crystalline form or dissolved in oil droplets carotenoids are far more bioavailable during digestion than when they are complexed to proteins in chloroplasts such as in green leafy vegetables (de Pee et al., 1995; Castenmiller and West, 1998). Cooking method is important as raw plant foods are suggested to be less bioavailable compared to thermally processed foods. Particle size is also important as reduced particle size improves bioavailability of carotenoids (Priyadarshani and Chandrika, 2007a). When these factors are taken into consideration, the actual value for RE and RAE will vary from situation to situation. As a result, the RE and RAE is only a rough guideline to a nutritionist.

RESEARCH CARRIED OUT ON CAROTENOIDS OF FRUITS IN SRI LANKA

Fruits are known to have complex and variable carotenoid composition with considerable qualitative and quantitative variations. Papaw (*Carica papaya*) is a widely recommended fruit for vitamin A deficiency worldwide. On the other hand, it is one of the fruits responsible for hypercarotenemia in Sri Lanka (Wageesha et al., 2008; Priyadarshani et al., 2009). In Sri Lanka, papaw has no agricultural cultivars. They are highly heterogeneous trees due to extensive cross-pollination, and these fruits are of different shapes, sizes, and flesh colors.

There have been three studies carried out on papaw in Sri Lanka with conflicting results. According to Atukorala (1985), β-carotene was the only carotenoid quantified, but no separation techniques were evident. The value reported was significantly higher than most of the other fruits given in the same reference: orange (*Citrus sinensis*) (2.06 $\pm$ 0.76 μg/g); plantains (*Musa sapientum*) variety "Ambul" (2.81 $\pm$ 0.42 μg/g), and variety "Kolikuttu" (2.58 $\pm$ 0.30 μg/g); and pineapple

(*Ananas comosus*) (5.15 ± 0.62 μg/g) (Atukorala, 1985). However, it is not clear if the results were expressed on fresh or dry weight. Chandrika et al. (2003) carried out a study on the carotenoids of two major types of papaw grown in Sri Lanka, namely the red- and yellow-fleshed varieties of papaw. The separation of carotenoids was done by MPLC packed with silica gel. They found that there are two provitamin A carotenoids detected from yellow-fleshed papaw, β-cryptoxanthin which is followed by β-carotene. In the red-fleshed variety, in addition to these two carotenoids, β-carotene-5,6-epoxide was reported as a provitamin A carotenoid. Lycopene was detected only from red-fleshed variety. Theoretically calculated RE values for yellow and red varieties were low: 1,516 ± 342 and 2,815 ± 305 μg/kg dry weight (DW), respectively. Priyadarshani et al. (2006) reported on the carotenoids of yellow-fleshed papaw collected from the Kurunegala district, this is the major papaw producing district of Sri Lanka; from this district the fruits are distributed to other parts of the country. As determined by OCC, spectrophotometrically and chemical tests, the carotenoid profile of papaw was composed of phytofluene, ζ-carotene, β-carotene, β-cryptoxanthin, β-cryptoxanthin 5,6-epoxide, and an unknown mono-epoxy-carotenoid. The results of this study have deviated from the other two studies as carotenoid contents varied markedly from specimen to specimen. The authors stated that therefore SD could not be calculated. Among six specimens, RE ranged from 50–313.3/100 g fresh weight (FW) (344.8–2410/100 g DW). However, these marked variations in carotenoid content of the latter study are compatible with studies done abroad on papaw; Rodriguez-Amaya (1999); Setiawan et al. (2001). Values reported from Sri Lankan studies are shown in Table 2.

Table 2 Carotenoid contents of papaw in Sri Lanka

Carotenoid	Carotenoid content
*Atukorala (1985)	
β-Carotene	22.4 ± 0.49
**Chandrika et al. (2003)	
Yellow-fleshed papaw:	
β-Cryptoxanthin	15.4 ± 3.3
β-Carotene	1.4 ± 0.4
ζ-Carotene	15.1 ± 3.4
Red-fleshed papaw:	
β-Cryptoxanthin	16.9 ± 2.9
β-Carotene	7.0 ± 0.7
β-Carotene-5,6-epoxide	2.9 ± 0.6
Lycopene	11.5 ± 1.8
ζ-Carotene	9.9 ± 1.1
***Priyadarshani et al. (2006)	
β-Cryptoxanthin	230–1510
β-Carotene	140–1030
β-Cryptoxanthin-5,6-epoxide	20–190
ζ-Carotene	29–238
Phytofluene	135–241
Unidentified carotenoid	62–223

*Locations of the samples: three different areas of Colombo, Sri Lanka, Mean ± SEM in three samples each in duplicate, values given μg/g, quantification by spectrophotometrically.
**Mean ± SD in ten samples, values given μg/g DW, quantification by spectrophotometrically.
***Locations of the specimens: Kurunegala, Sri Lanka, six specimens were analyzed each in duplicate, values given μg/100 g FW, quantification by HPLC.

It is felt that the underestimation of the carotenoid content in the study of Chandrika et al. (2003) may be due to the use of MPLC with silica gel. It is reported that silica gel causes degradation of carotenoids (Rodriguez-Amaya, 1999). The low SDs of both Atukorala (1985) and Chandrika et al. (2003) are difficult to explain especially as yellow-fleshed papaw has no agricultural selections and it is highly heterogeneous. It is concluded that inspite of the variations papaw is a good source of provitamin A carotenoids. In vitro bioaccessibility study showed that percentage bioaccessibility for β-carotene and β-cryptoxanthin were 50.5 and 45.7, respectively (Priyadarshani et al., 2006).

Palmyrah (*Borassus flabellifer*) fruit is a vastly underutilized fruit in Sri Lanka. The traditional cultivation of palmyrah has been in the North, East, and coastal North-West of Sri Lanka. There are many studies carried out in Sri Lanka on palmyrah fruits from different districts. Palmyrah fruits from Hambantota were studied for carotenoid profile and it was shown to contain four main carotenoids including α-carotene (8.1 μg/g DW) and β-zeacarotene (11.0 μg/g DW) as provitamin A carotenoids with an RE of 159.1/100 g DW. ζ-Carotene (8.1 μg/g DW) and lycopene (13.3 μg/g DW) were mentioned as non-provitamin A carotenoids (Chandrika, 2004). An inexplicably similar profile and concentrations of carotenoids had been reported using a bulk sample of palmyrah fruit pulp (PFP) from Kalpitiya (Samarasinghe and Jansz, 2001). According to Pathberiya and Jansz (2005) among the four types of palmyrah fruits collected from Mannar, β-carotene concentration was high only in type II B (43.8 μg/100 g FW, PFP). β-Zeacarotene was reported only from type II B (30 μg/100 g FW, PFP). The highest RE value reported was 9.8/100 g FW. This value was given by type II B. Other types—type I, type II A, and type III—had insignificant RE values. According to Wijemanne et al. (2006), RE values ranged from 75 to 969/100 g DW for palmyrah fruits collected from Kalpitiya. Due to high overall carotenoid content, some may contribute toward a significant RE. In this study, despite the low level of β-carotene compared to other carotenoids detected, RE of type I, IIB, III, and IV were in the range of 18–33/100 g FW, PFP which are comparable to the values reported by Chandrika (2004).

Priyadarshani (2007) studied the carotenoids of palmyrah fruit type IIB from the Hambantota district of Sri Lanka. It is because type II B trees dominated the plantation and in this case the genetic factor is minimized. However, here too SDs were extremely large showing the effect of environmental and soil factors. Carotenoid concentrations are as follows: phytofluene, 38.2 ± 9.2, phytoene, 77.0 ± 15.0, and unidentified carotenoids I, II, III, and IV; 11.7 ± 3.9, 18.5 ± 10.3, 99.7 ± 40.0, and 8.9 ± 2.7 μg/g DW, respectively. β-Carotene and ζ-carotene were present in traces. Other than β-carotene no provitamin A carotenoids were detected. Therefore, according to this study, RE is negligible in palmyrah fruit pulp. Results are puzzling as the palmyrah seeds obtained from Kalpitiya have been planted in the Hambantota district but the fruits from Kalpitiya had shown markedly different results (Wijemanne et al., 2006). Results were also different for carotenoid content of palmyrah fruit from Mannar (Pathberiya and Jansz, 2005). The results of Samarasinghe and Jansz (2001) and Chandrika (2004) markedly deviated from other studies on PFP, in that it reported the presence of lycopene. Other studies showed the absence of lycopene.

Fruits of the jak (*Artocarpus heterophyllus*) tree are readily available in rural Sri Lanka and the kernel of the ripe jakfruit is widely consumed by Sri Lankans. In Sri Lanka, jakfruit has no agricultural varieties. The yellow fruit kernel was found to contain carotenoids. According to Atukorala (1985), in ripe jakfruit β-carotene is the only carotenoid detected (Table 3). At variance to the above result, Chandrika et al. (2005a) showed that jakfruit contained four types of provitamin A carotenoids with β-carotene as the major provitamin A carotenoid (Table 3). The theoretically calculated RE was 141.6/100 g. Results suggested that jakfruit kernel is a good source of provitamin A carotenoids. Priyadarshani et al. (2007a) studied jakfruit from Matale and Kurunegala districts, which are the largest producers of jakfruit from where these fruits are transported to the North as far as the Jaffna peninsula and South to Colombo. However, results of Priyadarshani et al. (2007a) contrasted with the other two studies done on jakfruit as variations in the carotenoid contents have been observed among six specimens (Table 3). On the other hand, such variations in carotenoid content have been observed by the studies done abroad on carotenoids of jakfruit (Setiawan et al., 2001).

Table 3 Carotenoid contents of ripe jakfruit in Sri Lanka

Carotenoid	Carotenoid content
*Atukorala (1985)	
β-Carotene	2.71 ± 0.6
**Chandrika et al. (2005a)	
β-Carotene	5.6 ± 0.3
β-Carotene-5,6-epoxide	3.1 ± 0.3
α-Carotene	1.7 ± 0.1
β-Zeacarotene	3.1 ± 0.3
α-Zeacarotene	3.5 ± 0.2
Crocetin	2.1 ± 0.1
***Priyadarshani et al. (2007a)	
β-Carotene	Traces −50.0
α-Carotene	Traces −20.5
Lutein	2.7–221.5
Unidentified I	Traces −33.7
Unidentified II-*trans*	8.3–45.5
Unidentified II-*cis*	11.1–59.9
Unidentified III	Nondetectable level −32.2
Unidentified IV	Traces −to 32.8

*Locations of the samples: three different areas of Colombo, Sri Lanka, Mean ± SEM in three samples each in duplicate, values given μg/g, quantification by spectrophotometrically.

**Locations of the samples: Colombo and its suburbs, Sri Lanka, Mean ± SD in five samples each in duplicate, values given μg/g, quantification by spectrophotometrically.

***Locations of the specimens: Matale and Kurunegala districts, Sri Lanka, six specimens were analyzed each in duplicate, values given μg/100 g DW, quantification by HPLC.

In most specimens, lutein was the major contributor to the total carotenoid content of jakfruit. Theoretically calculated RE was 10/100 g DW and in vitro bioaccessibility of β-carotene was also very low (8%) due to the rubbery texture of the kernel. When these factors are taken into consideration, jakfruit does not appear to be a major contributor to the recommended daily allowance (RDA) of vitamin A (Priyadarshani et al., 2007a).

It is commonly stated that jakfruit kernel from the same home garden can vary in color. Furthermore, fruits from a single jak tree in different seasons vary slightly in color. Therefore, the very low coefficients of variation (CV) (4.8 to 9.7%) observed by Chandrika et al. (2005a) is difficult to explain if the samples had been collected from different locations, i.e., in and around the suburbs of Colombo, Sri Lanka.

In rarer species, accurate identification of plant material is important. For example, *lavalu* analyzed by Abeyesekara (1993) and Chandrika et al. (2005b) stated that the species was *Chrysophyllum roxburghii,* but this species is a green fruit with a white pulp (Dassanayake, 1995). de Lanerolle et al. (2008) had shown the common *lavalu* plant to be *Pouteria campechiana* (*ratalawulu*) by comparing National Herbarium specimens and authentic identification by an authority. As determined by taxonomical studies, this is the only yellow-fleshed *lavalu* found in Sri Lanka (Dassanayake, 1995).

In *lavalu*, violaxanthin was identified as the major carotenoid and its RE value was found to be very low (68 RE/100 g) (Chandrika et al., 2005b). Concentrations of the carotenoids detected are given in Table 4. In contrast to this result, de Lanerolle et al. (2008) had shown that RE values of the fruits of this plant collected from different districts of Sri Lanka ranged from traces to 759 to 11,813/100 g DW, as trees are extremely hybridized. Carotenoids were dominated by neoxanthin. As determined by HPLC, the individual carotenoid concentrations have varied markedly from specimen to specimen, as given in Table 4. Though Chandrika et al. (2005b) had not taken into

Table 4 Carotenoid contents of lavalu in Sri Lanka

Carotenoid	Carotenoid content
*Chandrika et al. (2005b)	
Violaxanthin	113.3 ± 17.2
Neoxanthin	31.3 ± 5.5
Lutein	0.4 ± 0.1
β-Carotene	1.4 ± 0.3
**de Lanerolle et al. (2008)	
β-Carotene	Traces −156
β-Cryptoxanthin	Traces −1,106
Violaxanthin	<188–1,151
Neoxanthin	1,594–19,270
Unidentified I	Traces −628
Unidentified II	68–1,162

*Locations of the samples: Piliyandala and Kadawata, Sri Lanka, Mean ± SD in four samples each in duplicate, values given mg/kg FW, quantification by HPLC.

**Locations of the specimen: Kadawata, Kurunegala, Maharagama, Makola, Peradeniya, Nawala, Sri Lanka, six specimens were analyzed each in duplicate, values given μg/g DW, quantification by HPLC.

account the provitamin A carotenoid; β-cryptoxanthin, in the study of de Lanerolle et al. (2008) β-cryptoxanthin was the major contributor to RE. de Lanerolle et al. (2008) also found that the procedure of carotenoid extraction had to be changed by including a water extraction step to remove a carbohydrate gum which had resulted in very low extraction of carotenoids.

Mango (*Mangifera indica*) is widely distributed in Sri Lanka with a number of varieties and hybrids. Among the local mango varieties, "Karuthacolomban" and "Beti Amba" are widely consumed by Sri Lankans. The HPLC analyses of crude extract of variety "Karuthacolomban" showed the presence of four main carotenoids: violaxanthin, neoxanthin, β-carotene, and α-cryptoxanthin, and variety "Beti Amba" highlighted the existence of three carotenoids including violaxanthin, neoxanthin, and β-carotene. "Karuthacolomban" and "Beti Amba" were reported to have β-carotene contents of 2.7 $\pm$ 0.3 and 2.6 $\pm$ 0.3 μg/g FW, respectively (Wansapala et al., 2008). According to Atukorala (1985) β-carotene concentration of mango and "Karthakolomban" collected from Colombo, Sri Lanka, were 20.96 $\pm$ 0.58 and 31.51 $\pm$ 1.1 μg/g, respectively. The amount of in vitro bioaccessible β-carotene was higher in "Beti Amba" (29.6%) than Karuthacolomban (24%) (Wansapala et al., 2008).

The most common watermelon (*Citrullus lanatus*) variety found in Sri Lanka, "Sugar Baby" purchased from in and around Colombo, Sri Lanka, was reported as a very good source of lycopene (39.5 $\pm$ 9.4 μg/g FW). The estimated in vitro bioaccessibility was also high (65.6 $\pm$ 24.3%) (Edirisinghe and Chandrika, 2006).

Local red-fleshed guava (*Psidium guajava*) variety "Horana Red" obtained from Horticultural Crop Research and Development Institute, Kandy, Sri Lanka, was a rich source of lycopene (45.3 $\pm$ 8.1 μg/g FW). Lutein (2.1 $\pm$ 0.6 μg/g FW) and β-carotene (2.0 $\pm$ 0.2 μg/g FW) have been reported as the minor components. The in vitro bioaccessibility of lycopene in guava was found to be very high 73% (Chandrika et al., 2007a). According to this study, guava can be ranked as a fruit that contains more lycopene than watermelon or red-fleshed papaw. Atukorala (1985) reported β-carotene as the only detected carotenoid from guava and had a concentration of 8.97 $\pm$ 0.86 μg/g.

Yellow passion fruit is a popular fruit in Sri Lanka. Variety derived from species *Passiflora edulis* showed that β-carotene (304.6 $\pm$ 24.5 μg/100 g FW) and β-cryptoxanthin (74.4 $\pm$ 28.5 μg/100 g FW) were the principal provitamin A carotenoids detected (Chandrapala and Chandrika, 2008).

Ambarella fruit is usually not a popular fruit among Sri Lankans, but it is a very popular vegetable curry. However, Fernando et al. (2006) reported the β-carotene content of two varieties of ambarella; *Spondias ceytherea* and *Spondias dulcis* as 0.7 $\pm$ 0.2 and 2.1 $\pm$ 0.2 μg/g. Lutein was found only in *Spondias ceytherea* in the concentration of 0.9 $\pm$ 0.7 μg/g.

Cantaloupe melon (*Cucumis melo reticulates*) is becoming a popular fruit eaten either raw when ripe or as dessert with sugar or ginger preserves. It had a high β-carotene level (26 $\pm$ 8 μg/g FW). In addition to that, it indicated the presence of small quantities of lutein (0.4 $\pm$ 0.1 μg/g FW) and lycopene (0.2 $\pm$ 0.1 μg/g FW). The in vitro bioaccessibility of cantaloupe melon was found to be high (71 $\pm$ 11%) (Chandrika et al., 2007b).

RESEARCH CARRIED OUT ON CAROTENOIDS OF NON-LEAFY VEGETABLES IN SRI LANKA

Carrot (*Daucus carota*) is the traditional example for a provitamin A carotenoid-rich food. Atukorala (1985) reported that β-carotene was the only carotenoid identified from carrot. According to Priyadarshani and Chandrika (2007b), there is only one variety of carrot found in Sri Lanka which is known as "New kuroda." This variety is very rich in β-carotene whose content is followed by α-carotene. Lutein, a non-provitamin A carotenoid is a minor constituent. Carotenoids levels are given in Table 5. Even though the genetic factor is constant for carrot, considerable standard deviations have been reported due to other factors.

Pumpkin (*Cucurbita maxima*) is easy to grow and is widely available during the entire year in Sri Lanka. Atukorala (1985) identified β-carotene as the only carotenoid in pumpkin but there was no variety identification. But according to Priyadarshani and Chandrika (2007b), there are five common pumpkin varieties found in Sri Lanka. They have been named as

Table 5 Carotenoid contents of some nonleafy vegetables in Sri Lanka

	Carotenoid content (μg/g)		
Carotenoid	β-Carotene	α-Carotene	Lutein
Carrot			
*Atukorala (1985)	40.39 $\pm$ 1.17	ND	ND
**Priyadarshani and Chandrika (2007b)	43.8 $\pm$ 5.6	20.5 $\pm$ 1.7	3.8 $\pm$ 0.4
Pumpkin			
*Atukorala (1985)	12.77 $\pm$ 0.89	ND	ND
**Priyadarshani and Chandrika (2007b)			
Variety:			
Arjuna	50.9 $\pm$ 5.7	27.3 $\pm$ 3.1	39.1 $\pm$ 4.7
Ruhunu	8.7 $\pm$ 1.2	6.2 $\pm$ 1.0	8.2 $\pm$ 1.2
Meemini	6.2 $\pm$ 2.1	11.8 $\pm$ 3.1	30.8 $\pm$ 4.7
Janani	3.0 $\pm$ 0.9	1.2 $\pm$ 0.3	31.2 $\pm$ 2.8
Samson	5.1 $\pm$ 0.8	2.0 $\pm$ 0.4	45.8 $\pm$ 4.8
Sweet potato			
*Atukorala (1985)	2.31 $\pm$ 0.47	ND	ND
**Priyadarshani and Chandrika (2007b)			
Variety:			
CARI-426	42.8 $\pm$ 4.3	ND	ND
P_2-20	35.4 $\pm$ 5.2	ND	ND
CIP-440060	37.5 $\pm$ 3.8	ND	ND
420027	59.0 $\pm$ 6.2	ND	ND
187617-1	14.7 $\pm$ 2.3	ND	ND

ND, not determined.

*Locations of the samples: three different areas of Colombo, Sri Lanka, Mean $\pm$ SEM in three samples each in duplicate, quantification by spectrophotometrically.

**Locations of the samples: Colombo and its suburbs, Sri Lanka, Mean $\pm$ SD in six specimens each in duplicate, values given on the basis of fresh weight, quantification by HPLC, variety identification was done by Horticultural Crop Research and Development Institute, Kandy, Sri Lanka.

"Arjuna," "Meemini," "Samson," "Ruhunu," and "Janani." The profile of pumpkin was characterized mainly by α-carotene, β-carotene, and lutein, as shown in Table 5. Significant quantitative variations in the carotenoid content of each variety have been observed. Within the five varieties, carotenoid concentrations ranged from 3.0 to 50.9 for β-carotene, from 1.2 to 27.3 for α-carotene, and from 8.2 to 45.8 μg/g FW for lutein. The flesh color of pumpkin varied from pale yellow to dark orange. Authors stated that this correlated well with the profile and the amount of carotenoid present in each variety.

In Sri Lanka, only one variety of squash (*Cucurbita moschata*) is found. Concentrations of the major carotenoids detected were β-carotene (6.0 $\pm$ 0.8 μg.g^{-1} FW) and α-carotene (5.1 $\pm$ 1.1 μg/g FW) (Priyadarshani and Chandrika, 2007b).

Many varieties of sweet potato (*Ipomaea batatas*) exist in Sri Lanka. Atukorala (1985) reported that only β-carotene was detected in sweet potato. According to Priyadarshani and Chandrika (2007b) among the yellow, orange-fleshed sweet potatoes, five varieties are commonly found in Sri Lanka. They are "CARI-426," "P_2-20," "420027," "CIP-440060," and "187617-1." Sweet potato had a simple profile and β-carotene was the only detectable carotenoid (Table 5).

Priyadarshani and Jansz (2006) studied carotenoids of stems of kohila (*Lasia spinosa*) in the course of a search for unconventional sources of provitamin A carotenoids. It is a common crop, especially in the rural areas. In the case of kohila, there are no agricultural varieties in Sri Lanka. Therefore, specimens purchased from markets of different locations of Colombo suburbs, Sri Lanka had a wide variation in carotenoid content; β-carotene and α-carotene contents ranged from 0.9 to 7.2 and 0.4 to 1.8 μg/g FW, respectively. This is probably due to genetic and environmental factors. Therefore in this case, SD could not be calculated.

Tomato and tomato-based products are very popular sources of lycopene worldwide. Thillan and Ekanayake (2009) carried out a study on carotenoids of locally grown tomato varieties "Mahesha," "Thilina," and "Tharindu." "Mahesha" has been identified as the variety with the highest amount of lycopene compared to other cultivars. The lycopene contents of "Thilina," "Mahesha," and "Tharindu" were 1.5 $\pm$ 0.2, 2.3 $\pm$ 0.5 and 1.5 $\pm$ 0.3 mg/100 g FW.

β-Carotene levels of some other nonleafy vegetables, legumes, starchy roots, and pulses collected from Colombo, Sri Lanka, are as follows: brinjal (*Solanum melongena*) 2.13 $\pm$ 0.65, snake gourd (*Trichosanthes anguina*) 5.14 $\pm$ 3.88, bitter gourd (*Mormordia charantia*) 2.34 $\pm$ 0.56, ladies fingers (*Hibiscus esculenteus*) 3.25 $\pm$ 0.98, ribbed gourd (*Luffa acutangula*) 1.82 $\pm$ 1.06, leeks (*Allium porrum*) 21.29 $\pm$ 6.01, cabbage (*Brassica oleracea*) 2.75 $\pm$ 0.65, spring onion (*Allium cepa*) 30.36 $\pm$ 0.73, beans (*Phaseolus vulgaris*) 18.60 $\pm$ 0.99, winged bean (*Phaseolus lunatus*) 17.69 $\pm$ 1.05, string bean (*Vigna cylindrica*) 7.59 $\pm$ 0.03, potato (*Solanum tuberosum*) 2.78 $\pm$ 0.4, innala (*Coleus rotundifolius*) 0.232 $\pm$ 0.081, kiriala (*Colacasia esculenta*) 0.119 $\pm$ 0.035, mysoor dhal (*Cajanus cajan*) 12.90 $\pm$ 3.4, cowpea (*Vigna unguiculata*) 2.50 $\pm$ 0.31, and mung bean (*Phaseolus aureus*) 7.22 $\pm$ 0.52μg/g. Quantification has been carried out spectrophotometrically (Atukorala (1985). According to Atukorala (1985), in the carotenoid analysis, the xanthophylls had been removed with 92% of methanol. This is the probable reason for the detection of β-carotene as the only carotenoid in all the plant materials analyzed.

Using two-dimensional thin-layer chromatography, Lord and Tirimanna (1976) showed presence of carotenoids in Sri Lankan chillies (*Capsicum annum*).

The percentage in vitro bioaccessibility of β-carotene and α-carotene of raw carrot was 12.1 and 9.4, respectively. But carrot salad, carrot curry, and boiled, homogenized carrot gave several fold higher values which ranged from 48.1 to 78.1% due to the effect of fat, heat treatment, and particle size on accessibility of carotenoids. In addition, the positive effect of the above-mentioned factors were shown also by the other nonleafy vegetables analyzed: pumpkin, squash, sweet potato, and stems of kohila (Priyadarshani and Jansz, 2006; Priyadarshani and Chandrika, 2007a).

RESEARCH CARRIED OUT ON CAROTENOIDS OF GREEN LEAFY VEGETABLES IN SRI LANKA

Sri Lanka has a wide variety of wild, cultivated, and commercially produced green leafy vegetables. They are one of the most accessible year-around sources of carotenoids in Sri Lanka. In contrast to fruits, green leafy vegetables are known to have a strikingly constant qualitative pattern of carotenoids as they are vegetatively propagated.

Besides β-carotene, other main carotenoid found in dark green leafy vegetables is lutein, which is more essential for the health of the eye. However, initial studies were focused on β-carotene. As determined spectrophotometrically, the β-carotene content of dark green leafy vegetables commonly consumed by the Sri Lankans-mukunuwenna (*Alternanthera sessilis*), kankun (*Ipomea aquatica*), gotukola (*Centella asiatica*), kathurumurunga (*Sesbania grandiflora*), spinach (*Basella alba*) and sarana (*Sesuvium portulacastrum*) (Atukorala, 1985) are given in Table 6.

A study carried out on gotukola (*Centella asiatica*) showed that β-carotene concentration of fresh leaves is markedly reduced when it is processed at 80°C. However, when it is prepared as "kolakenda" (extract of the pounded gotukola with grated coconut), there is no significant reduction in the β-carotene content (Panditharatne et al., 1992). Authors suggested that this is probably due to the fat content in coconut giving thermal stability to β-carotene at high temperature.

Another study including some uncommon green leafy vegetables showed that thebu (*Costus speciosus*) had the RE value of 18.7 μg/g which was followed by kiriala (*Colocasia esculenta*) (17.4 μg/g) and with intermediate values of 7 and 10 μg/g for anguna (*Dregia volubili*) and vel penela (*Cardiospermum helicacabum*), respectively. Other leafy vegetables, namely erabadu

Table 6 Carotenoid contents of green leafy vegetables in Sri Lanka

Green leafy vegetable	Carotenoid content (μg/g)		
	β-Carotene	Lutein	α-Carotene
*Atukorala (1985)			
Mukunuwenna	99.5 ± 16.7	ND	ND
Kankun	81.4 ± 12.0	ND	ND
Gotukola	87.1 ± 19.5	ND	ND
Kathurumurunga	75.8 ± 28.2	ND	ND
Spinach	61.9 ± 10.7	ND	ND
Sarana	76.5 ± 12.2	ND	ND
**Chandrika et al. (2005c)			
Mukunuwanna	252.7 ± 48.2	396.6 ± 16.2	ND
Katurumurunga	486.7 ± 39.7	497.7 ± 47.3	ND
Gotukola	297.4 ± 21.9	1014.8 ± 51.1	ND
Manioc	519.0 ± 176.8	1284.9 ± 63.1	ND
***Colombagama et al. (2006)			
Wel kohila	25.6 ± 4.5	4.1 ± 0.8	5.6 ± 4.2

ND, not determined.

*Locations of the samples: three different areas of Colombo, Sri Lanka, Mean ± SEM in three samples each in duplicate, quantification by spectrophotometrically

**Locations of the samples: Colombo and its suburbs, Sri Lanka, Mean ± SD in five samples each in duplicate, values given on the basis of dry weight, quantification by HPLC.

***Values given on the basis of fresh weight, quantification by HPLC.

(*Erythrina variegate*), ikiriya (*Hydrophila asiatica*), kankun (*Ipomea aquatica*), thampala (*Amaranthus oleraceus*), water spinach (*Basella alba*), and yellow pumpkin (*Cucurbita maxima*), had values between 2.2 and 5.6 μg/g for RE. Among the carotenoids, β-carotene was the major carotenoid detected, which was followed by α-carotene. γ-Carotene showed the lowest content except for kiriala where its concentration was higher than the α-carotene (Ratnayake et al., 1993).

Chandrika et al. (2005c) reported on the carotenoids of green leafy vegetables commonly consumed by Sri Lankans including mukunuwanna, katurumurunga, gotukola, and manioc (*Manihot esculenta*). The qualitative HPLC profiles of the extracts from these leafy vegetables are generally the same, and the major difference appeared to be the concentrations of the individual carotenoid. These leafy vegetables had been found to have markedly higher lutein contents. β-Carotene is the only provitamin A carotenoid detected. Manioc leaf reported the highest β-carotene content which is followed by katurumurunga. Mukunuwanna and gotukola showed the lowest values of β-carotene (Table 6).

Wel kohila (*Syngonium angustatum*) leaf contains β-carotene as well as lutein. This study revealed the presence of α-carotene as well (Colombagama et al., 2006).

In vitro studies carried out on traditional preparations of green leafy vegetables mentioned in Chandrika et al. (2005c), sarana (*Trianthema decandra*), thampala (*Amaranthus caudatus*), and nivithi (*Spinacea oleracea*), showed that fat promotes the in vitro bioaccessibility of carotenoids. The contribution to recommended daily allowance (RDA) of retinol from preparation methods with coconut ("malluma," "stir-fried," and "coconut milk curry" preparations) was significantly higher than the preparation methods without fat (Chandrika et al., 2006).

THE EFFECT OF OTHER FACTORS ON CAROTENOIDS OF PLANT MATERIALS

Kohila stem (stems of *Lasia spinosa*) had been chosen to study the effect of maturity on carotenoids, because it is a good nutritive food source that is consumed at all stages of maturity. In this case, "maturity factor" could easily be maintained as the only variable. Twelve-year-old stems of Type A (sagittate leaf type) and B (pinnatifid leaf type) from kohila were used in this study. Total provitamin A carotenoid content of the most immature stems was 18.8 and 101.0 μg/100 g FW for type A and B, respectively. There was a 14- and 2.3-fold higher total provitamin A carotenoid content in the edible part of the most mature stems of the type A and B, respectively, compared to the most immature stems. Thus RE increases with maturity (Priyadarshani and Jansz, 2006).

Priyadarshani et al. (2007b) has reported on the effect of storage on carotenoids of orange and yellow-fleshed sweet potato variety termed "Gannoruwa white." This was done to determine whether enzymes responsible for carotenogenesis were active during post-harvest storage. This study is important as sweet potato is a root crop that can be stored more than ten days at ambient conditions without spoilage. The storage method is compatible with that used by the vitamin A deficient groups not having refrigeration facilities.

Storage conditions were: (i) one lot was stored in a room (in the shade but open to the atmosphere), (ii) other lot was stored in a jutehessian (gunny) bag under similar atmospheric conditions. Storage was done at ambient temperature (25–30°C) and relative humidity varied from 70 to 90% in day and night time, respectively. Storage period was 12 days. The major carotenoid present was β-carotene. β-Carotene levels in open and bagged samples on first day of harvest were 80.1 and 82.6 μg/100 g DW, respectively. These levels increased by 2.2- and 2.3-fold for open and bagged samples, respectively, with storage. In addition, the content of an unidentified carotenoid increased by the same magnitude (2.4- and 3.0-fold for open and bagged samples, respectively). This indicated that the enzymes involved in biosynthesis of carotenoids were apparently functioning normally under the storage conditions showing carotenogenesis occurred during postharvest storage.

Another study has reported the effect of storage on lycopene of tomato. In this study, the samples were stored in the refrigerator. Storage period was 14 days. Results indicated that lycopene content increased with storage time for the tomato cultivars of "Thilina" and "Tharindu" where "Thilina" showed a significant increase in both from day 0–7 ($p = 0.02$) and day 7–14 ($p = 0.01$). However, "Tharindu" had not shown a significant increase from day 0–7 ($p > 0.05$) but showed a significant increase from day 7–14 ($p = 0.03$). In contrast to this, cultivar "Mahesha" showed no increase, instead showed a decline in lycopene content. The authors stated that these observations are due to the different effects of refrigeration temperature on the biosynthesis of carotenoids during storage (Thillan and Ekanayake, 2009).

OVER-FEEDING OF CAROTENOIDS

Feeding of high carotenoid containing fruits and vegetables is a recommended advice to low income groups in Sri Lanka. Over feeding of carotenoids develops "Hypercarotenemia" which is a benign condition characterized by carotenodermia caused by the deposition of carotenoids in the stratum corneum of the epidermis resulting in jaundice-like yellowing of the skin and high plasma carotenoid concentrations (Rock, 1997; Garrow et al., 2000). Failure to split provitamin A carotenoids due to genetic defects of the enzyme also can lead to metabolic carotenemia under less or normal intake of carotenoids, but this is very rare (Monk, 1982).

Research on hypercarotenemia is important as carotenoid food intake is sometimes very high in Sri Lanka giving rise to this problem. In Sri Lanka, there are only a few studies on hypercarotenemia. Priyadarshani et al. (2009) studied the serum carotenoids of eight cases of hypercarotenemia in Sri Lanka. In six of the cases, a common serum profile was shown with the contents of β-carotene: 9.9–35.7 μg/dL, β-cryptoxanthin and monohydroxy metabolites collectively 5.3–48.5 μg/dL, and six-eight metabolites (22.5–282.1 μg/dL) corresponding to di, tri, or polyhydroxy derivatives of the β-ionine rings. In all these cases, vitamin A levels were within normal range (32–61 μg/dL). The other two cases were abnormal. One showed low β-carotene (3.5 μg/dL) and neither β-cryptoxanthin nor monohydroxy metabolites but other normal di, tri, poly, etc., hydroxy metabolites were present (128.2 μg/dL). However, vitamin A level was high (75.2 μg/dL) but still lower than upper limit of normal. The other case showed high β-carotene (212.3 μg/dL) and β-cryptoxanthin (49.3 μg/dL) and absence of other normal hydroxy metabolites. Instead, there was a more hydrophobic metabolite (343.9 μg/dL) than β-cryptoxanthin as judged by higher retention time in HPLC. This study showed that excessive intake of boiled and homogenized carrot and ripe papaw are the main contributing factors to hypercarotenemia.

Wageesha et al. (2008) carried out a study to determine the food types that the hypercarotenemic infants or children had ingested from their serum carotenoid profile. α-Carotene and β-carotene peaks were observed from the HPLC profiles when carrot and pumpkin were high in the diet. Similarly, when papaw consumption was high, in addition to above two carotenoids, a peak corresponding to β-cryptoxanthin was observed. Out of the 15 hypercarotenemic subjects studied, 67% had a high amount of papaw in their diet. Therefore, the β-cryptoxanthin levels were high (mean = 30.9 μg/dL) compared to those who had not consumed papaw. For other subjects, the major contributors were carrot and/pumpkin as their α-carotene (mean = 36.3 μg/dL) and β-carotene (mean = 31.5 μg/dL) levels were high. Another finding was that in more than 80% of the subjects, α-carotene levels were higher than β-carotene levels. Authors suggest that this might be due to higher conversion efficiency of β-carotene compared to α-carotene.

CONCLUSION

Of the different studies conducted in Sri Lanka, among the fruits, papaw is a good source of provitamin A carotenoids and *Pouteria campechiana* is a good source of β-cryptoxanthin. Mango and cantaloupe melon are good sources of β-carotene, whereas red-fleshed guava and watermelon are rich sources of lycopene. Among the nonleafy vegetables carrot, pumpkin variety "Arjuna" and yellow, orange-fleshed sweet potato variety "420027" are rich sources of β-carotene, and tomato is a good source of lycopene. Green leafy vegetables are a very good source of β-carotene and lutein. Over-consumption of carotenoid-rich foods may give rise to complications in individuals who have defects in carotenoid metabolism. The major problem of compiling a country database is due to high biological variations caused by genetic, climatic, edaphic, storage, and maturity factors. Therefore, care in sampling is crucial. Bioavailability, RE, and RAE are difficult to predict as they are affected by many factors such as cooking method, matrix effects, and genetic effects in control of absorption. Therefore, RE and RAE should be calculated separately for different types of plant materials; may even be expanded so that a unique calculation depending on plant material and method of cooking could be devised.

ACKNOWLEDGMENT

Grant No: IPICS SRI: 07 is gratefully acknowledged.

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