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Advances in Food Carotenoid Research: Chemical and Technological Aspects, Implications in Human Health

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ABSTRACT

This article reviews advances on carotenoid analysis, carotenoid composition of foods and influencing factors, alterations during processing and storage, and the role of food carotenoids in human health. Substantial progress has been achieved in refining analytical methods to assure the accuracy of carotenoid data. Although carotenoid analysis is inherently difficult and error prone, more complete and reliable databases are now available, especially of carotenoids considered important to human health. The carotenoid composition of foods vary qualitatively and quantitatively. Even in a given food, compositional variability occurs because of factors such as maturity, variety/cultivar, climate or season, part of the plant consumed, production practices, post-harvest handling, processing and storage conditions. During processing, isomerisation of trans-carotenoids to the cis-forms occurs, with consequent alteration of the carotenoids' bioavailability and bioconversion. The principal cause of carotenoid loss during processing and storage of food is enzymatic or non-enzymatic oxidation. The bioavailability of carotenoids is influenced by many factors, and studies on this important topic have been confounded by wide variation in individual response and the existence of non-responders. In spite of reported evidence to the contrary and some weaknesses in earlier studies, the weight of evidence favours improvement of the vitamin A status of deficient children and adults on consumption of food sources of provitamin A. This is supported by more recent studies. More evidence has also been presented for the role of carotenoids, provitamins A or not, in reducing the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration.

INTRODUCTION

Among the bioactive phytochemicals, carotenoids have been the most studied from various aspects, including structure elucidation, analysis and composition, physico-chemical properties, stability and alterations during food processing and storage, kinetics and mechanism of degradation, biosynthesis and metabolism, carotenogenic food product development, chemical synthesis, biotechnological production, formulation and properties as natural food colourants, implications in

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human health and structure-function relationship. Numerous papers about these compounds are published each year, and there is a need to integrate the findings so that the knowledge acquired and the practical significance of the results are focalised. The present article focuses on the following topics: (a) progress achieved in carotenoid analysis to guarantee the accuracy of compositional data, (b) the carotenoid composition of foods and the factors responsible for variation in composition, (c) effects of processing and storage of food, and (d) the different roles of food carotenoids in human health.

Carotenoids are natural pigments synthesised by plants, algae, fungi, yeasts and bacteria, but are merely accumulated from the diet, unchanged or slightly modified, in some animals. Carotenoids in foods are C40 tetraterpenoids formed from eight C5 isoprenoid units joined head-to-tail, except at the center where a tail-to-tail linkage reverses the order and results in a symmetrical molecule. Lateral methyl groups near the center are separated by six carbon atoms and the others by five. The most prominent feature, responsible for their special properties and functions, is a centrally located, extended double-bond system. The basic skeleton may be modified in many ways, such as cyclisation, hydrogenation, dehydrogenation, introduction of oxygen-containing functions, rearrangement, chain shortening or combinations thereof, resulting in an immense array of structures. More than 650 carotenoids, exclusive of cis and trans isomers, have been isolated and characterised from natural sources (Kull & Pfander, 1995). These compounds are broadly classified into carotenes (hydrocarbons) and xanthophylls (carotenoids with one or more oxygen substituents).

In foods, about a hundred carotenoids have been found. Typically a food would have one to five major carotenoids with a series of minor carotenoids in trace or very small amounts (Rodriguez-Amaya, 1999a). The carotenoids most investigated in terms of human health are β-carotene (β,β-carotene), α-carotene (β,ε-carotene), β-cryptoxanthin (β,β-caroten-3-ol), lycopene (ψ,ψ-carotene), lutein (β,ε-carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol). These are also the principal carotenoids encountered in human blood (Epler, Ziegler & Craft, 1993) and, except for zeaxanthin, the carotenoids most commonly found in foods. Also widely distributed is violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol), but it is highly labile (i.e. it can be easily degraded during analysis and food processing and storage) and its human health implications have yet to be investigated.

CAROTENOID ANALYSIS

The need for reliable data on food carotenoids is widely acknowledged in the international literature in different fields. Some of the discrepant results in studies on the bioavailability and the effect of dietary carotenoids on vitamin A status, as well as in epidemiological studies correlating carotenoid intake and the incidence of diseases, can be attributed, at least in part, to unreliable data on the carotenoid contents of foods. Accurate carotenoid analysis has been pursued for years. Refinement of analytical methods, identification of sources of errors and means to avoid them, and implementation of quality assurance have been undertaken.

Trends in carotenoid analysis of foods reflects not only advances in analytical methodology and instrumentation, but also greater understanding of the role of these compounds in human health. Total carotenoids, used in earlier years mostly to assess carotenoids as natural colourants, is not adequate for health purposes. For a long time carotenoid analysis involved quantification of only the major provitamin A carotenoids. With the

mounting evidence on the importance of carotenoids in reducing the risk of degenerative diseases, such an action being independent of the provitamin A activity, both provitamin A and nonprovitamin A carotenoids are now being quantified, sometimes up to the separation of trans- and cis-isomers.

Several factors make this analysis inherently difficult: (a) existence of a large number of carotenoids, (b) qualitative and quantitative variation of the carotenoid composition of foods, (c) wide differences in carotenoid levels in a given food, (d) uneven distribution of carotenoids within a sample and between samples of a given food, (e) variation in the nature of the matrix, and (d) susceptibility of carotenoids to isomerisation and oxidation during analysis and during storage of samples prior to analysis (Rodriguez-Amaya, 1989, 1999a; Rodriguez-Amaya & Amaya-Farfan, 1992).

Typical sources of errors in carotenoid analysis are: analytical samples not representing the food lots under investigation, incomplete extraction, physical losses during the different steps, incomplete chromatographic separation, erroneous identification, faulty measurement and calculation, isomerisation and oxidative degradation during analysis and/or storage of the sample before analysis. The main problem in carotenoid analysis arises from their instability, thus, precautionary measures to avoid artifact formation and quantitative losses should be standard practice in the laboratory.

The current method of choice for carotenoids is high performance liquid chromatography (HPLC). Recent review articles cite the different HPLC methods that have been developed for qualitative and quantitative analyses of carotenoids in foods (Su, Rowley & Balazs, 2002; Quirós & Costa, 2006). But even this technique is subject to several sources of errors: (a) incompatibility of the injection solvent and the mobile phase, (b) erroneous identification, (c) impurity, instability and unavailability of standards, (d) quantification of highly overlapped peaks, (e) low recovery from the HPLC column, (f) inaccuracy in the preparation of standard solutions and in the calibration procedure, (g) calculation errors (Khachik *et al.*, 1988; Craft, 1992; Epler *et al.*, 1992, 1993; Scott, 1992; Hart & Scott, 1995; Kimura & Rodriguez-Amaya, 1999; Rodriguez-Amaya & Kimura, 2004).

The physico-chemical properties and the biological activities of carotenoids are intimately related to their structures. Thus, conclusive identification is a fundamental part of carotenoid analysis. Since inconclusive or even erroneous identifications could be found in the literature, Pfander, Riesen and Niggli (1994) and Schiedt and Liaaen-Jensen (1995) recommended the following minimum criteria for the identification of carotenoids: (1) the visible (or ultraviolet for shorter chromophores) absorption spectrum (λmax and fine structure) in at least two different solvents must be in agreement with the chromophore suggested; (2) chromatographic properties must be identical in two systems, preferably TLC (RF) and HPLC (tR), and co-chromatography with an authentic standard should be demonstrated; and (3) a mass spectrum should be obtained, which allows at least the confirmation of the molecular mass. The inclusion of a mass spectrum as a minimum requirement for identification will, however, limit carotenoid analysis to a very few laboratories around the world, precluding its execution in places where it is urgently needed.

Carotenoids with known structure can be conclusively identified by the combined use of chromatographic behavior, UV-visible absorption spectra and specific group chemical reactions to confirm the type, location and number of functional groups in xanthophylls (Azevedo-Meleiro & Rodriguez-Amaya, 2004). Alternatively, for well-equipped laboratories, conclusive

identification of these carotenoids can be achieved by the combined use of the retention times, co-chromatography with authentic carotenoid standards, the UV-visible spectra obtained with a photodiode array detector and the mass spectra obtained with a mass detector. The retention time together with co-chromatography with standards cannot be used as sole identification criteria. Mass spectrometry and nuclear magnetic resonance spectroscopy are indispensable in the elucidation of the structure of an unknown carotenoid.

Three interlaboratory studies on food carotenoids have been published. In an extensive study involving European laboratories experienced in carotenoid analysis, which spanned three years, analytical variability in the different stages of the analytical procedure was systematically verified (Scott *et al.*, 1996). A lyophilised vegetable mix was used as potential reference material and the laboratories employed both a common method and their own ("in-house") methods. Saponification was not carried out. The results suggested that the preparation of the extract might account for about 13% of the overall variance of around 23%. Analytical variability differed with different carotenoids, being highest with lycopene.

Another study organised by the US National Institute of Standards and Technology (NIST) (Sharpless *et al.*, 1999) used a baby food composite as reference material, and certified and reference values were obtained for several carotenoids. The relative expanded uncertainties were higher than those generally expected for certified values. The carotenoid concentrations obtained differed with different HPLC columns. Repeatibility of measurements made on directly saponified test material was better than that for test material that was saponified after extraction.

In a second European study, β-carotene and its cis-isomers were determined in commercial foods (margarine, vitamin drink, pudding powder, natural mixed vegetable) chosen according to type of matrix, range of concentration and availability in food stores (Schuep & Schierle, 1997). The supplemented drink showed the best repeatability and reproducibility while the pudding powder gave the worst results. Considerable differences were observed between total β-carotene and trans-β-carotene. These differences varied among laboratories, depending on the separation efficiency of the HPLC system used.

The analytical efforts through the years have resulted in better knowledge of the nature, distribution and levels of carotenoids in foods. Reliable databases are now available, although analytical errors can still be perceived in the literature.

FOOD SOURCES OF CAROTENOIDS

Because plants are able to synthesise carotenoids de novo, carotenoids are widely distributed in plant-derived foods and the composition is enriched by the presence of small amounts of biosynthetic precursors and derivatives of the major carotenoids (Rodriguez-Amaya, 1993). Incapable of carotenogenesis, animals depend on dietary carotenoids, thus the occurrence of these compounds is limited and the levels are much lower in foods of animal origin.

Some examples of food sources of the six carotenoids considered important to human health are shown in Table 1 (Holden *et al.*, 1999; Rodriguez-Amaya, 1999b; O'Neill *et al.*, 2001). β-Carotene is the most widespread food carotenoid. It is accompanied by α-carotene in a few foods, generally at much lower concentrations. β-Cryptoxanthin is the principal carotenoid of orange-fleshed fruits other

Table 1. Food sources of carotenoids considered important to human health[1]

Carotenoid	*Food sources*[2]
α-carotene	buriti (*Mauritia vinifera*), carrot, *Cucurbita moschata*, red palm oil
β-carotene	acerola (*Malpighia glabra*), apricot, bocaiúva (*Acronomia makayáyba*), broccoli, buriti, cantaloupe, carrot, *Cucurbita maxima*, *Cucurbita moschata*, green leafy vegetables, mamey (*Mammea Americana*), mango, peach palm (*Bactris gasipaes*), pink grapefruit, red palm oil, red pepper, yellow and orange-fleshed sweet potato, tucumã (*Astrocaryum vulgare*)
β-cryptoxanthin	caja (*Spondias lutea*), pitanga (*Eugenia uniflora*), red pepper, tree tomato (*Cyphomandra betacea*)
lycopene	pink grapefruit, pink-fleshed guava, red-fleshed papaya, pitanga (*Eugenia uniflora*), tomato, watermelon
lutein	green leafy vegetables, broccoli, Brussels sprout, corn, *Cucurbita maxima*
zeaxanthin	Buriti, corn

[1]Compiled from Holden *et al.* (1999), Rodriguez-Amaya (1999b) and O'Neil *et al.* (2001).
[2]Only foods containing 10 μg/g or more of the carotenoid are included. The scientific names are given for foods which are not so common.

than those mentioned in the table, such as nectarine, orange-fleshed papaya, orange and peach but at concentrations lower than 10 μg/g. Lutein, the dihydroxy derivative of β-carotene, is more widely distributed in foods than zeaxanthin, the dihydroxy derivative of β-carotene, contrary to the occurrence of their parent compounds. Zeaxanthin is the main carotenoid of the Brazilian fruit piqui (*Cariocar vilosium*) but the level is low. Although tomato and tomato products are the lycopene sources most focalised in terms of human health, lycopene is the predominating carotenoid in several fruits.

Carotenoids are invariably found in photosynthetic tissues in which they perform the dual role of being accessory light-harvesting pigments at wavelengths where chlorophyll is not absorbed, and of protecting chlorophyll from photodegradation. Dark green leafy or non-leafy vegetables are therefore rich sources of carotenoids with a defined qualitative pattern. Lutein predominates, followed by β-carotene, violaxanthin and neoxanthin as the major carotenoids. An exception is lettuce, where lactucaxanthin is also a principal carotenoid, and the lutein level is only slightly higher or almost the same as that of β-carotene (Kimura & Rodriguez-Amaya, 2002, 2003). Lutein had been underestimated in many published papers to date because it can be easily lost during analysis, especially in the saponification

step (Kimura, Rodriguez-Amaya & Godoy, 1990; Sá & Rodriguez-Amaya, 2004).

The carotenoid composition of fruits and fruit vegetables is much more complex and variable, with variations even in the principal carotenoids. The hydroxycarotenoids are mostly esterified with fatty acids. Carotenes predominate in the few carotenogenic roots (i.e. carrot, sweet potato). Lutein and/or zeaxanthin are major carotenoids in egg yolk, depending on the feed, and astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) is the red carotenoid of crustaceans, salmon and trout.

The importance of analysing indigenous foods is highlighted in Table 1. Many native Brazilian fruits and vegetables included in this table, e.g. acerola, bocaiúva, buriti, tucumã, pitanga, indigenous green leafy vegetables, indigenous varieties of squash and pumpkin, have higher carotenoid contents than internationally known commercial crops (Rodriguez-Amaya, 1999b).

COMPOSITIONAL VARIATION ALONG THE FOOD CHAIN

There is growing recognition that the composition of carotenoids and other bioactive compounds in foods can be optimised through agriculture (van den Berg *et al.*, 2000). This has to be complemented by post-harvest handling, processing and storage procedures that avoid loss of the desired food components (Rodriguez-Amaya, 1997). To put this strategy into practice, the compositional variation throughout the food chain has to be known. It is also important, for the accuracy of carotenoid data, that natural variability be distinguished from analytical inaccuracy.

Aside from the remarkable variation between foods, there is now ample demonstration that the carotenoid composition of a given food varies because of factors such as cultivar/variety, part of the plant consumed, maturity at harvest, climate/geographic site of production, farming practices, harvesting and post-harvest handling, processing method and conditions, storage conditions.

Maturation or ripening in fruits and fruit vegetables are generally accompanied by enhanced carotenogenesis, the carotenoids increasing markedly both in number and quantity (Rodriguez-Amaya, 1993, 1999b). In leafy vegetables, the ripening pattern is not as defined. The principal carotenoids of kale, endive and lettuce increased on maturation, but in New Zealand spinach, the young leaves had slightly higher carotenoid levels than the mature leaves (Azevedo and Rodriguez-Amaya, 2005a,b).

Exposure to sunlight and high temperature enhances carotenoid biosynthesis. Thus, tropical fruits are generally coloured by carotenoids, contrary to fruits from colder regions that owe their colour mostly to anthocyanins. Moreover, fruits (e.g. acerola, papaya and mango) of the same cultivar produced in the hot Northeastern states of Brazil were all shown to have markedly higher carotenoid contents than those produced in the temperate Southeastern state of São Paulo (Rodriguez-Amaya, 1999b).

Sunlight and high temperature, however, can also promote photodegradation. Leafy vegetables grown in open fields, for example, were found to have lower carotenoid levels in the summer than in the winter (Heinonen *et al.*, 1989; Ramos and Rodriguez-Amaya, 1987), indicating that photodegradation prevailed over increased carotenogenesis. On the other hand, leafy vegetables cultivated in plots protected with polyethylene roofing (Azevedo-Meleiro and Rodriguez-Amaya, 2005b) had higher carotenoid contents in the summer than in the winter. It is possible that the plants were protected from excessive sunlight during the summer, but might have restricted exposure to sunlight

in winter. In hydroponic curly lettuce, also cultivated under a polyethylene covering, the lutein, β-carotene, violaxanthin and neoxanthin contents were lower than those of the same variety of lettuce, at the same maturity, taken from a neighboring open field, both samples being collected at the same time in winter (Kimura & Rodriguez-Amaya, 2003). Tomatoes produced in greenhouses in winter had only one-third of the total carotenoid content of out-door produce (Muller, 1997).

Most fruits and fruit vegetables have higher carotenoid levels in the peel than in the pulp, with the exception of fruits like pink-fleshed guava, in which lycopene is concentrated in the pulp (Rodriguez-Amaya, 1993, 1999b).

Farming practices may also influence the carotenoid composition. For example, comparison of kale of the same cultivar at the same stage of maturity produced in neighbouring farms revealed significantly higher concentrations of all constituent carotenoids in samples collected from an organic farm than from a conventional farm where agrochemicals were utilised (Mercadante and Rodriguez-Amaya, 1991). In contrast, a study comparing conventionally produced and hydroponic leafy lettuce found no significant difference in the constituent carotenoids (Kimura and Rodriguez-Amaya, 2003).

During post-harvest transport or storage, carotenoid biosynthesis may continue, raising the carotenoid content, provided that the fruit, fruit vegetable or root is kept intact, preserving the enzyme system responsible for carotenogenesis (Rodriguez-Amaya, 1997). In leaves and other vegetables, post-harvest degradation of carotenoid may prevail, especially at high storage temperature and under conditions that favour wilting.

In processed foods, aside from the above factors that influence the raw material, the carotenoid composition will depend on the type and conditions of processing and storage.

ALTERATIONS DURING PROCESSING AND STORAGE OF FOODS

Alteration or loss of carotenoids occur during processing and storage of foods through physical removal (e.g. peeling), geometric isomerisation, and enzymatic or non-enzymatic oxidation (Rodriguez-Amaya, 1997, 1999c, 2002). Thus, retention of carotenoid is a major concern. Attention is often focused on industrial processing, but home preparation can also cause carotenoid losses, sometimes to a greater extent.

Being highly unsaturated, carotenoids are prone to isomerisation and oxidation during processing and storage of foods. Transformation of the *trans*-carotenoids, their usual form in nature, to the *cis*-isomers, is now well documented. It is promoted by acids, heat and light, and results in loss of provitamin A activity and alteration of bioavailability/metabolism. The release of organic acids during slicing, shredding, pulping or juicing is sufficient to provoke *trans-cis* isomerisation, but this isomerisation occurs to a greater extent during thermal processing.

Cis-provitamins A have long been attributed with lower vitamin A activity than their *trans*-isomers. More recently, *trans*-β-carotene was reported to be preferentially absorbed over 9-*cis*-β-carotene in humans (Gaziano *et al.*, 1995; Stahl *et al.*, 1995; Ben-Amotz & Levy, 1996) and ferrets (Erdman *et al.*, 1998). On the other hand, the *cis*-isomer of the vitamin A-inactive lycopene was found to be more bioavailable than *trans*-lycopene in ferrets (Boileau *et al.*, 1999).

The major cause of carotenoid loss is enzymatic or non-enzymatic oxidation, which depends on the availability of oxygen and the structure of the carotenoid. It is stimulated by light, heat, metals, enzymes and peroxides and is inhibited by antioxidants. Oxidative degradation is known to increase with the destruction of the food cellular structure,

greater surface area or porosity, length and severity of the processing conditions, length and temperature of storage, as well as use of packaging permeable to oxygen and light. Enzyme-catalysed oxidation can occur to a greater extent than thermal degradation. Typically, carotenoid loss in enzymatic oxidation happens rapidly, immediately after tissue disruption, after which the carotenoid concentrations stabilise. Non-enzymatic oxidation is usually characterised by a lag phase, followed by rapid decrease of carotenoids, coherent with a free radical mechanism.

Enzymatic oxidation can occur prior to heat treatment, during peeling, slicing, shredding, pulping or juicing. It is recommended that foods be consumed or thermally processed immediately after these operations. Enzyme-catalysed oxidation can also take place in minimally processed (Azevedo-Meleiro & Rodriguez-Amaya, 2005a,b) and in unblanched, frozen foods.

Contrary to lipid oxidation, for which details of the process are known, carotenoid oxidation is not well understood. Oxidation of both *trans*- and *cis*-carotenoids takes place, epoxidation and cleavage to apocarotenals being the initial steps (Figure 1). Subsequent fragmentations result in a series of low mass compounds. Completely losing their colour and biological activities, the carotenoids give rise to volatile compounds which contribute to the aroma/flavour, desirable in wine and tea, but undesirable in dehydrated carrot.

The cellular structure that protects carotenoids in nature limits their bioavailability. Recent studies have shown that processing (i.e. mechanical matrix disruption and/or heat treatment) can increase the bioavailability of carotenoids (Stahl & Sies, 1992; Gärtner, Stahl & Sies, 1997; Rock *et al.*, 1998; van het Hof *et al.*, 2000a) by softening or breaking the cell walls and denaturing proteins complexed with carotenoids, thus facilitating the release of carotenoids from the food matrices. Thus, processing conditions should be optimised to increase bioavailability without much degradation of the carotenoids.

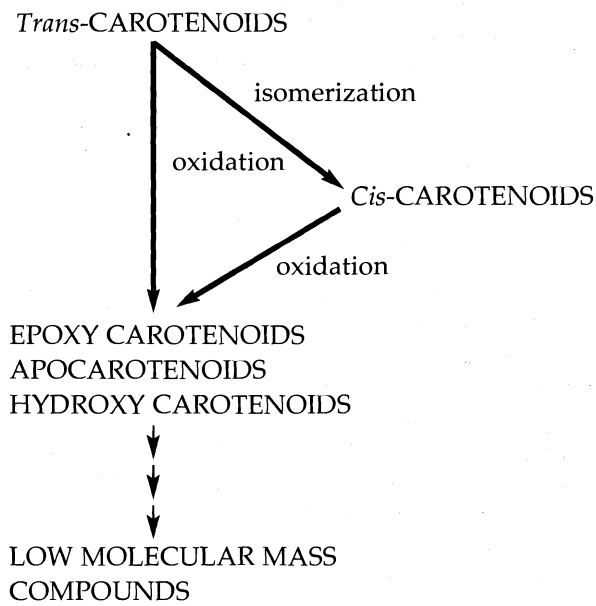

Figure 1. Possible scheme for the degradation of carotenoids (Rodriguez-Amaya, 1999c)

BIOAVAILABILITY, BIOCONVERSION, VITAMIN A STATUS

The provitamin A activity of some carotenoids has been known for a long time. Structurally, vitamin A (retinol) is essentially one-half of the molecule of -carotene with an added molecule of water at the end of the lateral polyene chain. Thus, this carotenoid is the most potent provitamin A, to which 100% activity has been attributed. The minimum requirement for a carotenoid to have provitamin A activity is an unsubstituted β-ring with a polyene chain of 11 carbon atoms. Thus, α-carotene and β-cryptoxanthin have about 50% of the activity of b-carotene while lutein and zeaxanthin are vitamin A-inactive.

There have been intense investigations into, and discussions of, the bioavailability of carotenoids, principally provitamin A carotenoids. Bioavailability refers to the amount of provitamin A that is absorbed from the gut and becomes available to target tissues. Conversion of absorbed provitamin A to retinol is called bioconversion. Adequate absorption of carotenoids from the diet requires: (a) digestion of the food matrix, (b) formation of lipid micelles in the gastrointestinal tract, (c) uptake of carotenoids by intestinal mucosal cells, and (d) transport of carotenoids and their metabolic products to the lymph and portal circulation (Erdman, Bierer & Gugger, 1993; Castenmiller & West, 1998).

The study of bioavailability is highly complicated because of the many factors that can affect it. Food-related factors are: the quantity and structure of the carotenoid, the nature of the matrix and the physical state of the carotenoid, food preparation or processing method, competition/interaction with other carotenoids, and the presence of other components of the diet (e.g. fat increases while fibre decreases bioavailability) (Castenmiller & West, 1998; van het Hof *et al.*, 2000b; Yeum & Russell, 2002). In relation to the individual, the influencing factors are nutritional state (e.g. vitamin A deficiency increases while protein deficiency lowers bioavailability), poor absorption of lipids, infections, parasite infestation and genetic factors. Bioavailability studies are confounded by very large variations in inter-individual responses and the existence of non-responders.

In 1995, de Pee *et al.* (1995) reported that the consumption of stir-fried leafy vegetables by Indonesian lactating mothers with low or marginal levels of serum retinol failed to improve the vitamin A status. Many studies previous to that of de Pee *et al.* showed improvement in the vitamin A status of vitamin A-deficient children in different countries with the consumption of provitamin A-rich foods (dark green leafy vegetables, buriti, carrot, red palm oil, papaya). De Pee and West (1996) pointed out weaknesses in these earlier studies: lack of negative and/or positive control groups, high drop-out rate, small number of individuals per treatment group with very variable response, no baseline data. Nevertheless, as grouped by Nestel and Trumbo (1999), of the 21 studies carried out with children from 1968 to 1994, improvement of vitamin A status was found in: 7 out of 9 studies that did not have adequate control groups; 4 out of 5 studies with positive control group; 1 out of 1 study with negative control group; 5 out of 6 studies with both negative and positive control groups. Of the only 4 studies that showed no effect, 2 included children who were overall not vitamin A-deficient. All of the above studies involved measurement of plasma retinol, which is homeostatically controlled. Thus, in spite of the experimental flaws, the weight of evidence favours β-carotene-rich fruit and vegetable intake improving vitamin A status in deficient children.

Subsequently, Jalal *et al.* (1998)

reported increased serum retinol in Indonesian children with the incorporation in the meals of β-carotene sources, principally sweet potato. Takyi (1999) found that consumption of dark green leafy vegetables with added fat-enhanced serum retinol in Ghanian children. Supplementation with dried mango and a source of fat resulted in a small increase in plasma retinol in Gambian children (Drammeh *et al.*, 2002). Using the modified-relative-dose-response test, van Jaarsveld *et al.*, (2005) observed that South African children had better vitamin A status after consumption of boiled and mashed orange-fleshed sweet potato.

Red palm oil supplementation improved the vitamin A status of pregnant women in Tanzania (Lietz *et al.*, 2000), and of both pregnant/lactating mothers and their infants in India (Radhika *et al.*, 2003) and Honduras (Canfield & Kaminsky, 2000). The latter authors found that the positive effect achieved with palm oil consumption was comparable to that from supplementation with purified β-carotene.

Studies using isotope dilution to determine vitamin A status, a more accurate assessment method than blood retinol levels, have also been concluded. Tang *et al.* (1999) confirmed that green-yellow vegetables maintained total body stores of vitamin A in Chinese children. In Filipino children, the bioconversion of plant carotenoids to vitamin A varied inversely with vitamin A status, improvement in status after dietary intervention being strongly influenced by total body stores of vitamin A but little or not at all by serum retinol (Ribaya-Mercado *et al.*, 2000). Daily consumption of cooked, puréed Indian spinach (*Basella alba*) or canned sweet potato had a positive effect on the total body stores in Bangladeshi men (Haskell *et al.*, 2004).

Studying anaemic Indonesian children, de Pee *et al.* (1998) calculated the relative vitamin A equivalency of β-carotene to be: 26 μg of β-carotene from leafy vegetables and carrots corresponded to 12 μg of β-carotene from fruit, and equaled 1 μg of pre-formed vitamin A in vitamin A-rich foods. Boileau *et al.* (1999) presented a bioavailability ranking scheme in which the highest bioavailability was attributed to formulated carotenoids in water-dispersible beadlets (formulated natural or synthetic carotenoids), followed in decreasing order of bioavailability by carotenoids in oil form (natural or synthetic), fruits (e.g., papaya, peach, melon), tubers (e.g., squash, yam, sweet potato), processed juice with fat containing meal (e.g., tomato juice), mild cooked yellow/orange vegetables (e.g., carrot, pepper), raw juice without fat (e.g. tomato) raw yellow/orange vegetables (e.g. carrot, pepper) and raw green leafy vegetables (e.g. spinach) with the lowest bioavailability. Bioavailability obviously varies with different foods, but it also depends on how the food is prepared. Interestingly, the recent study of Haskell *et al.* (2004), which used the deuterated-retinol dilution technique with Bangladeshi men, estimated the vitamin A equivalency factors (β-carotene:retinol, wt:wt) to be about 13:1 for sweet potato, 10:1 for Indian spinach and 6:1 for synthetic β-carotene.

As a consequence of the reported lower bioavailability of provitamin A carotenoids than previously thought, the conversion factors were increased from 6:1 to 12:1 for β-carotene [12 β-carotene = 1μg retinol = 1 retinol activity equivalent (RAE)] and from 12:1 to 24:1 for α-carotene, γ-carotene and β-cryptoxanthin (24 μg of other provitamin A carotenoid = 1 μg retinol = 1 RAE) (Institute of Medicine, 2001). However, the possibility of future changes in equivalency is recognised and it is recommended that the actual concentrations of the carotenoids be given in food composition tables (Trumbo *et al.*, 2001), as we have suggested earlier (Rodriguez-Amaya, 1989). Moreover, considering the wide variation in the bioavail-

ability of carotenoids in different foods, it is an oversimplification to have a single conversion factor for β-carotene and a single factor for the other provitamin A carotenoids (Rodriguez-Amaya, 1989, 1996). It is impossible to establish a factor for each food, but factors can be established for different food groups (e.g., fruits, raw leafy vegetables, cooked leafy vegetables). Since the factors cited above are estimated for healthy populations, and considering that bioavailability and bioconversion depend on the vitamin A status (Nestel and Trumbo, 1999; Ribaya-Mercado *et al.*, 2000), another issue is the applicability of these factors in developing countries. This issue merits in-depth study and discussion in the developing world.

CAROTENOIDS AND DEGENERATIVE DISEASES

In more recent years, food carotenoids have been credited with other health-promoting effects: immunoenhancement and reduction of the risk of developing degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration (Gaziano and Hennekens 1993; Krinsky 1993; Bendich 1994; Olson & Krinsky, 1995; Astorg, 1997; Olson, 1999). These physiological activities are unrelated to the provitamin A activity and have been attributed to an antioxidant property, specifically to the ability to quench singlet oxygen and interact with free radicals (Palozza and Krinsky, 1992). However, other mechanisms of action against chronic diseases have been demonstrated for carotenoids such as modulation of carcinogen metabolism, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of cell-to-cell communication and filtering of blue light (Olson and Krinsky, 1995; Astorg, 1997; Olson, 1999; Stahl, Ale-Agha & Polidori, 2002).

Because of the overwhelming emphasis on the antioxidant activity as the mode of action in relation to diseases, the antioxidant capacity of foods have been widely determined *in vitro,* sometimes correlated to the concentrations of the bioactive compounds in the food, and used to predict human health effects. Antioxidants, however, have different modes of action and the assays measure different actions and are carried out under different conditions. For example, the antioxidant capacities of nectarine, peach and plum cultivars were measured and the contribution of phenolic compounds to the antioxidant activity was found to be greater than those of vitamin C and carotenoids (Gil *et al.*, 2002). The two assays used, however, evaluated free radical scavenging and iron-reducing capacities but not singlet oxygen quenching. Carotenoids are the most efficient singlet oxygen quenchers while phenols are excellent chain-breaking antioxidants (Beutner *et al.*, 2001). Moreover, extrapolation of the results of the simple assays to human health effects is increasingly being questioned (Becker, Nissen & Skibested, 2004). Also, assessment of the antioxidant effect in the food itself and of the health effects of antioxidants should be clearly distinguished.

Similar considerations have been raised regarding antioxidant capacity measurement in biological samples (e.g. plasma antioxidant capacity) and it is admitted that no single *in vivo* assay of antioxidant status can be considered sufficient, several measurements being necessary to adequately assess oxidative stress in biological systems (Prior & Cao, 1999).

In the 1980s and early 1990s, numerous retrospective case-control and prospective cohort epidemiological studies in various countries had consistently and strongly shown that dietary intake of b-carotene or its plasma concentration was inversely associated with the incidence of cancer, particularly lung cancer (Ziegler 1991; Block, Patterson & Subar, 1992; van

Poppel and Goldbohm, 1995). This inverse relation was also seen with cardiovascular diseases (Gaziano and Hennekens, 1993; Manson *et al.*, 1993; Kohlmeier and Hasting, 1995). b-Carotene, however, fell into disrepute when intervention studies gave the unexpected finding that this carotenoid given to smokers and asbestos workers enhanced rather than reduced the incidence of lung cancer and cardiovascular disease (ATBC Study Group, 1994; Omenn *et al.*, 1996). These findings led Mayne (1996) to conclude that while the recommendation for increased consumption of carotenoid-rich fruits and vegetables still appeared to stand, the pharmacological use of supplemental b-carotene for the prevention of cardiovascular disease and lung cancer, particularly in smokers, could no longer be recommended.

It was later recognised that β-carotene was administered in the intervention studies at doses (20-30 mg) much higher than the optimum daily intake in the epidemiological studies (about 4 mg), in which the diets consisted of other carotenoids and other food constituents that could act jointly with β-carotene (CARIG, 1996). Moreover, the participants in the intervention studies were heavy smokers or workers exposed to asbestos for a long time; the oxidative stress or cancer process might have reached a stage at which the carotenoid could no longer be effective.

With the above considerations, carotenoids regained their prominence but the current emphasis is on carotenoids other than β-carotene. Moreover, it is increasingly recognised that the protective effect of foods against diseases is due not to a single class of compounds but to a number of bioactive components found in the food. The possibility of synergism between or among these components has been raised (Young & Lowe, 2001). But even if synergism does not exist, considering that the health-promoting food substances have varied modes of action, there would at least be complementation of the various actions, the different compounds acting in different manners and/or at different stages in the development of the disease.

The increase in lung cancer incidence in cigarette smokers and asbestos workers taking high supplemental doses of β-carotene mentioned above (ATBC Study Group, 1994; Omenn *et al.*, 1996) led several authors to consider a possible prooxidant behavior of this carotenoid. According to Palozza (1998) there was available evidence for a prooxidant activity of β-carotene and other carotenoids *in vitro* and *in vivo*, these compounds shifting from being antioxidants to prooxidants, depending on the redox potential of the carotenoid molecule and its biological environment. The prooxidant potency would be determined by several factors, including oxygen tension, carotenoid concentration and interactions with other antioxidants. The high oxygen tension and high carotenoid concentration that promoted prooxidant action *in vitro* are, however, much higher than those found under physiological conditions (Krinsky, 2001). Young and Lowe (2001) found no evidence to support the hypothesis that carotenoids may behave as prooxidants within a biological system. They found it more probable, on the basis of carotenoid performance *in vitro*, that a number of factors may reduce their antioxidant effectiveness *in vivo*, rendering them ineffective against certain reactive oxygen species.

The capacity of carotenoids to quench singlet oxygen has been associated with the conjugated double bond system, the maximum protection being given by those having nine or more double bonds (Foote, Chang & Denny, 1970). The acyclic lycopene was found to be more efficient than the dicyclic β-carotene (di Mascio, Kaiser & Sies, 1989), although both have 11 conjugated double bonds. Carotenoids can quench singlet oxygen in two ways. It can occur by a physical transfer of the excita-

tion energy from singlet oxygen to the carotenoid, resulting in the formation of triplet carotenoid; the subsequent dissipation of this energy as heat regenerates the original carotenoid molecule (Krinsky, 1989). It can also happen by a chemical reaction between singlet oxygen and the carotenoid, resulting in the irreversible destruction of the latter.

The role of lycopene in human health has drawn considerable attention in recent years (Stahl and Sies, 1996; Gerster, 1997; Kohlmeier *et al.*, 1997; Clinton, 1998; Giovannucci 1999; Rao & Agarwal, 1999; Hadley *et al.*, 2002; Khachik *et al.*, 2002; Rissanen *et al.*, 2002), the scientific evidence being stronger in relation to lung, oesophagus and prostate cancer. Lycopene was found to be a more potent inhibitor of the proliferation of various human cancer cells than α- or β-carotene (Levy *et al.*, 1995). A multi-center case-control study, involving 10 European countries, concluded that lycopene or some substance highly correlated might contribute to the protective effect of vegetable consumption on myocardial infarction risk, but associations for α- and β-carotene were largely eliminated (Kolhmeier *et al.*, 1997). A recent 12-year prospective study in the US, however, found that higher intake of foods rich in α- or β-carotene was associated with a reduction in the risk of coronary artery disease but no significant relation with intakes of lycopene, β-cryptoxanthin or lutein/zeaxanthin in women (Osganian *et al.*, 2003).

Lutein and zeaxanthin make up the yellow pigment in the macula of the human retina (Bone *et al.*, 1988; Handelman *et al.*, 1988; Landrum & Bone, 2001) and are believed to be responsible for the protective ophthalmologic effect of carotenoids, acting both as antioxidant and as filter of high energy blue light. Although not all studies showed such a relationship, dietary intake (e.g. consumption of spinach, collard greens, corn, eggs) and serum levels of these carotenoids were found to have inverse relation with the risk of age-related macular degeneration (EDCC, 1993; Seddon *et al.*, 1994; Snodderly, 1995; Moeller, Jacques & Blumberg, 2000), the principal cause of irreversible blindness in the elderly. It was also shown in a study that actually measured lutein and zeaxanthin in the central regions of the retina, that individuals possessing the highest concentrations were 82% less likely to have macular degeneration (Bone *et al.*, 2001). As reviewed by Moeller *et al.* (2000) and Alves-Rodrigues and Shao (2004), lutein has also been consistently linked to the lowering of the risk for cataract development, despite differences in study design, case definition and exposure measurement. Cataract extraction is one of the most frequently performed surgeries in the elderly.

Because of some inconsistencies in research findings and the apparent differences in the efficacy of the different carotenoids, along with the possibility of synergy or at least an additive effect, the prudent recommendation remains to be the increased consumption of a variety of carotenoid-rich fruits and vegetables.

CONCLUDING REMARKS

Food carotenoids will continue to be intensely investigated in the coming years. Although good results can be obtained with HPLC, less expensive, faster and simpler techniques, which do not generate so much used solvent waste (especially potentially toxic waste), are being sought. The need for determining the carotenoid composition of foods, especially in developing countries, is increasingly recognised, considering the diversity of carotenogenic foods in these regions that are not found in developed countries. Even with common foods, because of the natural variation in carotenoid composition, data obtained in one country may not

be relevant in another. Greater utilisation of indigenous carotenoid-rich crops is warranted. More studies will be carried out on the effects of processing and storage, especially with the emerging technologies. Many more studies on bioavailability and the health benefits of carotenoids are anticipated, hopefully clarifying current inconsistencies in research findings.

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REFERENCES

Alves-Rodrigues A & Shao A (2004). The science behind lutein. *Toxicol Lett* 150:57-83.

Astorg P (1997). Food carotenoids and cancer prevention: An overview of current research. *Trends Food Sci Technol* 8:406-413.

ATBC (Alpha-Tocopherol, Beta-carotene) Cancer Prevention Study Group (1994). The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in smokers. *N Engl J Med* 330:1029-1035.

Azevedo-Meleiro CH & Rodriguez-Amaya DB (2004). Confirmation of the identity of the carotenoids of tropical fruits by HPLC-DAD and HPLC-MS. *J Food Comp Anal* 17:385-396.

Azevedo-Meleiro CH & Rodriguez-Amaya DB (2005a). Carotenoid composition of kale as influenced by maturity, season and minimal processing. *J Sci Food Agric* 85: 591-597.

Azevedo-Meleiro CH & Rodriguez-Amaya DB (2005b). Carotenoids of endive and New Zealand spinach as affected by maturity, season and minimal processing. *J Food Comp Anal* 18: 845-855.

Becker EM, Nissen LR & Skibsted LH (2004). Antioxidant evaluation protocols: Food quality or health effects. *Eur Food Res Technol* 219:561-571.

Ben-Amotz A & Levy Y (1996). Bioavailability of a natural mixture compared with synthetic all-*trans* β-carotene in human serum. *Am J Clin Nutr* 63:729-734.

Bendich A (1994). Recent advances in clinical research involving carotenoids. *Pure Appl Chem* 66:1017-1024.

Beutner S, Bloedorn B, Frixel S, Blanco IH, Hoffman T, Martin HD, Mayer B Noack P, Ruck C, Scmidt M, Schülke I, Sell S, Ernst H, Haremza S, Seybold G, Sies H, Stahl W, Walsh R (2001). Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β-carotene in antioxidant functions. *J Sci Food Agric* 81:559-568.

Block G, Patterson B & Subar A (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutr Cancer* 18:1-29.

Boileau AC, Merchen NR, Wasson K, Atkinson CA & Erdman JW Jr (1999). *Cis*-lycopene is more bioavailable than *trans*-lycopene in vitro and in vivo in lymph-cannulated ferrets. *J Nutr* 129:1176-1181.

Bone RA, Landrum JT, Fernandez L, Tarsist SL (1988). Analysis of the macular pigment by HPLC: Retinal

distribution and age study. *Invest Ophthalmol Vis Sci.* 29:843-849.

Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE (2001). Macular pigment in donor eyes with and without AMD: A case-control study. *Invest Ophthalmol Vis Sci* 42:235-240.

Canfield LM & Kaminsky RG (2000). Red palm oil in the maternal diet improves the vitamin A status of lactating mothers and their infants. *Food Nutr Bull* 21:144-148.

CARIG (Carotenoid Research Interactive Group) (1996). Beta-carotene and the carotenoids: Beyond the intervention trials. *Nutr Rev* 54:185-188.

Castenmiller JJM & West CE (1998). Bioavailability and bioconversion of carotenoids. *Annu Rev Nutr* 18:19-38.

Clinton SK (1998). Lycopene: Chemistry, biology, and implications for human health and disease. *Nutr Rev* 56:35-51.

Craft NE (1992). Carotenoid reversed-phase high-performance liquid chromatography methods: Reference compendium. *Meth Enzymol* 213:185-205.

De Pee S & West CE (1996). Dietary carotenoids and their role in combatting vitamin A deficiency: A review of the literature. *Eur J Clin Nutr* 50:S38-53.

De Pee S, West CE, Muhilal, Karyadi D, Hautvast JGAJ (1995). Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. *Lancet* 345:75-81.

De Pee S, West CE, Permaesih D, Martuti S, Muhilal, Hautvast JGAJ (1998). Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and β-carotene in schoolchildren in Indonesia. *Am J Clin Nutr* 68:1058-1067.

Di Mascio P, Kaiser S & Sies H (1989). Lycopene as the most efficient biological singlet oxygen quencher. *Arch Biochem Biophys* 274:532-538.

Drammeh BS, Marquis GS, Funkhouser E, Bates C, Eto I & Stephensen CB (2002). A randomized, 4-month mango and fat supplementation trial improved vitamin A status among young Gambian children. *J Nutr* 132:3693-3699.

EDCC (Eye Disease Case-Control) Study Group (1993). Antioxidant status and neovascular age-related macular degeneration. *Arch. Opthalmol* 111:104-109.

Epler KS, Sander LC, Ziegler RG, Wise SA & Craft NE (1992). Evaluation of reversed-phase liquid chromatographic columns for recovery and selectivity of selected carotenoids. *J Chromatogr* 595:89-101.

Epler KS, Zeigler RG & Craft NE (1993). Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in foods. *J Chromatogr* 619:37-48.

Erdman JW Jr, Bierer TL & Gugger ET (1993). Absorption and transport of carotenoids. *Ann NY Acad Sci* 691:76-85.

Erdman JW Jr., Thatcher AJ, Hofmann NE, Lederman JD, Block SS, Lee CM &

Mokady S (1998). All-*trans* b-carotene is absorbed preferentially to 9-*cis*-β-carotene, but the latter accumulates in the tissues of domestic ferrets (*Mustela putorius puro*). *J Nutr* 128: 2009-2013.

Foote, CS, Chang YC & Denny RW (1970). Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection. *J Am Chem Soc* 92:5216-5218.

Gaziano JM & Hennekens CH (1993). The role of beta-carotene in the prevention of cardiovascular disease. *Ann N Y Acad Sci* 691:148-155.

Gaziano JM, Johnson EJ, Russell RM, Manson JE, Stampfer MJ, Ridker PM, Frei B, Hennekens CH & Krinsky NI (1995). Discrimination in absorption or transport of β-carotene isomers after oral supplementation with either all-*trans*- or 9-*cis*-β-carotene. *Am J Clin Nutr* 61:1248-1252.

Gerster H (1997). The potential role of lycopene for human health. *J Am Coll Nutr* 16 :109-126.

Gil MI, Tomás-Barberán, FA, Hess-Pierce B & Kader AA (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J Agric Food Chem* 50:4976-4982.

Giovannucci E (1999). Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiologic literature. *J Natl Cancer Inst* 91:317-331.

Hadley CW, Miller EC, Schwartz SJ & Clinton SK (2002). Tomatoes, lycopene, and prostate cancer: Progress and promise. *Exp Biol Med* 227:869-880.

Handelman GJ, Dratz EA, Reay CC, van Kujik FJGM (1988). Carotenoids in the human macula and whole retina. *Invest Ophthalmol Vis Sci* 29:850-855.

Hart DJ & Scott KJ (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem* 54:101-111.

Haskell MJ, Jamil KM, Hassan F, Peerson JM, Hossain MI, Fuchs GJ & Brown KH (2004). Daily consumption of Indian spinach (*Basella alba*) or sweet potatoes has a positive effect on total-body vitamin A stores in Bangladesh men. *Am J Clin Nutr* 80:705-714.

Heinonen MI, Ollilainen V, Linkola EK, Varo PT & Koivistoinen PE (1989). Carotenoids in Finnish foods: Vegetables, fruits and berries. *J Agric Food Chem* 37:655-659.

Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat S, Davis CS, Douglass LW, Gebhardt S, Haytowitz D & Schakel S (1999). Carotenoid content of U.S. foods: An update of the database. *J Food Comp Anal* 12:169-196.

Institute of Medicine (2001). Dietary reference intakes: Vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Washington DC.

Jalal F, Nesheim MC, Agus Z, Sanjur D & Habicht JP (1998). Serum retinol concentrations in childresn are affected by food sources of β-carotene, fat intake, and anthelmintic drug treatment. *Am J Clin Nutr* 68:623-629.

Khachik F, Beecher GR, Vanderslice JT & Furrow G (1988). Liquid chromatographic artifacts and peak distortion: sample-solvent interactions in the separation of carotenoids. *Anal Chem* 60:807-811.

Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao DY, Katz NB (2002). Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Proc Soc Exp Biol Med* 227:845-851.

Kimura M & Rodriguez-Amaya DB (1999). Sources of errors in the quantitative analysis of carotenoids. *Arch Latinoamer Nutr* 49:58S-66S.

Kimura M & Rodriguez-Amaya DB (2002). A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chem* 78:389-398.

Kimura M & Rodriguez-Amaya DB (2003). Carotenoid composition of hydroponic leafy vegetables. *J Agric Food Chem* 51:2603-2607.

Kimura M, Rodriguez-Amaya DB & Godoy HT (1990). Assessment of the saponification step in the quantitative determination of carotenoids and provitamins A. *Food Chem* 35:187-195.

Krinsky NI (1989). Antioxidant functions of carotenoids. *Free Radical Biol Med* 7:617-635.

Krinsky NI (1993). Actions of carotenoids in biological systems. *Annu Rev Nutr* 13: 561-587.

Krinsky NI (2001). Carotenoids as antioxidants. *Nutr* 17:815-817.

Kohlmeier L, Hastings SB (1995). Epidemiologic evidence of a role of carotenoids in cardiovascular disease prevention. *Am J Clin Nutr* 62:1370S-1376S.

Kohlmeier L, Kark JD, Gomez-Garcia E, Martin BC, Steck SE, Kardinaal AFM, Ringstad J, Thamm M, Masaev V, Riemersma R, Martin-Moreno JM, Huttunen JK & Kok FJ. Lycopene and myocardial infarction risk in the EURAMIC study. *Am J Epidemiol* 146:618-626.

Kull D & Pfander H (1995). Appendix: List of new carotenoids. In: Carotenoids Volume 1A: Isolation and Analysis. Britton G, Liaaen-Jensen S & Pfander H (eds). Birkhäuser, Basel, p. 295-317.

Landrum JT & Bone RA (2001). Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys* 385:28-40.

Levy J, Bosin E, Feldman B, Giat Y, Miinster A, Danilenko M, Sharoni Y (1995). Lycopene is a more potent inhibitor of human cancer cell proliferation than either α- or β-carotene. *Nutr Cancer* 24:257-266.

Lietz, G, Henry CJK, Mulokozi G, Mugyabuso J, Ballart A, Ndossi G, Lorri W & Tomkins A (2000). Use of red palm oil for the promotion of maternal vitamin A status. *Food Nutr Bull* 21: 215-218.

Manson JE, Gaziano JM, Jonas, MA, Hennekens CH (1993). Antioxidants and cardiovascular disease: A review. *J Am Coll Nutr* 12:426-432.

Mayne ST (1996). Beta-carotene, carotenoids, and disease prevention-in humans. *FASEB J* 10:690-701.

Mercadante AZ & Rodriguez-Amaya DB (1991). Carotenoid composition of a leafy vegetable in relation to some agricultural variables. *J Agric Food Chem* 39:1094-1097.

Moeller SM, Jacques PF, Blumberg JB (2000). The potential role of dietary xanthophylls in cataract and age-related macular degeneration. *J Am Coll Nutr* 19:522S-527S.

Müller H (1997). Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z Lebensm Unters Forsch A* 204:88-94.

Nestel P & Trumbo P (1999). The role of provitamin A carotenoids in the prevention and control of vitamin A deficiency. *Arch Latinoamer Nutr* 49:26S-33S.

Olson, JA (1999). Carotenoids and human health. *Arch Latinoamer Nutr* 49:7S-11S.

Olson JA & Krinsky NI (1995). The colorful, fascinating world of the carotenoids: Important physiologic modulators. *FASEB J* 9:1547-1550.

Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL JR., Valanis B, Williams JH JR, Barnhart S & Hammer S (1996). Effects of a combination of beta-carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 334:1150-1155.

O'Neill ME, Carroll Y, Corrida B, Olmedilla B, Granado F, Blanco I, van den Berg H, Hininiger I, Rousell AM, Chopra M, Southon S & Thurnham DI (2001). A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *Brit J Nutr* 85:499-507.

Osganian SK, Stampfer MJ, Rimm E, Spieglman D, Manson JE & Willett WC (2003). Dietary carotenoids and risk of coronary artery disease in women. *Am J Clin Nutr* 77:1390-1399.

Palozza P (1998). Prooxidant actions of carotenoids in biologic systems. *Nutr Rev* 56:257-265.

Palozza P & Krinsky NI (1992). Antioxidant effects of carotenoids *in vivo* and *in vitro*: An overview. *Meth Enzymol* 213:403-420.

Pfander H, Riesen R & Niggli U (1994). HPLC and SFC of carotenoids – scope and limitations. *Pure Appl Chem* 66:947-954.

Prior RL & Cao G (1999). In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biol Med* 27:1173-1181.

Quirós ARB & Costa HS (2006). Analysis of carotenoids in vegetable and plasma samples: A review. *J Food Comp Anal* 19:97-111.

Radhika MS, Bhaskaram P, Balakrishna N & Ramalakshmi BA (2003). Red palm oil supplementation: A feasible diet-based approach to improve the vitamin A status of pregnant women and their infants. *Food Nutr Bull* 24: 208-217.

Rao AV & Agarwal S (1999). Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. *Nutr Res* 19:305-323.

Ramos DMR & Rodriguez-Amaya DB (1987). Determination of the vitamin A value of common Brazilian leafy vegetables. *J Micronutr Anal* 3:147-155.

Ribaya-Mercado JD, Solon FS, Solon MA, Cabral-Barza MA, Perfecto CS, Tang GT, Solon JAA, Fjeld CR & Russell RM (2000). Bioconversion of plant carotenoids to vitamin A in Filipino school-aged children varies inversely

with vitamin A status. *Am J Clin Nutr* 72:455-465.

Rissanen T, Voutilainen S, Nyyssönen K & Salonen JT (2002). Lycopene, atherosclerosis, and coronary heart disease. *Exp Biol Med* 227:900-907.

Rock CL, Lovalvo JL, Emenhiser C, Ruffin MT, Flatt SW & Schwart SJ (1998). Bioavailability of b-carotene is lower in raw than in processed carrots and spinach in women. *J Nutr* 128:913-916.

Rodriguez-Amaya DB (1989). Critical review of provitamin A determination in plant foods. *J Micronutr Anal* 5:191-225.

Rodriguez-Amaya DB (1993). Nature and distribution of carotenoids in foods. In: Shelf-life Studies of Foods and Beverages. Chemical, Biological, Physical and Nutritional Aspects. Charalambous G (ed). Elsevier Science Publishers, Amsterdam, p. 547-589.

Rodriguez-Amaya DB (1996). Assessment of the provitamin A contents of foods - the Brazilian experience. *J Food Comp Anal* 9: 196-230.

Rodriguez-Amaya DB (1997). Carotenoids and Food Preparation: The Retention of Provitamin Carotenoids in Prepared, Processed, and Stored foods. USAID-OMNI, Washington DC.

Rodriguez-Amaya DB (1999a). A guide to carotenoid analysis in foods. International Life Sciences Institute (ILSI) Press, Washington DC.

Rodriguez-Amaya DB (1999b). Latin American food sources of carotenoids. *Arch Latinoamer Nutr* 49:74S-84S.

Rodriguez-Amaya DB (1999c). Changes in carotenoids during processing and storage of foods. *Arch Latinoamer Nutr* 49:38S-47S.

Rodriguez-Amaya DB (2002). Effects of processing and storage on food carotenoids. *Sight Life Newslett* 3 (Special Issue):25-35.

Rodriguez-Amaya DB & Amaya-Farfan J (1992). Estado actual de los metodos analiticos para determinar provitamina A. *Arch Latinoamer Nutr* 42: 180-191.

Rodriguez-Amaya DB & Kimura M (2004). HarvestPlus Handbook for Carotenoid Analysis. International Food Policy Research Institute, Washington DC.

Sá MC & Rodriguez-Amaya DB (2004). Optimization of HPLC quantification of carotenoids in cooked green vegetables - Comparison of analytical and calculated data. *J Food Comp Anal* 17:37-51.

Schiedt K & Liaaen-Jensen S (1995). Isolation and analysis. In Britton G, Liaaen-Jensen S, Pfander H (eds), Carotenoids, vol 1A: Isolation and analysis. Birkhäuser Verlag, Basel. pp. 81-108.

Schuep KE & Schierle J (1997). Determination of b-carotene in commercial foods: Interlaboratory study. *J Assoc Off Anal Chem* 80:1057-1064.

Scott KJ (1992). Observations on some of the problems associated with the analysis of carotenoids in foods by HPLC. *Food Chem* 45:357-364.

Scott KJ, Finglass PM, Seale R, Hart DJ & de Froidmont-Görtz I (1996). Interlaboratory studies of HPLC

procedures for the analysis of carotenoids in foods. *Food Chem* 57:85-90.

Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA & Willlet W (1994). Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *J Am Med Assoc* 272:1413-1420.

Sharpless KE, Arce-Osuna M, Thomas JB & Gill LM (1999). Value assignment of retinal, retinyl palmitate, tocopherol, and carotenoid concentrations in standard reference material 2383 (baby food composite). *J Assoc Off Anal Chem* 82: 288-296.

Snodderly DM (1995). Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am J Clin Nutr* 62:1448S-1461S.

Stahl W, Ale-Agha N & Polidori MC (2002). Non-antioxidant properties of carotenoids. *Biol Chem* 383:553-558.

Stahl W, Schwarz W, von Laar J & Sies H (1995). All-trans β-carotene preferentially accumulates in human chylomicrons and very low density lipoproteins compared with 9-cis geometrical isomer. *J Nutr* 125:2128-2133.

Stahl W & Sies H (1992). Uptake of lycopene and its geometrical isomers is greater from heat processed than from unprocessed tomato juice in humans. *J Nutr* 122:2161-2166.

Stahl W & Sies H (1996). Lycopene: A biologically important carotenoid for humans? *Arch Biochem Biophys* 336:1-9.

Su Q, Rowley KG & Balazs NDH (2002). Carotenoids: separation methods applicable to biological samples. *J Chromatogr B* 781:393-418.

Takyi EEK (1999). Children's consumption of dark green, leafy vegetables with added fat enhances serum retinol. *J Nutr* 129:1549-1554.

Tang G, Gu XF, Hu SM, Xu QM, Qin J, Dolnikowski GG, Fjeld CR, Gao X, Russell RM & Yin SA (1999). Green and yellow vegetables can maintain body stores of vitamin A in Chinese children. *Am J Clin Nutr* 70:1069-1076.

Trumbo P, Yates AA, Schlicker S & Poos M (2001). Dietary reference intakes: Vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc (commentary). *J Am Diet Assoc* 101:294-301.

Van den Berg H, Faulks R, Fernando Granado H, Hirschberg J, Olmedilla B, Sandmann G, Southon S & Stahl W (2000). The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J Sci Food Agric* 80:880-912.

Van het Hof KH, de Boer BCJ, Tijburg LBM, Lucius BRHM, Zijp I, West CE, Hautvast JGAJ & Weststrate JA (2000a). Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. *J Nutr* 130:1189-1196.

Van het Hof KH, West CE, Weststrate JA & Hautvast JGAJ (2000b). Dietary factors that affect the bioavailability of carotenoids. *J Nutr* 130:503-506.

Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard CJ & Benadé AJS (2005). β-carotene -rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *Am J Clin Nutr* 81:1080-1087.

Van Poppel G & Goldbohm RA (1995). Epidemiologic evidence for ß-carotene and cancer prevention. *Am J Clin Nutr* 62: 1393S-1402S.

Yeum KJ & Russell RM (2002). Carotenoid bioavailability and bioconversion. *Annu Rev Nutr* 22:483-504.

Young AJ & Lowe GM (2001). Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys* 385:20-27.

Ziegler RG (1991). Vegetables, fruits, and carotenoids and the risk of cancer. *Am J Clin Nutr* 53:251S-259S.