
2 Bioavailability of Carotenoids from Vegetables versus Supplements

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2.1 INTRODUCTION

Carotenoids are the widely distributed, naturally occurring pigments responsible for the yellow, orange, and red colors of fruits, roots, flowers, fish, invertebrates, and birds. They also occur in algae, bacteria, molds, and yeast. In higher plants, they occur in the photosynthetic tissue, the chloroplast, where their color is masked by that of the more predominant green chlorophyll.

The *basic* structure is a symmetric, linear, 40-carbon *tetraterpene*, built from eight five-carbon *isoprenoid* units, in such a way that the order is reversed at the center of the molecule. This basic skeleton could be modified in various ways, such as hydrogenation, dehydrogenation, cyclization, double-bond migration, chain short-

TABLE 2.1
Characteristics of Common Food Carotenes and Xanthophylls¹

Name	Characteristics	Vitamin A Activity (%) ²
CAROTENES		
Phytofluene	Acyclic, colorless	—
Lycopene	Acyclic, red	0
δ-carotene	Monocyclic (1β-ring), red-orange	42
β-carotene	Bicyclic (2β pt-rings), orange	100
α-carotene	Bicyclic (1β pt-ring, 1ε-ring), yellow	53
XANTHOPHYLLS		
β-cryptoxanthin	Bicyclic (2β-rings), orange	57
α-cryptoxanthin	Bicyclic (1β, 1ε-ring), yellow	—
Zeaxanthin	Bicyclic (2β-rings), yellow-orange	0
Lutein	Bicyclic (1β, 1ε-rings), yellow	0
Violaxanthin	Bicyclic, yellow	0
Astaxanthin	Bicyclic (2β-rings), red	0

Note: Unless otherwise stated, the carotenoids are in *trans* form.

ening or extension, rearrangement, isomerization or a combination of these processes, resulting in a great diversity of structures.¹

There are basically two types of carotenoids; those containing hydrocarbon (e.g., carotene) and those containing oxygen (e.g., *xanthophylls*). Both types of carotenoids may be acyclic (no ring), monocyclic (one ring), or bicyclic (two rings).

The distinctive building feature of carotenoids is an extensive conjugated double-bond system, which consists of alternating double and single carbon-carbon bonds, usually referred to as the polyene chain. This portion of the molecule (chromophore) is responsible for the ability of carotenoids to absorb light in the visible region of the spectrum. At least seven conjugated double bonds are needed for the carotenoids to impart color; phytofluene, with five such bonds, is colorless (Table 2.1). The color deepens as the conjugated system increases, thus lycopene (11 double bonds) is red.

Cyclization causes some limitations; hence even though β-carotene and α-carotene have the same number of conjugated double bonds (11) as lycopene, they are orange and orange-red, respectively. The intensity of food color depends on which carotenoids are present, their concentrations, physical states, as well as the presence or absence of any other plant pigments, such as chlorophyll.

2.2 LOCALIZATION OF CAROTENOIDS

Carotenoids are hydrophobic, lipophilic substances and are virtually insoluble in water. They dissolve in fat solvents such as alcohol, acetone, ethyl ether, and chloroform. Carotenoids are readily soluble in petroleum ether and hexane, while xanthophylls dissolve best in methanol and ethanol.

In plants and animals carotenoids occur as crystals or amorphous solids, in solution in lipid media, in colloidal dispersion, or combined with protein in an aqueous layer. Specifically, in green leaves, β -carotene molecules are organized in pigment-protein complexes located in cell chloroplasts. In fruits, the molecules are found in lipid droplets and chromoplasts.

The formation of carotenoid-protein complexes allows the carotenoids to have access to an aqueous environment, stabilizes the carotenoid, and changes its color. For example, in invertebrates such as crabs, shrimps, and lobsters, the carotenoid astaxanthin appears as blue, green, or purple carotenoprotein complexes. Upon cooking, denaturation of the protein releases the astaxanthin, revealing a red color.¹

2.3 IMPORTANCE OF CAROTENOIDS

The important physical and chemical properties of carotenoids are depicted in [Figure 2.1](#). It is necessary to highlight the physical-chemical properties of carotenoids since these ultimately confer the multifaceted functions and actions upon them.

The polyene chain is the cause of the instability of carotenoids, including their susceptibility to oxidation and geometric isomerization (change in geometry around a double bond). Heat, light, and acids promote isomerization of trans-carotenoids, their usual configuration in nature, to the cis-form. Oxidation, the major cause of carotenoid loss, depends on available oxygen, the carotenoid involved, and is stimulated by light, heat, peroxides, metals such as iron, and enzymes, while inhibited by antioxidants such as tocopherols (vitamin E) and ascorbic acid (vitamin C).

Oxidation therefore leads to complete loss of activity while isomerization leads to reduced activity. During isomerization, the carotenoid molecules fold back and change from the naturally occurring trans (linear) form to the cis (folded) form. The conditions necessary for the isomerization and oxidation of carotenoids are likely to exist in home preparations, industrial processing, and during storage of foods. The consequences are loss of color, vitamin A activity, and other biological activities. Furthermore, degradation of carotenoids has also been associated with the development of an off-flavor in foods such as in dehydrated carrot and sweet potato flakes.³

Many biological functions and actions have been and continue to be attributed to carotenoids. The more important of these functions are depicted in [Figure 2.2](#). Of the more than 600 carotenoids now known, about 50 could be precursors of vitamin A, based on structural considerations. Vitamin A is provided in our diet as preformed vitamin A (retinyl ester, retinol, retinal, 3-dehydroretinol, and retinoic acid) from foods of animal origin such as liver, milk and milk products, fish and meat (liver and organelles), or as carotenoids, generally from plant sources, that can be biologically converted to vitamin A. Globally about 60% of dietary vitamin A is estimated to come from plant foods,⁴ however, due to the prohibitive cost of animal foods, the dietary contribution of provitamin A could rise to 80 to 90% in developing countries.

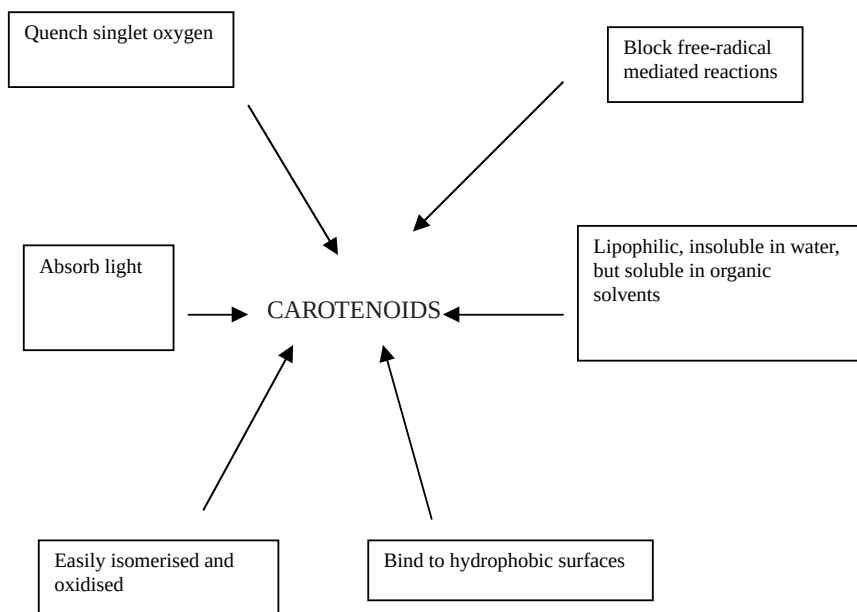


FIGURE 2.1 Important physical and chemical properties of carotenoids.¹

In developed countries, vitamin A deficiency (VAD) has been largely eliminated, except in people who suffer from lipid malabsorption which interferes with the absorption of fat-soluble vitamins such as vitamin A. While the absence of VAD is largely due to the consumption of vitamin A-fortified foods, there is growing interest in fruits and vegetables because of a negative association between their consumption and the incidence of cancers⁵ and coronary heart diseases.⁶ This negative association could be due to the carotenoids and/or other antioxidants, both of which normally exist in fruits and vegetables.⁷ Thus, carotenoids are important dietary components because of their provitamin A activity as well as their possible roles in the prevention of degenerative diseases.

Provitamin A carotenoids have the advantage of being converted to vitamin A only when vitamin A is needed in the body, thus avoiding potential toxicity due to excesses. On the other hand, many factors influence the absorption and utilization of provitamin A carotenoids, thus the bioavailability of carotenoids is variable and difficult to appraise.

The relative biopotencies (vitamin A activity) of only a few forms of provitamin A have been determined by rat assays (Table 2.1). In terms of biological activity and widespread occurrence, the most important provitamin A carotenoid is β -carotene. Virtually all carotogenic plant foods analyzed to date contain β -carotene as a major or minor constituent. β -Carotene is a potent provitamin A carotenoid to which 100% activity is assigned (Table 2.1). The activity of the other provitamin A carotenoids are ranked on the activity of β -carotene (Table 2.1).

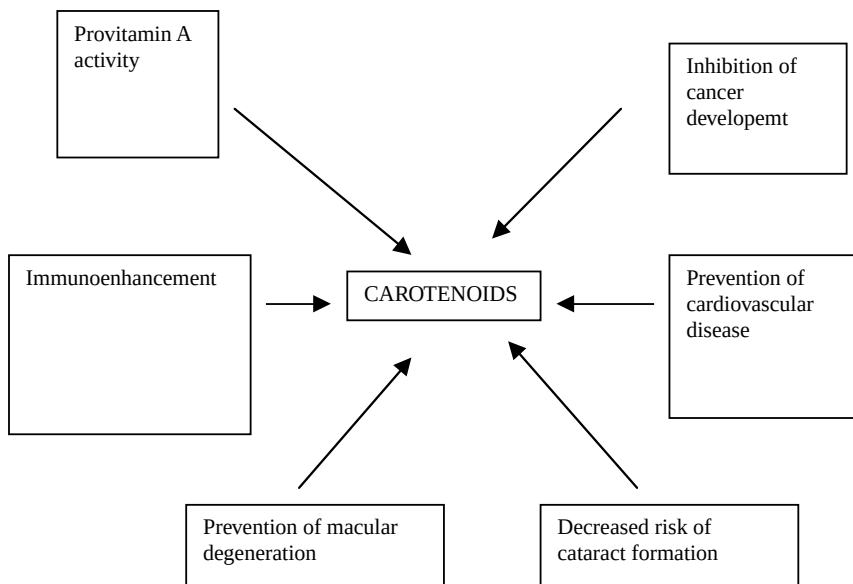


FIGURE 2.2 Health-promoting functions attributed to carotenoids.¹

Carotenoids have also been associated with enhancement of the immune system and decreased risk of degenerative diseases such as cancer, cardiovascular disease, age-related macular degeneration, and cataract formation.⁸⁻¹⁶ These functions are attributed to the antioxidant property of carotenoids through deactivation of free radicals and singlet oxygen quenching.¹⁷⁻¹⁹

The ability of carotenoids to quench singlet oxygen is related to the conjugated double bond system. Those having nine or more double bonds give maximum protection.²⁰ The acyclic provitamin A-inactive lycopene was more effective than bicyclic β -carotene;²¹ canthaxanthin and astaxanthin, both with conjugated keto groups, were better antioxidants than β -carotene and zeaxanthin.²²

2.4 IMPORTANT FOOD SOURCES OF PROVITAMIN A

Surveys conducted in different countries show that in terms of provitamin A carotenoid content, the most important sources are dark green leafy vegetables, red palm oil, palm fruits, carrot, orange, sweet potatoes, mature squashes and pumpkins, and other yellow/orange tropical fruits.

2.4.1 LEAFY VEGETABLES

In many countries in the developing world, dark green leafy vegetables are the most common and relatively abundant sources of provitamin A carotenoids. Due to their relative ease of cultivation and their availability practically all year round (except

in arid and semi-arid areas, where their availability is seasonal and limited to the short rainy seasons only), they are an inexpensive and accessible source of provitamin A carotenoids.

For most of the developing countries which lie in subtropical and tropical areas, β -carotene is essentially the most important source of vitamin A activity, with α -carotene and α - or β -cryptoxanthin reported occasionally at relatively low levels. However, the β -carotene content of leafy vegetables varies markedly.

Based on the β -carotene content in leafy vegetables, Begum and Pereira²³ classified Indian leafy vegetables into three groups thus:

- Those with a high β -carotene level: 46 to 74 $\mu\text{g/g}$ leaf
- Those with a moderate β -carotene level: 25 to 39 $\mu\text{g/g}$ leaf
- Those with a low β -carotene level: 12 to 23 $\mu\text{g/g}$ leaf

Seasonal variations were noted but no consistent pattern could be seen, with some of the leaves being higher in β -carotene content in the summer and others in the colder months. Recently, analyses using HPLC showed higher results — ranging from 20 to 197 $\mu\text{g/g}$.²⁴

Leafy vegetables have been analyzed by HPLC in several other countries.²⁵ In Malaysia, of 27 leaves examined 3 had a β -carotene level between 114 and 136 $\mu\text{g/g}$ and 16 contained between 30 and 93 $\mu\text{g/g}$ β -carotene.²⁶ The commonly consumed green leaves in Bangladesh had β -carotene content between 54 to 100 $\mu\text{g/g}$ ²⁷ while three of the leaves analyzed in Taiwan had β -carotene levels of 70, 92, and 105 $\mu\text{g/g}$, respectively.²⁸ The highest level of β -carotene found in leaves analyzed in Nepal was 58 $\mu\text{g/g}$,²⁹ 187 $\mu\text{g/g}$ from Japan,³⁰ 40 $\mu\text{g/g}$ from Ghana,³¹ 60 $\mu\text{g/g}$ from Brazil,¹ while all 78 Australian leaves had <30 $\mu\text{g/g}$ β -carotene.³²

2.4.2 ROOT CROPS

Carrot (*Daucus carota*) and yellow-to-orange sweet potatoes (*Ipomoea batatas*) are available throughout the world and are important, rich sources of carotenoids, even though their concentrations vary widely from one locality to another.¹ The average β -carotene level ranged from 36³³ to 182 $\mu\text{g/g}$,³⁴ while the average for α -carotene varied from 5.3 $\mu\text{g/g}$ in Finland³⁵ to 106 $\mu\text{g/g}$ in the U.S.³⁴

Dark-orange carrots, consisting mainly of β -carotene and α -carotene, ranged from 63 to 584 $\mu\text{g/g}$.¹ The carotene content of sweet potatoes varied from 0.2 to 218 $\mu\text{g/g}$.³⁶ Japan has established a strong sweet potato breeding program³⁷ due to the fact that the sweet potato is tolerant to typhoons, droughts, pests, and diseases, and is important as a source of starch and vitamins, including β -carotene.

2.4.3 FRUITS

Even though fruits generally have lower provitamin A carotenoids than leafy vegetables, they are usually more readily accepted by both children and adults and their provitamin A content is believed to be more bioavailable.^{37,38} Tropical and subtropical fruits have an advantage over temperate fruits in that they are more carotenogenic

compared to temperate fruits in which the anthocyanin pigments (noncarotenogenic) predominate. Popular tropical fruits such as mango and papaya are important sources of provitamin A in developing countries. The β -carotene content of mangoes varied from 0.6 mg/g in Thailand³⁹ to 29 μ g/g in India.⁴⁰ Papaya also represents important sources of both β -carotene and β -cryptoxanthin (having only one half the bioactivity of β -carotene).¹ Red-fleshed Brazilian papayas analyzed by HPLC contained 1.2 ± 0.3 μ g/g β -carotene and 6.7 ± 0.9 μ g/g β -cryptoxanthin.⁴¹

2.4.4 PALM OIL

Crude red palm oil, obtained from the mesocarp of the oil palm (*Elaeis guineensis*) is considered the world's richest plant source of provitamin A carotenoids.⁴¹ The provitamin A content of oil from varieties of *E. guineensis* and *E. oleifera*, ranged from 142 to 1854 μ g/g for α -carotene and 377 to 2483 μ g/g for β -carotene. Palm fruits are especially important not only because of their high provitamin A content, but also because of the co-occurrence of fat, which results in higher bioavailability of provitamin A most probably due to higher absorption.

In Brazil, the palm fruit *buriti* is the richest source of provitamin A carotenoids, having a β -carotene content of 360 μ g/g, an α -carotene content of 80 μ g/g, and a gamma-carotene content of 37 μ g/g. In most carotenogenic fruits, ripening is accompanied by enhanced carotenoid biosynthesis, which considerably raises the levels of carotenoids, including provitamin A carotenoids. However, in fruits that remain green when ripe and in those that owe their color to anthocyanins, the small amounts of carotenoids tend to decrease during ripening. Also carotenogenesis may continue in intact fruits, fruit vegetables, and root crops after harvest, but in leaves and some other vegetables degradation prevails during post-harvest storage, especially at elevated temperatures and under conditions favorable to wilting.¹

2.5 FACTORS AFFECTING THE BIOAVAILABILITY OF CAROTENOIDS IN VEGETABLES

Bioavailability is defined as the proportion of carotenoids ingested that is absorbed and converted to vitamin A in the body. Bioconversion is the proportion of bioavailable carotenoids converted to vitamin A. Both absorption and conversion are affected by many complex factors. This chapter presents a summary of the factors which affect the bioavailability of carotenoids.

Early studies which investigated the bioavailability of dietary carotenoids concluded that purified carotene in oil is more bioavailable than carotene from leafy vegetables and carrots, and that grinding and homogenizing foods increase carotene bioavailability. de Pee et al.³⁸ have developed a mnemonic word "Slamanghi," to describe factors which influence the bioavailability of carotenoids (β -carotene). In this chapter two more factors have been added. The list is discussed briefly below.

S = Species of carotenoids — The vitamin A activity of β -carotene has been set on a weight basis, as one-fourth to one-tenth of that of retinol, depending on the amount of β -carotene in a meal, while that of other provitamin carotenoids has been set at one-twelfth. This appears to be an oversimplification since several stereois-

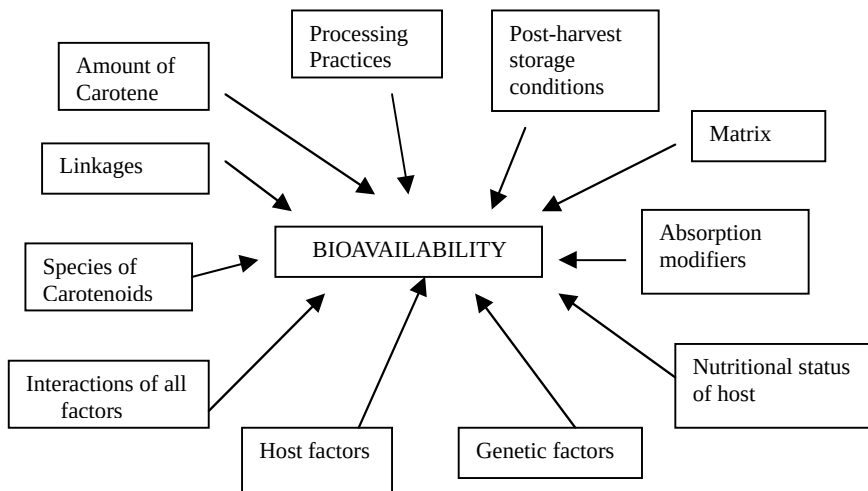


FIGURE 2.3 Factors affecting the bioavailability of dietary carotenoids.

mers exist for each carotenoid and trans-isomers have higher provitamin A activity than cis-isomers. Many factors, such as heat, light, pH, and cooking cause isomerization, leading to the production of species with lower provitamin A activity.

L = Linkages at molecular levels — Some carotenoids such as β -cryptoxanthin exist in plants in both esterified and nonesterified forms. There may be differences in the bioavailability of these two forms.

A = Amount of Carotene — Carotenoids are absorbed through passive diffusion and the proportion absorbed decreases as the amount of carotenoids in a meal increases. FAO/WHO guidelines propose that the amount of dietary β -carotene equivalent to 1 mg retinol is 4, 6, or 10 mg, depending on whether the amount of β -carotene in a meal is <1 mg, 1 – 4 mg, or >4 mg, respectively. Also the presence of some carotenoids in a medium might inhibit the absorption of other carotenoids.

M = Matrix — The matrix in which the carotenoids is embedded in foods seems to be a very important determinant of its bioavailability. As stated earlier, in green leaves carotenoids are organized in pigment-protein complexes located in cell chloroplasts. In other vegetables and fruits, they are located in the chromoplasts of cells, often found in lipid droplets or bound to protein. To become bioavailable, the carotenoid must be freed from its ligands and other matrixes. The cells must first be disrupted by a process such as homogenization. Releasing the carotenoid from a pigment-protein complex is more difficult than freeing it from a lipid droplet. Thus, carotenoids in a fat matrix are more bioavailable than from vegetables and fruits. Cooking and reduction of particle size by grinding or homogenization can reduce matrix effects. However, since a substantial destruction of the matrix, e.g., through cooking, could also destroy the carotenoids, a compromise must be made between maximal destruction of the matrix effect and minimum destruction and/or isomerization of carotenoids.

A = Absorption modifiers — Since the mucosal cells absorb β -carotene from lipid micelles, the diet should contain a sufficient amount and the right type of fat for micelle formation. Unsaturated fats are more efficient than saturated fats. While the absorption of vitamin A is not significantly affected by its food sources or by the amount ingested, or by gastrointestinal infections,⁴² the absorption of dietary carotenoids, on the other hand, is affected by a large number of factors. These include the chemical nature, physical binding within the food, presence of dietary fat, conjugated bile salts, pancreatic enzymes in the intestinal lumen, the presence of other food components that inhibit their absorption (such as fiber, pectin, cellulose, and chlorophyll), the amount ingested, the relative size of the food particles ingested, and food preparation practices that disrupt the food to different degrees. Systemic and parasitic infections reduce carotenoid absorption. Also vitamin A deficiency enhances, whereas protein deficiency reduces, the conversion of β -carotene to vitamin A. Other factors include the amount of vitamin E, the zinc status, and nonprovitamin A carotenoids such as lycopene. Most of these factors are present in appreciable amounts in vegetables.

N = Nutrient status — Even though the absorption of carotenoids is not influenced by carotene status or vitamin A status because absorption occurs through passive diffusion, conversion of carotene to retinol is influenced by serum retinol concentration. An adequate serum retinol level has an inhibiting effect on the enzyme that cleaves carotene into retinol. Also, by improving an impaired zinc status the vitamin A status can also improve since some enzymes involved in vitamin A metabolism are zinc dependent. Protein and iodine status should also be adequate in order to ensure that metabolism of carotene and retinol is normal.

G = Genetics — The bioconversion of β -carotene to retinol is mediated by a cleavage enzyme and there is evidence that some people have a genetic defect which renders them unable to convert β -carotene to retinol. Others have an inherited fat malabsorption, low enzymatic digestion, or poor synthesis of thyroid hormones, proteolytic enzymes, and/or bile salts, which ultimately affect the metabolism of carotenoids and/or vitamin A.

H = Host-related factors — The age of an individual could be an important factor in the bioavailability of carotenoids. Infants handle carotenoids differently from adults or the elderly. Gastrointestinal infections such as *Helicobacter pylori* and parasites (*Giardia lamblia*, *Ascaris lumbricoides*, and hookworm) can cause maldigestion, malabsorption, and excessive loss of dietary carotenoids.

I = Interactions — All the factors discussed can interact with one another to affect carotenoid availability.

Processing practices — Processing of β -carotene-rich foods can actually make the nutrient more available than it would be if the foods were left raw. For example, the bioavailability of carotenoids in raw carrots can be as low as 1%; mild heating greatly enhances digestibility. Some of the carotenoids in leafy vegetables are bound up in protein complexes, which cooking releases. Blanching — the brief (1 to 3 min) exposure of food to boiling water, steam, or hot air — does not reduce the carotene content appreciably and may make it more bioavailable.

Post-harvest storage — A lot of physiological and biochemical processes take place during post-harvest storage. The type and extent of these processes depend on

the storage conditions, such as the temperature and the presence or absence of light and oxygen. An ideal storage condition could lead to carotenoid products with increased bioavailability when the products are finally consumed.

In summary, the bioavailability of carotenoids is influenced by complex and varied factors and is difficult to predict. Even conditions such as the species of leafy vegetables, soil in which the leaves were cultivated, climate, age, and post-harvest storage conditions can affect bioavailability. Thus, one needs caution in extrapolating results from one leaf to another, or even from the same type of leafy vegetable grown in different seasons, since seasonal variations could influence the type and extent of carotenoid-protein complex formation.

2.6 RESEARCH STUDIES

Many research studies have been carried out to determine whether the consumption of dark green leafy vegetables, as the sole source of provitamin A, could raise the serum retinol status of individuals to acceptable levels. While some of the studies showed that serum retinol levels are raised after consumption of the vegetables,^{31,41} others showed no significant rise.⁴²⁻⁴⁵

Though it is true that some of the studies suffer from poor experimental design, the disparities in the results could be due to how well the factors which affect the bioavailability of provitamin A carotenoids have been met in a particular experimental design. It is reasonable to believe that the more factors that are met in a particular study, the more pronounced is the response of serum retinol levels.

de Pee et al.⁴⁵ investigated in the Bogor District, West Java, Indonesia the effect of an additional daily portion of dark green leafy vegetables on the vitamin A and iron status in women with low hemoglobin concentration (<130 g/l) who were breast-feeding children 3 to 17 months old.

In the study, 5 days a week for 12 weeks a group of women (n = 57) received a stir-fried vegetable; a second group (n = 62) received as a supplement a wafer enriched with β -carotene, iron, vitamin C, and folic acid; and a third group (n = 56) received a nonenriched wafer. The vegetable-supplemented and the enriched-wafer groups consumed 3.5 mg β -carotene, 5.2 and 4.8 mg iron, and 7.5 and 4.4 g fat, respectively. In the β -carotene-supplemented group (wafer group) there were increases in the serum retinol (mean increase 0.32 $\mu\text{mol/l}$ (95% CI 0.23 to 0.46), breast milk retinol, 0.59 $\mu\text{mol/l}$ (95% CI 0.35–0.84), and serum β -carotene 0.73 $\mu\text{mol/l}$ (95% CI 0.59–0.88). These figures were significantly greater than in the other two groups in which the only changes were small increases in breast milk retinol in the control-wafer group (0.16 $\mu\text{mol/l}$, 95% CI 0.02–0.30) and in serum β -carotene in the vegetable group, 0.03 $\mu\text{mol/l}$ (95% CI 0–0.06).

It was concluded that a daily portion of dark green leafy vegetables did not improve vitamin A status, whereas a similar amount of β -carotene from a simpler matrix produced a profound improvement.

Takyi³¹ carried out a similar study in northern Ghana in which 519 preschool children were randomly assigned to 5 feeding groups and fed once daily for 12 weeks. Two groups were fed 400 RE of dark green leafy vegetable plus and minus

10% fat (groups 1 and 2, respectively), a third group was fed on a home diet containing 10 RE, while the fourth supplemented group was given 400 RE of synthetic β -carotene plus fat, and a fifth group was fed on dark green leafy vegetables (400 RE) after the children were dewormed with mebendazole (Table 2.2).

Relative to the baseline serum retinol value, consumption of dark green leafy vegetables (*Manihot* sp. and *Ceiba* sp.) with fat (10%) significantly ($p < .05$) enhanced the serum retinol; consequently, the percentage of children with adequate retinol status increased from 28.2 to 48.2% after feeding.

The details of the diets are shown in Table 2.2. On the stew basis, there were significant differences in both the change in the retinol level as a result of feeding and the efficiency of each stew in enhancing retinol status. Diets 4 and 5, representing the β -carotene-supplemented group and the dewormed group, respectively, gave similar values. When the percentage efficiency of the stews were computed, the supplemented group (group 4) gave the highest value followed by the dewormed group (group 5). However, analysis of the efficiency values using the chi-square test showed that there were no significant differences between these two groups. This unequivocally demonstrates the extreme importance of deworming in carotenoid bioavailability and further strengthens the recommendation of Jafal et al.⁴⁴ that food-based intervention in vitamin A-deficient areas could be successful, but other interventions, such as increasing dietary fat intake and anthelmintic treatment should be considered along with increasing the consumption of β -carotene, for maximum results.

The differences in response to feeding with dark green leafy vegetables in the two studies could be due to the following: (1) de Pee et al.⁴⁸ used a leafy vegetable supplement consisting of cassava leaves, water spinach (*Ipomea* sp.), spinach (*Amaranthus* sp.), and carrot (*Daucus* sp.), while (2) Takyi³¹ used cassava and kapok (*Ceiba* sp.) leaves. Apart from the cassava leaf, which is common to both studies, the other leaves were different. The bioavailability of provitamin A carotenoids will differ due to the different varieties/species used, as well as differences in the climatic conditions during cultivation. It is known that the provitamin A content of leaves varies markedly because of the cultivar used, growth conditions, and stage of maturity. Carotenoid content increases with age up to a limit⁴⁶ and then declines slightly.⁴⁷ Second, de Pee et al.⁴⁸ used stir-fried leaves in feeding while Takyi³¹ used leaves which were first pounded in a wooden mortar and then homogenized in a high-speed homogenizer. As discussed earlier, provitamin A carotenoids are in the form of pigment-protein complexes located in the chloroplast of cells. These are indeed complex, strong ligands, needing strong sheering forces to break up and liberate the provitamin A carotenoid molecules from the ligands and other matrixes to make them bioavailable. While pounding followed by high-speed homogenization is considered effective in achieving this, it is not known if stir-frying could achieve similar results. In addition, it is known that frying (excessive heat) could destroy and/or cause isomerization of trans-provitamin A carotenoids into their less active cis-isomers (13-*cis* β -carotene and 9-*cis* β -carotene).

These isomers are about 50% as active as their trans-isomers. Indeed the authors reported that the level of cis-isomers of β -carotene in the supplemented leafy vegetable diet was 30 to 35%, compared to a raw tissue level of 15%. Third, de Pee fed

TABLE 2.2
Characteristics of the Various Stews Fed to Children in Saboba,
Northern Ghana

Feeding Group	Composition of Stew	Change in Serum Retinol (post-feeding value – baseline value) ($\mu\text{mol/l}$)	% Efficiency
1	DVL (400 RE) + dry fish + 10% fat + onion + tomatoes	0.15	26.5 ^c
2	DVL (400 RE) + dry fish + onion + tomatoes	0.14	23.4 ^b
3	Home-type stew (10 RE) + dry fish + onion + tomatoes	0.02	48.1 ^a
4	β -carotene (400 RE) + dry fish + 10% fat + onion + tomatoes	0.22	44.1 ^d
5	DVL (400 RE) + dry fish + 10% fat + onion + tomatoes + mebendazole	0.22	38.8 ^d

Note: In each group, the condiments were made into stews using a minimum of groundnut oil and served with the main carbohydrate meal of either rice, yam, gari and beans, or gari. The dry fish did not contain any vitamin A. The leafy vegetable was either cassava or kapok leaves. These were fed on alternate days. DVL = dark green leafy vegetable; the fat used was shea butter, a local fat widely used in cooking. The efficiency of each of the five stews in enhancing the vitamin A status of the children was determined by subtracting the retinol level at baseline from the values after feeding and dividing the difference by the retinol level at baseline. Values within each column with the same superscript letter were not significantly different.

the leafy supplements for 5 days per week for 12 weeks while Takyi fed it for 7 days per week for 12 weeks. Perhaps it is necessary to feed the subjects for a longer period in order to effect increases in the serum retinol levels. Fourth, preschool children were used in the study by Takyi while de Pee et al. studied lactating women. It can be expected that there will be qualitative and quantitative differences in the manner in which these two groups respond to ingested carotenoids. It is expected that there will be a higher increment in serum retinol level in preschool children than in lactating women since the physiological demands of the former group will be less than that of the latter group. Fifth, there is no indication as to the iodine status of subjects in the de Pee et al. study, while Takyi sought to increase iodine status in his subjects by using iodized salt in food preparation. It is known that an adequate iodine status is necessary for conversion of β -carotene to vitamin A in humans.

Furthermore, the fat content of meals fed by Takyi was 10%, compared to 7.8% used by de Pee et al. The 10% fat content significantly enhanced ($p < .05$) the bioavailability of carotenoids (group 1) compared to the nonfat group (group 2, Table 2.2).

2.7 RECOMMENDATIONS

Carotenoids are affected chiefly by exposure to air, light, excessive heat, or presence of acids. They are therefore vulnerable during food processing, storage, and cooking, unless certain precautions are taken. These precautions can be summarized as follows. Protect β -carotene-rich foods from light, particularly sunlight; store them in a manner which excludes light and air and in the cold; cook them with the least amount of water and heat, in the shortest time possible, or use a microwave oven; and cook in closed containers. If they must be preserved by drying, use solar drying, away from direct sunlight.

2.8 CONCLUSIONS

The bioavailability of provitamin A carotenoids in humans can be compared to that of β -carotene (supplement) if the factors which affect the bioavailability are met. These conditions, even though numerous, can be achieved in homes and public feeding programs through concerted and conscious effort. All that is necessary is the willingness of all those involved in the production, harvesting, storage, transportation, and diet preparation of leafy vegetables to realize and ensure that we maintain a dietary approach as the most reliable, natural, and sustainable means of alleviating vitamin A deficiency and degenerative diseases in both the developed and the developing world.

ACKNOWLEDGMENTS

Parts of this chapter were obtained from OMNI publications. These were studies produced with funds from the Office of Health and Nutrition, Bureau for Global Programs, Field Support and Research, U.S. Agency for International Development, under the terms of Contract No. HRN-C-00-93-00025-08. Other parts came from the research of the following: S. de Pee, Jakarta, Indonesia and The Vitamin A Sieve.

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