Carotenoids and Food Preparation: The Retention of Provitamin A Carotenoids in Prepared, Processed, and Stored Foods

by

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INTRODUCTION

Carotenoids have attracted the interest of researchers from diverse fields including chemistry, biochemistry, biology, food science and technology, medicine, pharmacy, and nutrition for more than a century, and these fascinating compounds continue to be intensely investigated. Carotenoids are widely distributed natural pigments responsible for the yellow, orange, and red colors of fruits, roots, flowers, fish, invertebrates, and birds. They invariably occur in the chloroplasts of higher plants, although in this photosynthetic tissue their color is masked by that of chlorophyll. They are also found in algae, bacteria, molds, and yeasts. It is estimated that nature produces about 100 million tons of carotenoids annually.

The basic carotenoid structure is a symmetrical, linear, 40-carbon tetraterpene built from eight 5-carbon isoprenoid units joined in such a way that the order is reversed at the center. This basic skeleton may be modified in various manners, such as hydrogenation, dehydrogenation, cyclization, double bond migration, chain shortening or extension, rearrangement, isomerization, introduction of oxygen functions, or combinations of these processes, resulting in a great diversity of structures. More than 600 naturally occurring carotenoids have been isolated and characterized. The number of carotenoids so far encountered in foods is much lower; nevertheless, the carotenoid composition of a given food can be quite complex.

Hydrocarbon carotenoids are collectively termed carotenes (Table 1); those containing oxygen are called xanthophylls (Table 2). The most common oxygen functions are hydroxy (OH) and epoxy (5,6- or 5,8-epoxides) groups. Also found are aldehyde (CHO), keto (C=O), carboxy (CO_2H), carbomethoxy (CO_2Me), and methoxy (OMe) groups.

Carotenoids, whether carotenes or xanthophylls, may be acyclic (e.g., phytofluene, æcarotene, lycopene), monocyclic, or bicyclic. Cyclization occurs at one or both ends of the molecule, forming one or two six-membered â-rings (sometimes called â-ionone) or g-rings (sometimes referred to as á-ionone). Thus, the monocyclic ã-carotene has one â-ring while the bicyclic â-carotene, â-cryptoxanthin, zeaxanthin, and astaxanthin have two of these rings. The bicyclic á-carotene and lutein each have one â-ring and one g-ring.

Table 1: Structures and Characteristics of Common Food Carotenes

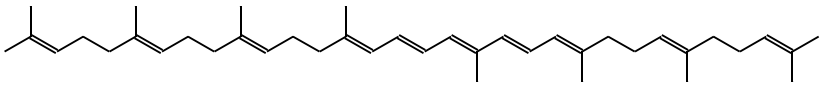
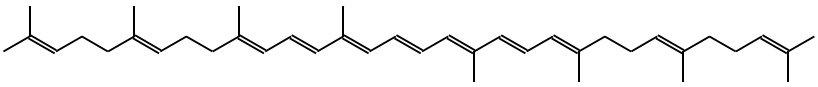
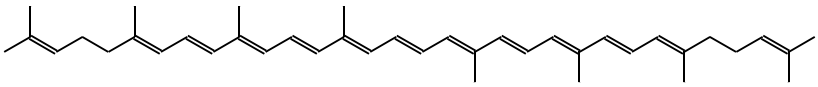
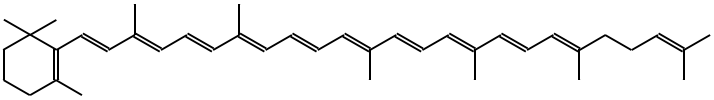
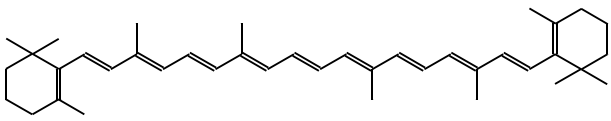
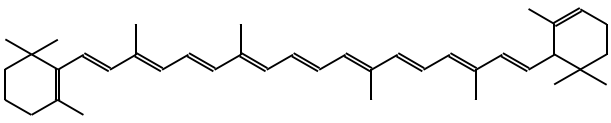
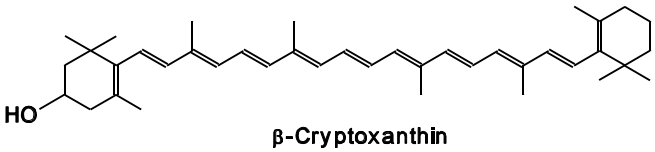
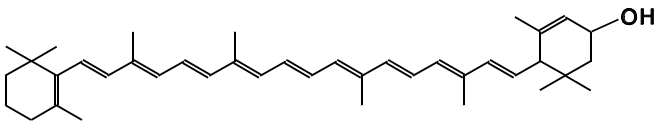
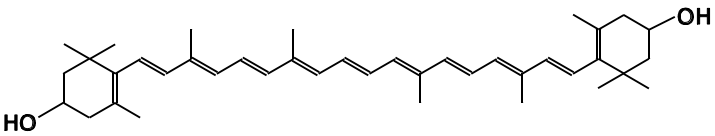
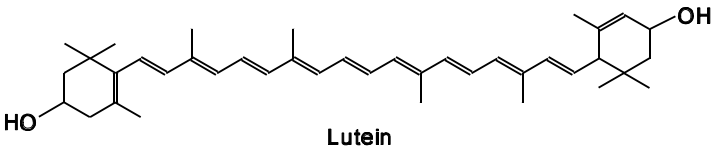
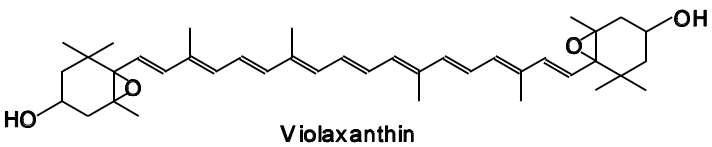
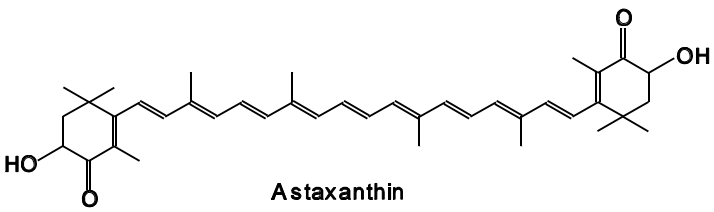
Structure	Characteristics
 Phytofluene	acyclic, colorless
 ζ–Carotene	acyclic, light yellow
 Lycopene	acyclic, red
 γ-Carotene	monocyclic (1â-ring), red-orange
 β-Carotene	bicyclic (2â-ring), orange
 α-Carotene	bicyclic (1â-ring, 1 ĝ-ring), yellow

Table 2: Structures and Characteristics of Common Food Xanthophylls

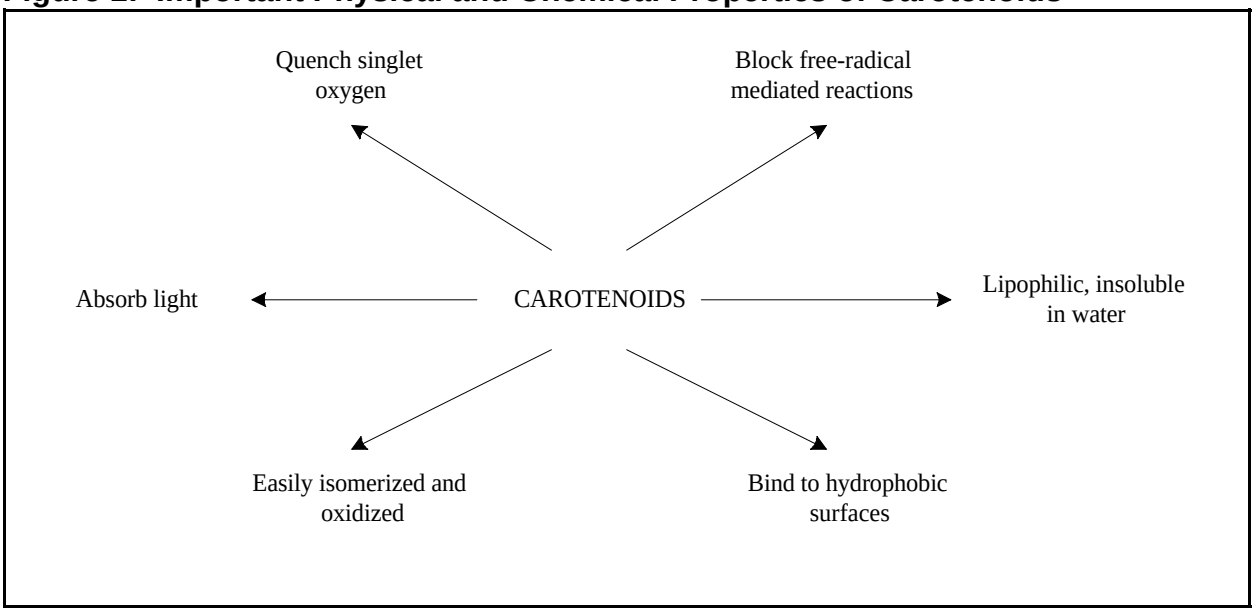
Structure	Characteristics	Oxygen function
 β-Cryptoxanthin	bicyclic (2â-rings), orange	1 hydroxy-group
 α-Cryptoxanthin	bicyclic (1â-, 1 ģ-ring), yellow	1 hydroxy-group
 Zeaxanthin	bicyclic (2â-rings), yellow-orange	2 hydroxy-groups
 Lutein	bicyclic (1â-, 1 ģ-ring), yellow	2 hydroxy-groups
 Violaxanthin	bicyclic, yellow	2 hydroxy-groups, 2 epoxy-groups
 Astaxanthin	bicyclic (2â-rings), red	2 hydroxy-groups, 2 keto-groups

PROPERTIES, FUNCTIONS, AND ACTIONS OF CAROTENOIDS

A review of the structures and physico-chemical properties of carotenoids provides the background needed to understand their multifaceted functions and actions.

Important physical and chemical properties of carotenoids are summarized in Figure 1. The distinctive structural feature of carotenoids is an extensive conjugated double bond system, which consists of alternating double and single carbon-carbon bonds. It is usually referred to as the polyene chain. This portion of the molecule, known as the chromophore, is responsible for the ability of carotenoids to absorb light in the visible region, consequently their strong coloring capability. At least seven conjugated double bonds are needed for a carotenoid to impart color, as in æcarotene which is light yellow. Phytofluene, with five such bonds, is colorless. The color deepens as the conjugated system is extended, thus lycopene is red. Cyclization causes some impediment, so â-carotene and ã-carotene are orange and red-orange, respectively, although they have the same number of conjugated double bonds (eleven) as lycopene. The intensity and hues of food colors depend on which carotenoids are present, their concentrations, and their physical state.

Figure 1: Important Physical and Chemical Properties of Carotenoids



Carotenoids are hydrophobic, lipophilic substances, and are virtually insoluble in water. They dissolve in fat solvents such as acetone, alcohol, ethyl ether, tetrahydrofuran, and chloroform. Carotenes are readily soluble in petroleum ether and hexane. 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 phase. Aside from permitting access to aqueous environments, the association of carotenoids with proteins stabilizes the pigment and changes its color. In invertebrates such as shrimp, crab, and lobster, for example, the carotenoid astaxanthin appears as blue, green, or purple carotenoprotein complexes. Upon cooking, denaturation of the protein releases astaxanthin and reveals a red color.

The importance of carotenoids in foods goes beyond their role as natural pigments. Biological functions and actions have been increasingly attributed to these compounds. Indeed, the provitamin A activity of carotenoids has been known for a long time. Vitamin A is provided in the 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 or as carotenoids that can be biologically transformed to vitamin A (provitamins A), generally from plant foods. On a worldwide basis, about 60 percent of dietary vitamin A is estimated to come from provitamins A (Simpson 1983). Because of the usually prohibitive cost of animal foods, the dietary contribution of provitamin A rises to 82 percent in developing countries. Provitamin A also has the advantage of being converted to vitamin A only when needed by the body; thus avoiding potential toxicity from an overdose of vitamin A. On the other hand, many factors influence the absorption and utilization of provitamin A, such as amount, type, and physical form of the carotenoids in the diet; intake of fat, vitamin E, and fiber; protein and zinc status; existence of certain diseases; and parasite infestation. Thus, the bioavailability of carotenoids is variable and difficult to appraise.

Of the more than 600 carotenoids now known, about 50 would be precursors of vitamin A based on structural considerations. The relative biopotencies of only a few of these provitamins have been estimated by rat assays and some examples are given in Table 3. The most important provitamin A is â-carotene both in terms of its bioactivity and widespread occurrence. Virtually all samples of carotenogenic plant foods analyzed to date contain â-carotene as a principal or minor constituent. Structurally, vitamin A is essentially one-half of the molecule of â-carotene with an added molecule of water at the end of the lateral chain. Thus, â-carotene is a potent provitamin A to which 100 percent activity is assigned (Table 3). An unsubstituted â-ring with an 11-carbon polyene chain is the minimum requirement for vitamin A activity. Therefore, phytofluene, æcarotene, and lycopene (Table 1), which are devoid of â-rings, and zeaxanthin, lutein, violaxanthin, and astaxanthin (Table 2), in which both â-rings have hydroxy, epoxy, or keto substituents, are not provitamins A. However, ã-carotene, á-carotene (Table 1), â-cryptoxanthin, and á-cryptoxanthin (Table 2), all of which have one unsubstituted â-ring, are vitamin A active (Table 3), having about one-half of the bioactivity of â-carotene. Notwithstanding its lower biopotency compared with â-carotene, â-cryptoxanthin also merits attention because it is the major carotenoid of many fruits including peach, nectarine, orange-fleshed papaya, persimmon, mombin, and the fruit of the tree tomato. Some fruits and vegetables have appreciable amounts of á-carotene, such as carrot, some varieties of squash and pumpkin, red palm, and the Brazilian palm fruit buriti (*Mauritia vinifera*). High amounts of ã-carotene are found in buriti, and the Brazilian fruits peach palm (*Bactris gasipaes*), piqui (*Cariocar villosium*), and pitanga (*Eugenia uniflora*). The provitamin á-cryptoxanthin has been shown to be widely distributed in Brazilian fruits and vegetables, but only at low levels (Rodriguez-Amaya 1996).

Table 3: Relative Bioactivities of Some Provitamins A

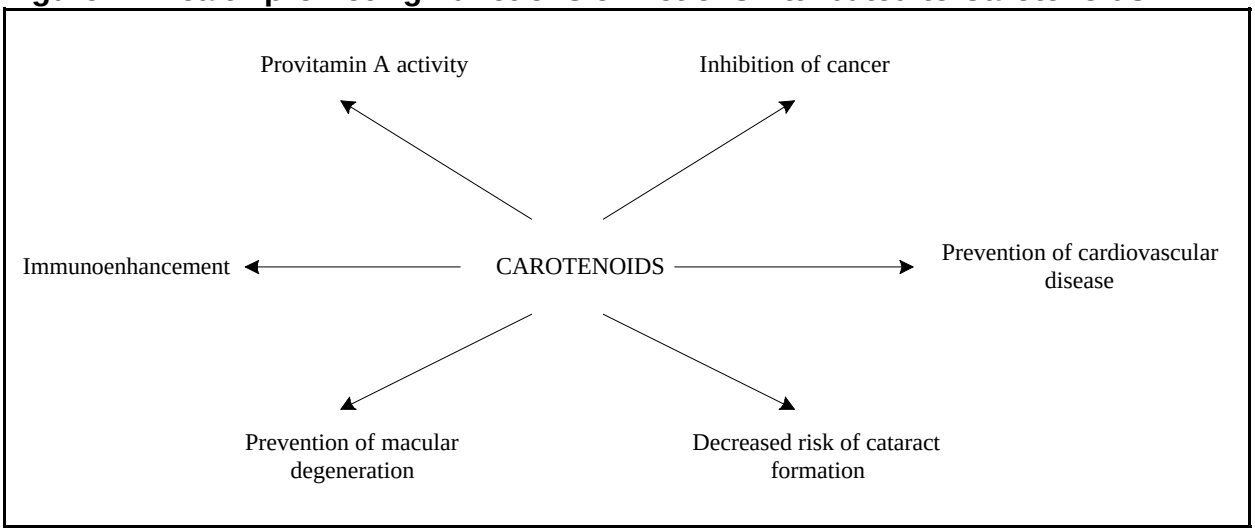
Provitamin A	Relative activity (%) Bauernfeind (1972)	Relative activity (%) Zechmeister (1949)
â-carotene	100	100
13-*cis*-â-carotene	—	53
9-*cis*-â-carotene	—	38
á-carotene	50-54	53
cis-á-carotene (13-*cis* ?)	—	16
cis-á-carotene (9-*cis* ?)	—	13
â-zeacarotene	20-40	—
ã-carotene	42-50	42
cis-ã-carotene	—	19
5,6-monoepoxy-â-carotene	21	—
mutatochrome	50	—
â-cryptoxanthin	50-60	57
cis-â-cryptoxanthin (9-*cis* ?)	—	27
cis-â-cryptoxanthin (15-*cis* ?)	—	42
â-apo-8'-carotenal	72	—

Unless otherwise stated, the carotenoids are in *trans*-form

Carotenoids have also been linked with enhancement of the immune system and decreased risk of degenerative diseases such as cancer, cardiovascular disease, age-related macular degeneration, and cataract formation (Mathews-Roth 1985, 1991; Bendich and Olson 1989; Bendich 1990, 1994; Krinsky 1990, 1994; Ziegler 1991; Gerster 1991; Byers and Perry 1992) (Figure 2). These biological effects are independent of the provitamin A activity and have been attributed to an antioxidant property of carotenoids, through deactivation of free radicals (atoms or groups of atoms possessing an odd, unshared electron), and singlet oxygen quenching (Burton 1989; Krinsky 1989; Palozza and Krinsky 1992). The ability of carotenoids to quench singlet oxygen is related to the conjugated double bond system, and maximum protection is given by those having nine or more double bonds (Foote et al. 1970). The acyclic lycopene was observed to be more effective than the bicyclic â-carotene (Di Mascio et al. 1989). Results obtained with a free radical-initiated system also suggested that canthaxanthin and astaxanthin, both with conjugated keto groups, were better antioxidants than â-carotene and zeaxanthin (Terão 1989).

Because vitamin A deficiency remains a serious public health problem in developing countries, dietary sources and adequacy of provitamins A continue to be the major concern. On the other hand, the focus in the developed world has shifted to the other health-promoting effects of carotenoids.

Figure 2: Health-promoting Functions or Actions Attributed to Carotenoids



The polyene chain is, ironically, also the cause of the instability of carotenoids including their susceptibility to oxidation (combination with oxygen) and geometric isomerization (change of geometry about 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 losses, depends on available oxygen, the carotenoids involved, and their physical condition. Oxidation is stimulated by light, heat, metals, enzymes, and peroxides and is inhibited by antioxidants, such as tocopherols (vitamin E) and ascorbic acid (vitamin C). Epoxides and apocarotenoids (carotenoids with the carbon skeleton shortened) are believed to be the initial products (Hunter and Krakenberger 1947; El-Tinay and Chichester 1970; Ramakrishnan and Francis 1979; Marty and Berset 1988). Subsequent fragmentations result in a series of low-molecular weight compounds similar to those obtained in fatty acid oxidation. Conditions necessary for the isomerization and oxidation of carotenoids are likely to exist in home preparation, industrial processing, and storage of foods. The consequences are loss of color and of vitamin A and other biological activities. Degradation of carotenoids has also been associated with the development of off-flavor in foods, such as in dehydrated carrot and sweet potato flakes (Falconer et al. 1964).

DIFFICULTIES IN MEASURING PROVITAMIN A LEVELS

As the inherent difficulties in carotenoid analysis are sometimes not perceived by the analysts themselves, the reliability of a substantial part of existing data still appears questionable. The analyst must be aware of the importance of proper sampling and sample preparation, necessary precautions to avoid isomerization and oxidation of the carotenoids during analysis, minimum criteria for conclusive identification, and measures to avoid quantification errors, in order to eliminate the discrepancy of results and misinformation that persist in the literature.

As the importance of determining the provitamin A content of foods is becoming more widely recognized, more effort has been directed at improving the validity/reliability of the data. Because of the inherent difficulties in carotenoid analyses, which are sometimes not perceived by the analysts themselves, the reliability of a substantial part of existing data still appears to be questionable. A good understanding of the nature of carotenoids is certainly a prerequisite to a well executed analysis, and helps the researcher avoid the types of result discrepancies and misinformation that persist in the literature.

The magnitude of the analytical challenge can be better comprehended if the following factors are considered (Rodriguez-Amaya 1989, 1990; Rodriguez-Amaya and Amaya-Farfan 1992):

- there are many natural carotenoids
- the carotenoid composition of foods varies both qualitatively and quantitatively
- the concentrations of carotenoids in a given food cover a wide range
- only a few carotenoids are provitamins A and those that are vary in their bioactivity
- carotenoids are prone to isomerization and oxidation.

Thus, problems in separating, identifying, and quantifying the provitamins are likely to occur, along with their degradation during analysis. In addition, it is well documented that carotenoid composition varies as a function of factors such as variety or cultivar, stage of maturity, climate or geographic location, portion analyzed, post-harvest handling, and storage (Gross 1987, 1991; Rodriguez-Amaya 1993a). The importance of proper sampling cannot be overemphasized and pertinent information such as citing the

variety, stage of maturity, season and geographical origin, and portion analyzed should accompany the results. Errors incurred in sampling can easily surpass those from the analysis itself.

Whatever the analytical method chosen, it is essential that the necessary precautions be taken to avoid transformations and quantitative losses of carotenoids during analysis. These include principally (Davies 1976; Schiedt and Liaaen-Jensen 1995):

- completion of the analysis within the shortest possible time
- use of reagent-grade or distilled solvents free from harmful impurities, such as peroxide-free ethyl ether and tetrahydrofuran, or acid-free chloroform
- protection from light
- exclusion of oxygen, for example by using a vacuum, nitrogen, or argon atmosphere
- avoiding high temperature.

Antioxidants and neutralizing agents may also be needed, especially when the analysis is prolonged. Samples, after being cut or minced, should immediately be extracted to avoid enzymatic oxidation. In fact, sample disintegration and extraction with an organic solvent are usually carried out simultaneously. It is a good policy to begin analysis as soon as the samples are collected because it is difficult to preserve samples without changing their composition.

The general analytical procedure for carotenoid analysis consists of:

- sampling and sample preparation
- extraction
- transfer (partition) to petroleum ether, hexane, or ethyl ether
- saponification and washing
- concentration in a rotary evaporator ($\leq 35^{o}C$)
- chromatographic separation
- identification and quantification.

Partition may be omitted in high performance liquid chromatography (HPLC) methods. Saponification is considered the best means of removing chlorophylls and unwanted lipids and of hydrolyzing carotenoid esters. However, this extends the time required to do the analysis, and may form artifacts and degrade carotenoids; the extent to which this happens depends on the conditions used (Kimura et al. 1990). Saponification should, therefore, be eliminated from the procedure whenever possible; it was shown to be

unnecessary for leafy vegetables and tomatoes, for example (Mercadante and Rodriguez-Amaya 1989; Rodriguez-Amaya and Tavares 1992).

Typical sources of errors in carotenoid analysis are:

- samples not representing the food lots under investigation
- incomplete extraction
- losses upon partition, saponification, and washing
- incomplete separation
- misidentification
- faulty quantification and calculation
- isomerization and oxidation of the carotenoids during analysis.

Often given only cursory attention, the prechromatographic steps can introduce significant errors, which cannot be compensated for in the measurement step, no matter how sophisticated the analytical instrument may be. The results obtained are only as good as the extract that is chromatographed.

Provitamin A analysis is evidently simpler than the determination of the complete spectrum of carotenoids. Nevertheless, it is necessary to separate the provitamins A from the inactive carotenoids and from each other so they can be quantified separately. Chromatographic separation of carotenoids can be carried out either by classical open column chromatography (OCC) or by HPLC. Thin layer chromatography (TLC), although useful in identifying carotenoids, is not recommended for quantitative analysis because of the difficulty in recovering the separated carotenoids from the plates and the possibility of isomerization and oxidation on the highly exposed surface. Gas chromatography (GC) is also not appropriate, because of the thermolability and low volatility of carotenoids.

Too often it has been stated that provitamin A determination should be done by HPLC, OCC being considered inadequate. Three papers, however, have shown that either of these two chromatographic methods can be reliably used (Carvalho et al. 1992; Adewusi and Bradbury 1993; Wilberg and Rodriguez-Amaya 1995), provided that the analysis is done under optimum conditions. The accuracy and reproducibility of results using OCC depend on the analyst's skill in packing the column and in adjusting the volumes and proportions of the solvents used for elution (removal from the adsorbent), as well as acuity in visualizing the separation. The chromatographic separation should proceed until the provitamins A are separated, not until a fraction of a mixture of carotenoids is collected, as in the Association of Official Analytical Chemists (AOAC) and European Cooperation for Scientific and Technological Research (COST) methods (Brubacher et al. 1985; Deutsch 1990). The main problems in HPLC are obtaining and maintaining carotenoid standards for quantification, the extremely high capital outlay, and the maintenance and operational costs. Projects using HPLC in developing countries are usually supported by external funding and the issue of sustainability cannot be ignored.

In OCC, the carotenoids are applied in a glass column packed with an adsorbent (usually MgO:Hyflosupercel or neutral, deactivated alumina). Separation and elution is accomplished by increasing the polarity of the solvent (usually petroleum ether or hexane with increasing amounts of ethyl ether or acetone). The separated (conclusively identified) provitamins A are collected and quantified spectrophotometrically. HPLC is generally carried out using a C_{18} column with mixtures of acetonitrile, methanol, ethyl acetate, chloroform, or tetrahydrofuran as mobile phases.

Because carotenoids absorb maximally at different wavelengths, and have different absorption coefficients, the results obtained from normalization (area percentages) can only be taken as approximate relative proportions. For food science and nutrition purposes, however, the results are only useful if presented in terms of concentration, that is, weight of the provitamin per unit weight of sample. This can be done in HPLC by means of internal or external calibration curves, for which the concentrations of the standards are also determined spectrophotometrically.

A constant and reliable source of pure provitamin A standards is, therefore, needed especially for external standardization. The accuracy of the results is directly related to how accurately the concentrations of the standard solutions are known. Unfortunately, only á- and â-carotene standards are available commercially. Moreover, commercial â-carotene standards have been shown to have widely varying purity (Quackenbush and Smallidge 1986; Craft et al. 1990), which means that the purity of the commercial carotenoid standard must be verified before use and purification may be necessary. The instability of carotenoids is another problem in maintaining standards for quantification. Other provitamins, such as â-cryptoxanthin, have to be isolated by the analyst from natural sources.

IMPORTANT FOOD SOURCES OF PROVITAMINS A

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

Data on the provitamin A content of foods are still not considered ideal. The discrepancy of results often exceeds expected natural variation, indicating that analytical errors are still prevalent and collaborative interlaboratory studies to validate methods and evaluate laboratory performance are urgently needed. Nonetheless, important food sources of provitamin A around the world can now be pinpointed.

Leafy Vegetables

In surveys done in different countries (Table 4), dark green, leafy vegetables figure as the most common rich sources of provitamin A. Relatively easy to produce, and available practically all year round, they are inexpensive, accessible sources of provitamin A for most of the developing world. In these vegetables, â-carotene is essentially the sole contributor to vitamin A activity, with á-carotene and á- or â-cryptoxanthin being reported only occasionally and at very low levels. However, the â-carotene content of leafy vegetables varies markedly.

Table 4: Important Provitamin A Sources in Different Countries[a]

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
INDIA				
Begum and Pereira 1977	Leafy vegetables	Nerringi keerai	*Tribulus terrestris*	74±9
		Kuppai keerai	*Amaranthus viridis*	72±28
		Manathakkali keerai	*Solanum nigrum*	70±37
		Agathi keerai	*Sesbania grandiflora*	66±22
		Pacharisi keerai	*Euphorbia hirta*	62±6
		Puliara keerai	*Oxalis corniculata*	60±22
		Mullu keerai	*Amaranthus spinosus*	57±28
		Kasini keerai	*Raphanus sp*	56±12
		Sirukeerai	*Amaranthus polygonoides*	53±14

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
		Poonanganni keerai	*Alternanthera sessilis*	52±5
		Muringa keerai	*Moringa oleifera*	52±22
		Seemai Poonanganni	*Alternanthera sp*	46±8
		Chakravathy keerai	*Amaranthus sp*	39±3
		Nadirsan keerai	*Portulaca wightiana*	36±10
		Modakathan keerai	*Cardiospermum helicacabum*	35±7
		Knol khol keerai	*Brassica oleracea* var. *caulorapa*	35±7
		Arai keerai	*Amaranthus tristis*	34±10
		Panna keerai	*Celosia sp*	34±10
		Pulichai keerai	*Hibiscus cannabinus*	34±20
		Thandu keerai	*Amaranthus gangeticus*	32±11
		Molai keerai	*Amaranthus sp*	31±9
Bhaskarachary et al. 1995; Reddy et al. 1995	Common leafy vegetables	Drumstick	*Moringa oleifera*	197±55
		Agathi	*Sesbania grandiflora*	154±69
		Methi	*Trigonella foenum graecum*	92±15
		Amaranth	*Amaranthus gangeticus*	86±28
		Curry leaf	*Murraya koenigii*	71±24
		Gogu	*Hibiscus sabdariffa*	58±28
		Chammakura	*Colocasia antiquoram*	55±19
		Onion leaf	*Allium cepa*	49±2
		Koyyalakura	*Sueda monoica*	48±6
		Coriander	*Coriandum sativum*	48±2
		Pudina (mint)	*Menta arvensis*	43±20
		Palak	*Spinacea oleracea*	32±12
		Ceylon bacchali	*Talinum triangulare*	30±6
Bhaskarachary et al. 1995; Reddy et al. 1995	Less familiar leafy vegetables	Botla benda	*Abutilon indicum*	126±15
		Chennangiaku	*Cassia sp*	119±22
		Yerramolakakaura	*Amaranthus sp*	119±15
		Mulla thotakura	*Amaranthus spinosus*	109±12
		Tulasi	*Ocimum sanctum*	82±11
		Chirrakura	not cited	71±11
		Harichandamkura	not cited	75±14
		Betel leaf	*Piper beetle*	59±10
		Ponnagantikura	*Alternanthera sessilis*	57±16
		Uttareni	*Achyranthes aspera*	43±7
		Tummikura	*Leucas aspera*	41±9
		Chitramulan	*Plumbago zeylanica*	39±11

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
Bhaskarachary et al. 1995; Reddy et al. 1995	Non-leafy vegetables	Carrot	*Daucus carota*	65±15
Bhaskarachary et al. 1995; Reddy et al. 1995	Fruit	Indian dates	*Phoenix sp*	30±3
THAILAND				
Speek et al. 1988	Leafy vegetables	Common fennel	*Eryngium foetidum*	44
		Bitter melon leaves	*Momordica charantia*	34
Wasantwisut et al. 1995	Leafy vegetables	Garlic leaves	*Allium sativum*	50
		Ivy gourd	*Coccinia arandis L. Voist*	32-41
		Lead tree leaves	*Leucaena glauca*	31-33
		Amaranth	*Amaranthus viridis*	15-39
		Chinese swamp cabbage	*Ipomoea reptans*	12-42
MALAYSIA				
Tee and Lim 1991	Leafy vegetables	Daun turi	*Sesbania grandiflora*	136
		Cekur manis	*Sauropus androgynus*	133
		Tanki	*Neptunia oleracea*	114
		Curry leaves	*Murray koenigii*	93
		Drumstick leaves	*Moringa oleifera*	75
		Ranti	*Solanum nigrum*	70
		Wolfberry leaves	*Lycium chinense*	59
		Tapioca shoots	*Manihot utilissima*	57
		Spinach, red	*Amaranthus gangeticus*	51
		Mint leaves	*Mentha arvensis*	48
		Chinese kale	*Brassica alboglabra*	41
		Pegaga gajah	*Hydrocotyle javanica*	38
		Ceylon spinach	*Basella rubra*	35
		Chinese chives	*Allium odorum*	35
		Salted vegetable	————	34
		Spinach	*Amaranthus viridis*	32
		Coriander leaves	*Coriandrum sativum*	32
		Cemperai	*Champereia griffithii*	32
		Daun mengkudu	*Morinda citrifolia*	31
		Chinese cabbage	*Brassica chinensis*	30
Tee and Lim 1991	Non-leafy vegetables	Carrot	*Daucus carota*	68[b]

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
Choo 1994	Oil	Red palm oil Tenera	*Elaeis guineensis*	377[c]
BANGLADESH				
Rahman et al. 1990, 1995	Leafy vegetables	Pat sak	*Chorchorus capsularis*	100±11
		Motor sak	*Pisum sativus*	89±9
		Sharisha sak	*Brassica campestris*	88±7
		Kalmi sak	*Ipomoea reptans*	83±4
		Helencha sak	*Enhydra fluctuans*	82±3
		Kachu sak	*Colocasia antiquoram*	80±10
		Lal sak	*Amaranthus gangeticus*	80±9
		Lau sak	*Lagenaria vulgaris*	66±3
		Palang sak	*Spinacea oleracea*	64±10
		Mula sak	*Raphanus sativus*	63±6
		Thankuni sak	*Centella asiatica*	61±1
		Pui sak	*Basella alba*	56±7
		Nunia sak	*Portulaca oleracea*	54±3
Rahman et al. 1990, 1995	Flower	Sharisha phool	*Brassica campestris*	160±10
TAIWAN				
Chen et al. 1993	Leafy vegetables	Basil	not cited	105±28
		Green onion fragrant	not cited	92±9
		Kale	*Brassica oleracea* var. *acephala*	70±11
Chen et al. 1993	Non-leafy vegetable	Carrot	*Daucus carota*	54±6[b]
NEPAL				
Vaidya 1995	Common leafy vegetables	Garden cress	*Lepidium sativum*	58
		Spinach	*Spinacea oleracea*	30
Vaidya 1995	Leafy portion of other vegetable	Carrot leaves	*Daucus carota*	42
Vaidya 1995	Traditional/wild leaves	Pahadi pig weed	*Amaranthus blitum*	92
		Pig weed	*Amaranthus viridis*	75
		Edible fern	*Dryopteris cochleate*	44
		Mint	*Mentha aquatica*	37
		Prostrate yarba-detegra	*Eclipta prostrate*	36
		Lamb's quarter	*Chenopodium album*	33
		Amaranth	*Amaranthus leucocarpus*	33

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
Vaidya 1995	Non-leafy vegetable	Carrot	*Daucus carota*	43
JAPAN				
Izaki et al. 1986	Vegetables	Perilla green leaves	Not cited	187
		Komatsuna	*Brassica campestris*	63
		Celery leaves	*Apium graveolens*	61
		Parsley	*Petroselineum hortense*	58
		Collard	*Brassica oleracea var. acephala*	54
		Ashitaba	*Angelica keiskei*	51
		Mitsuba	*Cryptotaenia japonica*	48
		Turnip leaves	*Brassica rapa*	46
		Hatakena	*Brassica campestris var. oleifera*	46
		Chinese chive	*Allium odorum*	46
		Spinach	*Spinacea oleracea*	44
		Carrot Kintoki	*Daucus carota*	43
		Sugukina	*Brassica rapa var. neo suguki*	41
		Shungiku	*Chrysanthemum coronarium*	41
		Water dropwort	*Oenanthe javanica*	40
		Malabar spinach	*Basella alba*	39
		Vitamin-na	*Brassica campestris var. narinosa*	37
		Hiroshimana	*Brassica campestris var. pekinensis*	36
		Broad-leaved mustard	*Brassica juncea*	35
		Water cress	*Nastrutium officinale*	31
		Leaf mustard	*Brassica juncea*	31
		Hamaboufuu	*Glehnia littoralis*	30
Kon 1989	Green vegetables	Chingentsuai leaves	*Brassica campestris var. chinensis*	91
		Komatsuna leaves	*Brassica campestris*	86
		Perilla, leaves	Not cited	72
		Spinach leaves	*Spinacea oleracea*	66
		Leaf-blade, green	Not cited	52
		Spinach, whole	*Spinacea oleracea*	35
		Komatsuna, whole	*Brassica campestris*	33
FINLAND				
Heinonen et al. 1989	Leafy vegetables	Parsley	*Petroselinum hortense*	56
		Dill	*Anethum graveolens*	45

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
		Spinach	*Spinacea oleracea*	33
Heinonen et al. 1989	Non-leafy vegetable	Carrot	*Daucus carota*	76[b]
TANZANIA				
Pepping et al. 1988	Leafy vegetables	"Kayeba" leaves	not cited	35
		Dried cowpea leaves	*Vigna spp*	23-57
		Dried pumpkin leaves	*Cucurbita moschata*	90-102
Pepping et al. 1988	Oil	Red palm oil	*Elaeis guineensis*	93-330[c]
UNITED STATES				
Khachik et al. 1986; Bureau and Bushway 1986; Bushway 1986; Bushway and Wilson 1982; Bushway et al. 1986; Quackenbush 1987	Leafy vegetables	Kale	*Brassica oleracea var. acephala*	82-146
		Spinach	*Spinacea oleracea*	43-82
		Beet greens	*Beta vulgaris*	19-50
		Swiss chard	*Beta vulgaris*	21-46
Bureau and Bushway 1986; Bushway 1986; Bushway and Wilson 1982; Bushway et al. 1986; Quackenbush 1987; Khachik and Beecher 1987	Non-leafy vegetables	Carrot	*Daucus carota*	36-182[b]
		Sweet potato	*Ipomoea batatas*	50-160
		Squash	*Cucurbita maxima*	24-84
		Pumpkin	*Cucurbita moschata*	80[d]
Bureau and Bushway 1986; Bushway 1986; Bushway and Wilson 1982; Bushway et al. 1986; Quackenbush 1987; Khachik et al. 1989	Fruit	Cantaloupe	*Cucumis melo*	16-216
BRAZIL				
Southeastern Region				

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
Ramos and Rodriguez-Amaya 1987; Minazzi-Rodrigues and Penteado 1989; Godoy and Rodriguez-Amaya 1996	Commercial leafy vegetables	Mustard leaves	*Brassica juncea*	60±15
		Parsley	*Petroselinum hortense*	50±15
		Coriander leaves	*Coriandrum sativum*	47±5
		Cress	*Nastrutium officinale*	42±10
		Kale	*Brassica oleracea* var. *acephala*	35±13
		Roquette	*Eruca sativa*	35±13
		Common chicory	*Chicorium intybus*	34±10
Minazzi-Rodrigues and Penteado 1989; Mercadente and Rodriguez-Amaya 1990	Native or uncultivated leafy vegetables	Caruru	*Amaranthus viridis*	110±6
		Mentruz	*Lepidium pseudodidymum*	85±19
		Taioba	*Xanthosoma spp*	67±21
		Serralha	*Sonchus oleraceus*	63±14
		Beldroega	*Portulaca oleracea*	30±8
Godoy and Rodriguez-Amaya 1996; Arima and Rodriguez-Amaya 1988; Almeida and Penteado 1987; Godoy and Rodriguez-Amaya 1993	Non-leafy vegetabless	Pumpkin Menina Verde	*Cucurbita moschata*	39±12[d]
		Carrot	*Daucus carota*	34±15[b]
Northern Region				
Penteado et al. 1986	Leafy vegetables	Quiabo	*Hibiscus esculentus*	116
		Macaxeira	*Manihot esculenta*	129
		Vinagreira branca	*Hibiscus sabdariffa*	86
		Vinagreira roxa	*Hibiscus acetosela*	78
		Indian spinach	*Amaranthus sp*.	63
		Taioba	*Colocasia esculenta*	57
		Bertalha	*Basella rubra*	55
		Mentruz	*Chenopodium ambrosioides*	55
		Tomato	*Lycopersicum esculentum*	55
		Chinese kale	*Brassica chinensis*	49
		Orelha de macaco	*Alternanthera sp.*	46
		African spinach	*Amaranthus sp*.	39
		Jambu branco	*Spilanthes acmella*	39
Rodriguez-Amaya et al. 1995a	Fruit	Tucumã	*Astrocaryun vulgaris*	107±31
Northeastern Region				

Country/Reference	Food	Common name	Scientific name	â-carotene content (µg/g edible portion)
Arima and Rodriguez-Amaya 1990	Non-leafy vegetable	Pumpkin Baianinha	*Cucurbita moschata*	235[d]
Godoy and Rodriguez-Amaya 1994	Fruit	Buriti	*Mauritia vinifera*	360±32[e]
Trujillo-Quijano et al. 1990	Oil	Red palm oil Tenera	*Elaeis guineensis*	363[c]
Centralwestern Region				
Hiani and Penteado 1989b	Fruit	Bocaiúva	*Acrocomia makayáyba*	55
EGYPT				
Abdel-Kader 1991	Leafy vegetables	Spinach	*Spinacea oleracea*	53
Abdel-Kader 1991	Root crops	Carrot	*Daucus carota*	63[b]
		Sweet potato	*Ipomoea batatas*	64

[a] Only foods with â-carotene content equal to or higher than 30 µg/g are included.

[b] Carrots from Malaysia, Taiwan, Finland, United States, Brazil, and Egypt also had 34, 28±3, 5.3, 20-106, 22±10, and 34 µg/g á-carotene, respectively.

[c] Red palm oil from Malaysia, Tanzania, and Brazil also contained 237, 58-144, and 164 µg/g *trans* á-carotene, respectively, and small amounts of *cis*-isomers of á- and â-carotene.

[d] The U.S. pumpkin and the Brazilian pumpkins Menina Verde and Baianinha also had 55, 23, and 47 µg/g á-carotene, respectively.

[e] Buriti also contained 80±9 µg/g á-carotene and 37±4 µg/g ã-carotene.

Begum and Pereira (1977) classified Indian leafy vegetables into three groups:

- those with high â-carotene content (46 to 74 µg/g)
- those with moderate â-carotene level (25 to 39 µg/g)
- those with low â-carotene content (12 to 23 µg/g).

Of 32 leafy vegetables analyzed by OCC, twelve belonged to the first category, twelve to the second, and eight to the third. Twenty-one leaves had a â-carotene level greater than 30 µg/g (Table 4). Some

seasonal differences were seen, but no consistent pattern could be discerned, with some leafy vegetables being higher in â-carotene in the summer and others in the colder months. Edible leaves in India were recently analyzed, this time by HPLC (Bhaskarachy et al. 1995; Reddy et al. 1995). Of 17 common leafy vegetables, 13 contained 30 to197 µg/g of â-carotene; 12 out of 20 less familiar leaves contained 39 to 126 µg/g. The HPLC results were higher than the OCC results, for the same vegetables such as drumstick (197 compared with 52 µg/g), agathi (154 compared with 66 µg/g), amaranth (86 compared with 32 µg/g), chammakura (55 compared with 20 µg/g).

In a recent HPLC study undertaken in Northeast Thailand (Wasantwisut et al. 1995), 22 leafy vegetables were analyzed across different seasons (winter, summer, early rainy, and late rainy), although only nine were available during all four seasons. No definite seasonal trend in â-carotene content could be observed; some leaves had a lower â-carotene content in the summer while others had lower levels in the late rainy season or in winter. Five of the 22 leaves had, on average, a â-carotene level of about 30 µg/g or slightly higher (Table 4). An earlier investigation (Speek et al. 1988), also conducted in Northeast Thailand using HPLC, found the â-carotene level in leaves to be even lower, with only two leaves out of 33 — common fennel and bitter melon leaves — to have moderate amounts of â-carotene (44 and 34 µg/g, respectively). The â-carotene concentration for the same leafy vegetables were noticeably lower in the older study than in the more recent one: Chinese swamp cabbage (10 compared with 12 to 42 µg/g), Asiatic pennywort (1.0 compared with 13 µg/g), and kale (2.3 compared with 11 to 26 µg/g).

Leafy vegetables have also been analyzed by HPLC in several other countries (Table 4). In Malaysia, of 27 leaves examined, three had a â-carotene concentration between 114 and 136 µg/g, and 16 contained between 30 and 93 µg/g (Tee and Lim 1991). Thirteen commonly eaten green leaves in Bangladesh presented 54 to 100 µg/g of â-carotene (Rahman et al. 1990, 1995). The flower of one of the vegetables (*Brassica campestris*) surpassed this range, having 160 µg/g of â-carotene. Of seven leafy vegetables submitted for analysis in Taiwan, three contained 70, 92, and 105 µg/g â-carotene (Chen et al. 1993). Only two out of 12 common leafy vegetables analyzed in Nepal contained 30 µg/g or higher (58 µg/g) of â-carotene (Vaidya 1995). One of six leafy portions not usually consumed as vegetables (such as the leaves of carrot, beet, or turnip) and seven of 15 wild, traditional leafy foods had 33 to 92 µg/g â-carotene. Of 69 vegetables analyzed in Japan, 22 had 30 to 187 µg/g of â-carotene (Izaki et al. 1986). In another study, the â-carotene content of seven out of 24 green Japanese vegetables surpassed 30 µg/g (33 to 91 µg/g) (Kon 1989). As in studies carried out in other countries, some differences could be seen in the values obtained in the two Japanese investigations for the same vegetables, and the leaves turned out to be the richest sources. On the other hand, none of 78 Australian vegetables reached 30 µg/g of â-carotene (Wills 1987).

The â-carotene concentration of leaves investigated in Finland were comparatively low. Of 10 leaves analyzed, the highest levels were obtained in parsley (56 µg/g), dill (45 µg/g), and spinach (33 µg/g) (Heinonen et al. 1989). In Tanzania, of five fresh leaves studied, only one had a â-carotene level higher than 30 µg/g (Pepping et al. 1988). In the U.S., kale, spinach, beet greens, and Swiss chard are good or rich sources of â-carotene (Bushway and Wilson 1982; Khachik et al. 1986; Bureau and Bushway 1986; Bushway 1986; Bushway et al. 1986; Quackenbush 1987).

Of 15 leafy vegetables commercially produced in Brazil, only seven had a â-carotene content between 34 and 60 µg/g (Ramos and Rodriguez-Amaya 1987; Minazzi-Rodrigues and Penteado 1989; Godoy and Rodriguez-Amaya 1996) (Table 4). However, 15 wild or indigenous edible leaves showed a generally

higher â-carotene content (Penteado et al. 1986; Minazzi-Rodrigues and Penteado 1989; Mercadante and Rodriguez-Amaya 1990). Of two varieties of kale, grown in the same farm, one was significantly (statistically) higher in â-carotene in the winter than in the summer (54 compared with 44 µg/g) (Mercadente and Rodriguez-Amaya 1991). All of these Brazilian results were obtained by OCC.

Root Crops

Two root crops, carrot and yellow-to-orange sweet potato, are available throughout the world and are important sources of carotenoids (Table 4). Widely varying provitamin A concentrations have been reported, however, for both roots. Carrot — from which the carotenoids derived their name — is the traditional example of a provitamin A-rich food, and is among the most analyzed food in terms of carotenoids, both by HPLC and OCC (Bushway and Wilson 1982; Bureau and Bushway 1986; Bushway 1986; Bushway et al. 1986; Almeida and Penteado 1987; Khachik and Beecher 1987; Quackenbush 1987; Simon and Wolff 1987; Heinonen et al. 1989; Heinonen 1990; Abdel-Kader 1991; Tee and Lim 1991; Portocarrero et al. 1992; Chen et al. 1993; Godoy and Rodriguez-Amaya 1993; Reddy et al. 1995; Vaidya 1995; Godoy and Rodriguez-Amaya 1996). The average á-carotene level in carrot varied from 5.3 µg/g (Finland) (Heinonen 1989) to 106 µg/g in the U.S. (Khachik and Beecher 1987), while â-carotene ranged from 36 µg/g (U.S.) (Bushway et al. 1986) to 182 µg/g (U.S.) (Khachik and Beecher 1987). These lower and upper limits were obtained by HPLC. Most of the investigations placed the concentration of á-carotene at about 30 µg/g and â-carotene at 60 to 70 µg/g. Heinonen (1990) determined the provitamin A content of 19 cultivars of orange carrot and found the á-carotene varied from 22 to 49 µg/g, â-carotene from 46 to 103 µg/g, and ã-carotene from 6.3 to 27 µg/g. Simon and Wolff (1987) studied seven typical and dark orange carrots; the total carotene content, consisting mostly of â-carotene and á-carotene, ranged from 63 to 548 µg/g. The very dark orange line HCM contained more than twice as much total carotene as any other line tested.

Sweet potato has also been analyzed in several countries (Bureau and Bushway 1986; Bushway 1986; Khachik and Beecher 1987; Quackenbush 1987; Almeida and Penteado 1988; Pepping et al. 1988; Abdel-Kader 1991; Almeida-Muradian and Penteado 1992; Chen et al. 1993; Reddy et al. 1995; Wasantwisut et al. 1995) and the â-carotene content varied from 0.2 µg/g (white-fleshed, Nepal) (Vaidya 1995) to 218 µg/g (orange-fleshed, Brazil) (Almeida-Muradian and Penteado 1992). Almeida-Muradian and Penteado (1992) compared the carotenoid composition of 10 sweet potato cultivars produced in Brazil and found three cultivars, originating from the U.S., rich in â-carotene: Heart Gold, 52 µg/g; Centennial, 149 µg/g; and Acadian, 218 µg/g. Unfortunately, the sweet potato usually produced and commercialized in Brazil is not the â-carotene-rich cultivar. Of ten fruits and vegetables sampled from supermarkets in Egypt, sweet potato (64 µg/g â-carotene), carrot, and spinach were considered the best sources of provitamin A (Abdel-Kader 1991) (Table 4). In the U.S., sweet potato was found to contain between 50 and 160 µg/g of â-carotene (Bureau and Bushway 1986; Bushway 1986; Quackenbush 1987; Khachik and Beecher 1987). Because of their tolerance against typhoon, drought, pests, and diseases and their importance as a source of starch and vitamins, Japan has established a strong sweet potato breeding program (Kukimura et al. 1988, 1990). The germplasm collections include Japanese local cultivars; breeding lines from China, Fiji, Indonesia, New Zealand, Papua New Guinea, the Philippines, Solomon Islands, and the U.S.; and wild sweet potatoes from Latin America.

Squashes and Pumpkins

Other potentially important provitamin A sources are squashes and pumpkins (Table 4), especially if their worldwide availability, ease of production, and long shelf-life are considered. In some countries, the flowers and the leaves of these fruit vegetables are also consumed (Pepping et al. 1988; Wasantwisut et al. 1995; Vaidya 1995). Although data from several countries (Bureau and Bushway 1986; Bushway et al. 1986; Khachik et al. 1986; Hidaka et al. 1987; Khachik and Beecher 1988; Pepping et al. 1988; Speek et al. 1988; Tee and Lim 1991; Reddy et al. 1995; Vaidya 1995; Wasantwisut 1995) show the á-carotene (from undetected or not determined to 12 µg/g) and â-carotene (from 0.5 to 15 µg/g) contents of squashes and pumpkins to be very low to low, some studies show moderate to high provitamin A levels in these vegetables. Of four commercial Brazilian cucurbits analyzed by OCC (Arima and Rodriguez-Amaya 1988), the native *Cucurbita moschata* Menina Verde presented the highest mean levels for á-carotene (23 µg/g) and â-carotene (39 µg/g) at the mature stage. An indigenous variety from Northeastern Brazil, *C. moschata* Baianinha, presented much higher average values: 47 µg/g á-carotene and 235 µg/g â-carotene (Arima and Rodriguez-Amaya 1990). The marked variations observed in the provitamin contents, even between samples of the same cucurbit variety, may be attributed to the long period during which these vegetables can be harvested and to their extended shelf life. In fact, some of the low levels reported may be due to the analyses of immature squashes and pumpkins. In the U.S., some samples of squash and pumpkin analyzed by HPLC contained 24 to 84 µg/g of â-carotene (Bushway 1986; Quackenbush 1987; Khachik and Beecher 1987); one sample of pumpkin contained 55 µg/g á-carotene (Khachik and Beecher 1987). Lee et al. (1984) examined six cultivars of winter squash by OCC and found that â-carotene varied from 7.0 to 17 µg/g while á-carotene ranged from trace to 2.1 µg/g.

Fruits

Fruits generally have lower provitamin A levels than leafy vegetables. However, they are usually better accepted by both children and adults and their provitamins A are believed to be more bioavailable (Olson 1996; de Pee et al. 1996). Unlike temperate fruits where the anthocyanin pigments predominate, many tropical and sub-tropical fruits are carotenogenic. Popular tropical fruits, such as mango and papaya, are considered important provitamin A sources in developing countries. The â-carotene content of mango obtained in some countries (Speek et al. 1988; Godoy and Rodriguez-Amaya 1989, 1994; Wilberg and Rodriguez-Amaya 1995; Reddy et al. 1995; Wasantwisut et al. 1995; Vaidya 1995) varied from 0.6 µg/g in Thailand (Speek 1988) to 29 µg/g in India (Reddy et al. 1995). Five mango cultivars commercially produced in Brazil and analyzed by OCC contained 8.1 to 25 µg/g of â-carotene, on average (Godoy and Rodriguez-Amaya 1989, 1994). Brazilian mangoes of unknown varieties were also analyzed by HPLC, and found to contain 15±7 µg/g of â-carotene (Wilberg and Rodriguez-Amaya 1995). The â-carotene range of papaya is lower: 0.4 to 10 µg/g (Pepping et al. 1988; Speek et al. 1988; Kimura et al. 1991; Godoy and Rodriguez-Amaya 1994; Reddy et al. 1995; Vaidya 1995; Wasantwisut et al. 1995).

Although â-cryptoxanthin is not determined in most studies, and its bioactivity is only one-half that of â-carotene, it is the major provitamin A in papaya. Studies that do not include â-cryptoxanthin's determination underestimate the provitamin A content in papaya. The â-carotene and â-cryptoxanthin ranges obtained by OCC in four commercial Brazilian orange and red papaya cultivars were 1.2 to 6.1 µg/g and 2.1 to 10 µg/g, respectively (Kimura et al. 1991; Godoy and Rodriguez-Amaya 1994). Notably, papaya Formosa from the hot, sunny Northeastern state of Bahia was shown to have higher â-carotene

(6.1 compared with 1.4 µg/g) and â-cryptoxanthin (8.6 compared with 5.3 µg/g) contents than the same papaya cultivar from the temperate São Paulo state. Red-fleshed Brazilian papayas of unknown varieties, analyzed by HPLC, contained 1.2±0.3 µg/g â-carotene and 6.7±0.9 µg/g â-cryptoxanthin (Wilberg and Rodriguez-Amaya 1995).

Other indigenous, less known fruits deserve investigation. In Brazil, for example, the fruit of the tree tomato (*Cyphomandra betacea*) (Rodriguez-Amaya et al. 1983), mamey (*Mammea americana*) (Godoy and Rodriguez-Amaya 1994), and pitanga (*Eugenia uniflora*), especially from the hot Northeastern states of Ceará and Pernambuco (Cavalcante and Rodriguez-Amaya 1992; Godoy and Rodriguez-Amaya 1994), have a comparable or higher vitamin A value than mango. Palm fruits merit special mention, not only because they usually have higher provitamin A content than non-palm fruits, but also because the co-occurrence of the provitamin with fat may result in better bioavailability, an important consideration especially in countries where fat intake is low. In Brazil, the palm fruit buriti proved to be the richest source of provitamins A (Mariath et al. 1989; Godoy and Rodriguez-Amaya 1994, 1995), mainly â-carotene (360 µg/g), á-carotene (80 µg/g), and ã-carotene (37 µg/g) (Godoy and Rodriguez-Amaya 1994, 1995). The palm fruits bocaiúva (*Acrocomia makayáyba*) with 59 µg/g â-carotene (Hiani and Penteado 1989a); peach palm (*Bactris gasipaes*) with 3.2 µg/g á-carotene, 22 µg/g â-carotene, and 18 µg/g ã-carotene (Rodriguez-Amaya et al. 1995a); and tucumã (*Astrocaryum vulgare*) with 107 µg/g â-carotene (Rodriguez-Amaya et al. 1995a) are also good sources of provitamins A.

One laboratory in the U.S. reported a 216 µg/g concentration for â-carotene in cantaloupe obtained by HPLC (Khachik et al. 1989) (Table 4). Another U.S. laboratory (Bureau and Bushway 1986; Bushway et al. 1986), also using HPLC, found a much lower level (16 to 21 µg/g). This illustrates the need for interlaboratory studies even within the U.S. to ascertain whether the difference is natural or analytical. In Japan (Watanabe et al. 1991), the orange-fleshed melon cultivars Iroquois, Blenheim Orange, Birdie Red, Quincy, and Tiffany contained 9.2 to18.0 µg/g â-carotene and the light orange Hale's Best contained 4.0 µg/g.

Palm Oils

Crude red palm oil, extracted from the mesocarp of the oil palm *Elaeis guineensis*, is considered the world's richest plant source of provitamin A (Choo 1994; Rukmini 1994). It has been consumed in some African countries for many centuries, was brought to Brazil, and recently has been produced in India (Arumugam 1989). Unfortunately, the carotenoids are destroyed when the oil is refined. Recently, however, there have been efforts to develop technology that would permit the utilization of this plant material both as a source of oil and of carotenoids. The á- and â-carotene concentrations of oil from three varieties of *E. guineensis*, (Tenera, Psifera, and Dura Dumpy), and of *E. oleifera*, (a South American oil palm) produced in Malaysia (Choo 1994) and Brazil (Trujillo-Quijano et al. 1990) were determined by HPLC and OCC, respectively, and are shown in Table 5.

Table 5: Provitamin A Content (µg/g) of Palm Oil

Species/Variety	Malaysian study/HPLC[a]		Brazilian study/OCC	
	á-carotene[b]	â-carotene[b]	á-carotene[b]	â-carotene[b]

E. guineensis Tenera	237	377	164	363
E. guineensis Psifera	142	233	18	202
E. guineensis Dura Dumpy	243	558	296	576
E. oleifera	1,854	2,483	425	1,026
E. guineensis Psifera x *E. oleifera* hybrid	469	866	—	—
E. guineensis Dura Dumpy x *E. oleifera* hybrid	846	1,311	—	—

[a] Estimated from the percentages and total carotenoid contents reported in the paper.

[b] All-*trans* configuration.

Small amounts of *cis*-isomers of á- and â-carotene were encountered in both the Malaysia and Brazil investigations. Low levels of ã-carotene and â-zeacarotene were also reported in the Malaysian work, while â-cryptoxanthin was detected, especially in *E. oleifera* in the Brazilian study. The common commercial variety in both countries is Tenera (see Table 4). Hybrids were also analyzed in Malaysia, and the results for á-and â-carotene are also shown in Table 5. In comparison, a single sample of the Brazilian buriti oil contained 3,040 µg/g of â-carotene (Mariath et al. 1989).

RIPENING AND POST-HARVEST CHANGES IN CAROTENOIDS

In most carotenogenic fruits, ripening is accompanied by enhanced carotenoid biosynthesis, which considerably raises the levels of carotenoids, including provitamins A. In fruits that remain green when ripe and in those that owe their color to anthocyanins, however, the small amount of carotenoids tends to decrease during ripening.

Carotenoid changes during fruit ripening have been well documented, demonstrating divergent patterns in different fruits (Gross 1987). Some examples of the effect of the stage of maturity on the provitamin A carotenoids are shown in Table 6. The provitamin A levels do not necessarily reflect the overall carotenoid trend because nonprovitamin A carotenoids are also present, sometimes as principal carotenoids.

Table 6: Provitamin A Levels in Some Ripening/Maturing Fruits and Fruit Vegetables

Reference	Fruit/Carotenoid	Concentration (µg/g pulp)		
		Unripe	Partially ripe/mature[a]	Ripe/mature[a]
Gross 1985	Cherry, yellow[b]			
	â-carotene	1.3	0.6	0.4
Gross 1982/3	Curant, red			
	â-carotene	2.4	2.7	0.6
Mínguez-Mosquera and Garrido-Fernandez 1989	Olive Hojiblanca[b]			
	â-carotene	8.4	5.1	3.7
Mínguez-Mosquera and Garrido-Fernandez 1989	Olive Manzilla[b]			
	â-carotene	7.3	3.7	2.1
Gross 1982a	Strawberry Tenira			
	â-carotene	0.4	0.2	tr
Gross 1982b	Kiwi[b]			
	â-carotene	1.3	0.8	0.1
Huyskens et al. 1985	Kumquat Nagami[b]			

Reference	Fruit/Carotenoid	Concentration (µg/g pulp)		
		Unripe	Partially ripe/mature[a]	Ripe/mature[a]
	á-carotene	0.3	0.3	0.1
	â-carotene	2.8	1.8	0.2
	á-cryptoxanthin	0.1	0.1	—
	â-cryptoxanthin	0.3	0.5	3.2
	cryptoflavin	0.2	0.4	0.7
Farin et al. 1983	Mandarin hybrid Michal juice[b]			
	á-carotene	0.1	0.1	0.1
	â-carotene	0.1	0.1	0.3
	á-cryptoxanthin	0.1	0.2	0.1
	â-cryptoxanthin	2.6	3.4	5.6
John et al. 1970	Mango Badami[b]			
	â-carotene	0.2	11.3	45.2
	cis-â-carotene	tr	tr	1.2
	ã-carotene	tr	0.1	0.2
	5,6-monoepoxy-â-carotene	tr	tr	0.7
	mutatochrome	tr	0.4	1.0
	â-cryptoxanthin	tr	0.5	0.4
Mercadente and Rodriguez-Amaya 1993	Mango Keitt			
	â-carotene	1.7	4.2	6.7
	â-cryptoxanthin	tr	tr	0.2
Mercadente and Rodriguez-Amaya 1993	Mango Tommy Atkins			
	â-carotene	2.0	4.0	5.8
	cis-â-cryptoxanthin	0.1	0.1	0.1
	â-cryptoxanthin	0.1	0.1	0.3
Wilberg and Rodriguez-Amaya 1995	Papaya			
	â-carotene	0.6	n.d.	1.2
	â-cryptoxanthin	1.7	n.d.	6.7
Rahman and Buckle 1980	Pepper Long Red Cayenne			
	á-carotene	1.0	5.0	9.0
	â-carotene	8.0	40.0	108.0
	mutatochrome	—	2.0	9.0
	â-cryptoxanthin	—	4.0	37.0
Rahman and Buckle 1980	Pepper Pacific Bell			
	á-carotene	—	0.4	1.0
	â-carotene	2.0	3.0	16.0
	mutatochrome	—	1.0	4.0
	â-cryptoxanthin	—	1.0	5.0

Reference	Fruit/Carotenoid	Concentration (µg/g pulp)		
		Unripe	Partially ripe/mature[a]	Ripe/mature[a]
Rahman and Buckle 1980	Pepper Ram Horn			
	á-carotene	0.1	0.4	1.0
	â-carotene	0.8	7.0	28.0
	5,6-monoepoxy-â-carotene	0.1	0.2	—
	mutatochrome	—	1.0	3.0
	â-cryptoxanthin	—	4.0	15.0
Rahman and Buckle 1980	Pepper College Gold			
	â-carotene	0.4	3.0	36.0
	5,6-monoepoxy-â-carotene	0.1	0.2	—
	mutatochrome	—	0.6	2.0
	â-cryptoxanthin	—	4.0	18.0
Rahman and Buckle 1980	Pepper Golden California Wonder			
	á-carotene	—	0.4	2.0
	â-carotene	2.0	9.0	16.0
	5,6-monoepoxy-â-carotene	tr	0.2	0.4
	â-cryptoxanthin	—	0.4	7.0
	5,6-monoepoxy-â-cryptoxanthin	—	1.0	3.0
Mínguez-Mosquera and Hornero-Mendéz 1994a	Pepper Agridulce			
	1st year			
	â-carotene	8.0	18.0	99.0
	â-cryptoxanthin	—	12.0	78.0
	2nd year			
	â-carotene	4.7	14.0	106.0
	â-cryptoxanthin	—	9.3	76.0
Mínguez-Mosquera and Hornero-Mendéz 1994a	Pepper Bola			
	1st year			
	â-carotene	6.4	8.3	52.0
	â-cryptoxanthin	—	7.7	37.0
	2nd year			
	â-carotene	6.0	17.0	71.0
	â-cryptoxanthin	—	9.1	26.0
Kon and Shimba 1987	Persimmon Yotsumizo			
	á-carotene	0.1	0.5	0.5
	â-carotene	1.6	5.5	8.5
	â-cryptoxanthin	0.2	1.6	13.0
	5,6-monoepoxy-â-cryptoxanthin	—	—	1.8

Reference	Fruit/Carotenoid	Concentration (µg/g pulp)		
		Unripe	Partially ripe/mature[a]	Ripe/mature[a]
Homnava et al. 1991	Persimmon Fuyu			
	á-carotene	0.1	0.2	0.3
	â-carotene	0.4	0.6	1.0
	â-cryptoxanthin	0.1	0.5	0.6
Homnava et al. 1991	Persimmon Sheng			
	á-carotene	0.2	0.3	0.4
	â-carotene	0.9	1.0	1.6
	â-cryptoxanthin	0.1	0.5	1.3
Arima and Rodriquez-Amaya 1988	Pumpkin Menina Verde			
	á-carotene	0.1	n.d.	23.0
	â-carotene	1.5	n.d.	39.0
	mutatochrome	tr	n.d.	0.5
	á-cryptoxanthin	0.2	n.d.	1.5
Ramos and Rodriguez-Amaya 1987	Endive			
	â-carotene	4.2	n.d.	14.0
Ramos and Rodriguez-Amaya 1987	Lettuce			
	â-carotene	3.5	n.d.	12.0

[a] Mature for squash and the leafy vegetables endive and lettuce, ripe for the other foods

[b] Estimated from the relative percentages and total carotenoid content reported in the paper

n.d. – not determined

tr – trace

Carotenoid Levels During Ripening

In fruits where the color at the ripe stage is due to anthocyanins, such as yellow cherry (Gross 1985), red currant (Gross 1982/83), olive fruit (Mínguez-Mosquera and Garrido-Fernandez 1989), and strawberry (Woodward 1972; Gross 1982a), and in fruits which retain the green color, such as kiwi (Gross 1982b), the carotenoid content decreases during ripening (Table 6). The same trend is seen with some fruits, such as banana (Giami and Alu 1994), which undergo yellowing simply because chlorophyll degradation unmasks the carotenoids.

In some fruits, the carotenoid level decreases at mid-season and increases in quantity and diversity thereafter. This behavior was observed in kumquat (Huyskens et al. 1985) (Table 6), in the flavedo of tangerine cultivars Dancy and Clementine (Gross 1981), and in the peel of a mandarin hybrid (Farin et al. 1983).

In most carotenoid-containing fruits and fruit vegetables, ripening is accompanied by enhanced carotenoid biosynthesis, such as in apricot (Katayama et al. 1971), mango (John et al. 1970; Mercadante and Rodriguez-Amaya 1993), orange (Rotstein et al. 1972), muskmelon (Reid et al. 1970), papaya (Wilberg and Rodriguez-Amaya 1995), pepper (Rahman and Buckle 1980; Mattus et al. 1991; Deli et al. 1992; Howard et al. 1994; Moya et al. 1994; Mínguez-Mosquera and Hornero-Méndez 1994), persimmon (Kon

and Shimba 1987; Homnava et al. 1991), mandarin juice (Farin et al. 1993), tangerine juice (Gross 1982c), and tomato (Koskitalo and Ormrod 1972; Raymundo et al. 1976). As chlorophyll decomposes and chloroplasts are converted to chromoplasts, the chloroplast carotenoid pattern is transformed into a complex composition and the carotenoids increase significantly both in number and in quantity, especially in the principal pigments. The carotenoids of the â,ğ-type, particularly lutein, decline as carotenoids of the â,â-type take over.

Apricot and Mango

In apricot, the predominant pigment, â-carotene, accumulates rapidly during ripening. One study found it accumulated from trace amounts in the immature fruit to about 22 µg/g in the ripe stage (Katayama et al. 1971). Similarly, the â-carotene in mango increases significantly as ripening takes place, although an earlier study (John et al. 1970) places the concentration much higher than that found in more recent work (Mercadante and Rodriguez-Amaya 1993) (Table 6).

Pepper

In red and black pepper, there is a massive increase of the vitamin A-inactive, red pigments capsanthin and capsorubin during ripening (Rahman and Buckle 1980; Deli et al. 1992; Mínguez-Mosquera and Hornero-Méndez 1994). There is also a significant increase in the provitamins A â-carotene and â-cryptoxanthin. In an Australian study (Rahman and Buckle 1980), for example, â-carotene increased from a range between 0.4 and 8 µg/g in the immature stage to 16 to 108 µg/g in the fully ripe stage in four red cultivars and one yellow cultivar (Table 6). The study did not detect â-cryptoxanthin at the immature stage in any of the cultivars, but it reached 5 to 37 µg/g in the fully ripe stage. In the Spanish cultivar Agridulce, analyzed at harvest in two different years, the â-carotene content increased from 8.0 and 4.7 µg/g at the green stage to 99 and 106 µg/g, respectively, at the red stage (Mínguez-Mosquera and Hornero-Méndez 1994). In the same cultivar, â-cryptoxanthin went from not being detected at either harvest time to 78 and 76 µg/g. In the Bola Variety, the â-carotene concentrations went from 6.4 and 6.0 µg/g at the green stage to 52 and 71 µg/g, respectively, at the red stage. Similarly, â-cryptoxanthin increased from undetected in the green stage in both years to 37 and 26 µg/g at harvest. In a Hungarian yellow pepper, â-carotene was found to increase 8- to 9-fold during ripening (Matus et al. 1991). Marked increases were also observed in the á-carotene, á-cryptoxanthin and â-cryptoxanthin content in the yellow pepper. A similar trend was seen in black paprika (Deli et al. 1992).

In the capsicum variety Anaheim, the á-carotene and â-carotene levels rose from 0.3 and 4.4 µg/g to 1.2 and 12.4 µg/g, respectively, from the immature to the mature stage (Moya et al. 1994). Both â-cryptoxanthin and ã-carotene were undetected in the immature fruit, but reached 1.8 and 0.8 µg/g in the mature pepper.

In three cultivars of Jalapeño, two cultivars of Chile, two cultivars of Bell, and one cultivar of Serano, á-carotene increased from 0.1 to 0.6 µg/g at the green stage to 0.3 to 2.8 µg/g in the red fruits (Howard et al. 1994). The corresponding changes in â-carotene were 1.9 to 8.2 µg/g to 3.7 to 27 µg/g.

Persimmon and Tangerine

From the unripe, green stage to the tree-ripe stage, the â-carotene concentration in the Japanese persimmon Yotsumizo increased from 11 to 73 µg/g in the peel and from 1.6 to 8.5 µg/g in the flesh (Kon and Shimba 1987) (Table 6). Concentrations of â-cryptoxanthin went up from not detected to 53 µg/g in the peel, and from 0.2 to 13 µg/g in the pulp. In the persimmon cultivar Fuyu, â-cryptoxanthin increased from 0.1 to 0.6 µg/g; á-carotene from 0.1 to 0.3 µg/g; and â-carotene from 0.4 to 1.0 µg/g in the edible portion (including the skin) from the fully mature, green stage to the tree-ripe stage (Homnava et al. 1991). In the persimmon cultivar Sheng, â-cryptoxanthin rose from 0.1 to 1.3 µg/g; á-carotene from 0.2 to 0.4 µg/g; and â-carotene from 0.9 to 1.6 µg/g. In the Dancy tangerine juice (Gross 1982c), â-cryptoxanthin increased from 0.9 to 4.9 µg/ml, and â-carotene went from undetected to 0.6 µg/ml during ripening.

Tomato

There is a considerable increase in carotenoid content, particularly in the principal pigment lycopene, during tomato ripening. The carotenoids of dark-grown, dark-ripened tomatoes were found to be qualitatively similar to those of dark-grown or light-grown tomatoes that ripened exposed to light (Raymundo et al. 1976). The concentrations of all carotenoids, however, were higher in the light-grown, light-ripened fruits. Ripening temperature and date of harvest (days after onset of initial coloration) also had a pronounced effect on tomato carotenoids, with the vitamin A-inactive lycopene behaving differently from â-carotene (Koskitalo and Ormrod 1972). At a diurnal temperature range of 17.8/25.6ºC (minimum-maximum temperatures), seven days past breaker stage, the lycopene level was 44 µg/g while that of â-carotene was 3.0 µg/g. After 21 days, lycopene reached 65 µg/g while â-carotene diminished slightly to 2.2 µg/g. At a lower diurnal temperature range of 2.8/13.9ºC, seven days past breaker stage, the lycopene content was only 9.3 µg/g while the â-carotene concentration was 3.6 µg/g. After 21 days, the lycopene concentration reached 24 µg/g while that for â-carotene was essentially maintained (3.7 µg/g).

Green Leafy Vegetables

The á- and â-carotene levels of the *C. moschata* Menina Verde rose from 0.1 and 1.5 µg/g, respectively, at the immature stage, to 23 and 39 µg/g in the mature vegetable (Arima and Rodriguez-Amaya 1988) (Table 6). In lettuce and endive, the â-carotene content of the mature leaves was found to be three-times greater than that of the young leaves taken from the same bunches of vegetables (Ramos and Rodriguez-Amaya 1987).

Carrot

In three carrot cultivars, analyzed periodically starting from 60 days after planting, á-carotene in Nantes and â-carotene in Spartan Classic increased until harvest time (140 days after planting) (Lee 1986). However, â-carotene in the K-strain and Nantes, and á-carotene in the K-strain and Spartan Classic, reached a maximum at 110 days and decreased slightly thereafter.

Post-Harvest Carotenogenesis

Carotenogenesis may continue in intact fruits, fruit vegetables, and root crops after harvest, but in leaves and some other vegetables, degradation prevails during postharvest storage, especially at elevated temperature and under conditions favorable to wilting.

Carotenogenesis may continue even after harvest, provided the fruit or vegetable remains intact. African mango, picked at the mature, hard green stage, continued to ripen on ambient storage, with concomitant increase in the total carotenoid content (Aina 1990). Data from three harvest seasons and two different growing areas demonstrated the importance of post-harvest temperature (Thomas and Janave 1975). Biosynthesis of carotenoids in the flesh of ripening Indian Alphonso mango was observed to be maximal at tropical ambient temperatures (28 to 32°C). Storage at 7 to 20°C for 16 to 43 days caused a substantial reduction in the total carotenoid content, even when the fruits were subsequently ripened at optimal conditions.

The â-carotene content in the peel of Japanese persimmon rose from 73 to 423 µg/g, while the â-cryptoxanthin level decreased from 53 to 21 µg/g, during storage (Kon and Shimba 1987). The â-carotene level in the flesh of Japanese persimmon increased from 8.5 to 18.5 and the â-cryptoxanthin went down from 13 to 4.5 µg/g (Table 7). In mature squashes and pumpkins stored for 70 days at ambient temperature (about 20°C), â-carotene also increased (Pedrosa et al. 1983).

The carotene content of red capsicums that were not irradiated or were irradiated at doses suitable for disinfestation, increased slightly during storage at 5°C for three weeks (Mitchell et al. 1990). An Indian potato tuber showed irregular behavior (Bhushan and Thomas 1990) as its carotenoid content increased with storage at 4°C and 25 to 30°C, but decreased at 15°C and 20°C.

In Nantes carrot, stored at 2°C and 90 percent relative humidity, á-carotene and â-carotene levels increased slowly up through 100 to 125 days and then decreased (Lee 1986) (Table 7). Some changes in the á-carotene and â-carotene contents of carrot could be discerned under dark and lighted cold storage (4°C), but these changes were not found to be statistically significant, except for the 47 percent loss of 9-*cis*-â-carotene under lighted storage (Kopas-Lane and Warthesen 1995). It was concluded that neither lighted or dark cold storage affected the major carotenoids of carrot.

Losses in total carotenoid content were reported in some vegetables, especially leaves. Both sweet pepper and parsley lost over 20 percent of their total carotenoid content at cold room storage (7°C) for nine days (Takama and Saito 1974). Carotenoid losses amounted to 60 and 80 percent at 15°C and 17°C, respectively. Leek lost about 53 percent of its total carotenoid content within three days at both temperatures. Kale, collard, turnip greens, and rape underwent more rapid losses of carotenes under conditions favorable to wilting; high storage temperature aggravated these losses (Ezell and Wilcox 1962). In kale, average carotene losses were 1.6 percent at 0°C, 22.4 percent at 10°C, and 66.7 percent at 21°C after four days of storage. The corresponding losses for collard were 7.5, 34.3, and 67.9 percent.

Table 7: Retention of Provitamins A in Intact Fruits and Vegetables During Post-harvest Storage

Reference	Post-harvest storage condition	Fruit or vegetable	Carotenoid	Retention (%)
Kon and Shimba 1987	not specified	Japanese Persimmon	â-carotene	218
			á-cryptoxanthin	35
Lee 1986	2EC, 90% RH, 100 days	Carrot	á-carotene	121
			â-carotene	114
Lee 1986	2EC, 90% RH, 155 days	Carrot	á-carotene	88
			â-carotene	106
Kopas-Lane and Warthesen 1995	4EC, dark, 12 days	Carrot	á-carotene	96
			all-*trans*-â-carotene	95
			9-*cis*-â-carotene	73
			13-*cis*-â-carotene	129
Kopas-Lane and Warthesen 1995	4EC, light, 12 days	Carrot	á-carotene	118
			all-*trans*-â-carotene	100
			9-*cis*-â-carotene	53
			13-*cis*-â-carotene	136
Simonetti et al. 1991	4-6EC, 95% RH, 21 days	Spinach	â-carotene	89
Simonetti et al. 1991	4-6EC, 95% RH, 21 days	Peas	â-carotene	54
Kopas-Lane and Warthesen 1995	4EC, dark, 8 days	Spinach	all-*trans*-â-carotene	82
			9-*cis*-â-carotene	90
			13-*cis*-â-carotene	88
Kopas-Lane and Warthesen 1995	4EC, light, 8 days	Spinach	all-*trans*-â-carotene	59
			9-*cis*-â-carotene	52
			13-*cis*-â-carotene	55
Wu et al. 1992	4EC, 3 days	Green beans	â-carotene	114
Wu et al. 1992	4EC, 3 days, then 10-16EC under fluorescent light, 4 days	Green beans	â-carotene	110
Wu et al. 1992	4EC, 3 days, then 10-16EC under light, 3 days, then 4EC, 3 days	Green beans	â-carotene	103
Wu et al. 1992	4EC, 3 days	Broccoli	â-carotene	117
Wu et al. 1992	4EC, 3 days, then 10EC in lighted walk-in cooler, 4 days	Broccoli	â-carotene	100
Wu et al. 1992	4EC, 3 days, then 10EC in lighted walk-in cooler, 3 days, then 4EC, 3 days	Broccoli	â-carotene	88

Simonetti et al. (1991) found that â-carotene levels decreased in spinach and peas stored in boxes for 21 days at 4ºC to 6ºC and 95 percent relative humidity (Table 7), although the loss in spinach was not statistically significant. In another study (Kopas-Lane and Warthesen 1995), the all-*trans*-â-carotene in spinach showed an 18 percent loss after eight days in the dark at 4ºC. Losses were greater under light

exposure at the same temperature, amounting to 41 percent for all-*trans*-â-carotene, 48 percent for 9-*cis*-â-carotene, and 45 percent for 13-*cis*-â-carotene after eight days.

Wu et al. (1992) simulated retail market conditions in the U.S. for green beans and broccoli. The vegetables were held for five hours post-harvest at ambient temperature (20 to 25°C) and refrigerated at 4°C for three days to simulate transport time and temperature. The green beans were then subjected to display conditions at 10 to 16°C under fluorescent light, while the broccoli was held at 10°C in a lighted walk-in cooler for four days, resembling the holding conditions in supermarkets. A portion of the vegetables was removed after three days of storage in a display case or cooler and placed in a home refrigerator at 4°C for three days to simulate consumers' storage conditions at home after purchase. No statistically significant changes were seen in the â-carotene level under the different simulated retail market conditions (Table 7).

APPRAISING PROVITAMIN A RETENTION

Conflicting results for carotenoid retention have been reported even for the same food and the same type and conditions of processing and storage. This may be due in part to the analysis and calculation of retention rather than real changes occurring in foods. Alleged increases in the provitamin A concentration after thermal treatment, for example, may be due to the greater ease with which the carotenoids of cooked or processed samples can be extracted compared with raw samples; to enzymatic degradation of the raw samples during analysis; and to unaccounted losses of moisture and soluble solids that concentrate and elevate the carotenoid content per unit weight. On the other hand, degradation of carotenoids during analysis may be erroneously attributed to processing and storage.

The problems with carotenoid assays are compounded when the objective is to evaluate the effects of home or industrial processing and storage on the provitamins. Although the literature on the retention or loss of carotenoids during processing and storage appears to be extensive, most of the papers involved measurements of total carotenoid or carotene content. Aside from the fact that total values are only gross estimates, the influence of different factors associated with processing and storage can only be adequately observed if the provitamins A are specifically and individually quantified.

Highly conflicting results have even been reported for the same food and the same type and conditions of processing and storage. This can be due, at least in part, not to real changes in the food but to the analysis and calculation of retention. For example, claimed increases in the concentrations of â-carotene and other provitamins during thermal processing cannot be true increases because the enzyme system responsible for their biosynthesis has already been inactivated. Chemical transformations that occur on heat treatment involve isomerization and epoxidation of provitamins A, not their formation. The alleged increases of â-carotene could simply be due to the greater ease with which carotenoids of cooked or processed samples can be extracted compared with those of fresh foods, where the carotenoids are physically protected and/or are combined with other food components that impede solvent penetration and extraction. They might also be due to unaccounted moisture and soluble solid losses, which would concentrate and increase the provitamin A levels per unit weight of food. On the other hand, absorption of water or oil, which would dilute the provitamins and decrease their concentrations per unit weight, might not be taken into consideration. Degradation of provitamins A during analysis may also be erroneously attributed to cooking, processing, or storage.

Although it is important to report the provitamin A content per unit weight of cooked or processed food (as the food is eaten) in food composition tables, the calculations for the retention of nutrients should refer to the original sample weight (original fresh weight basis) to take into account weight changes (water and

soluble solid losses or water and oil gains). Alternatively, the calculations can be done on the dry weight (when soluble solid loss is negligible) or on the insoluble solid basis (when the soluble solid loss is significant). Preferentially, the percent true retention (TR) should be calculated according to Murphy et al. (1975):

$$\%TR = \frac{\text{nutrient content per g of cooked food} \times \text{g of food after cooking}}{\text{nutrient content per g of raw food} \times \text{g of food before cooking}} \times 100$$

Comparing the retention of several nutrients under different situations of weight changes, Murphy et al. found that when retention was calculated on the dry weight basis, the true retention was overestimated in nearly all instances. It is not always feasible, however, to obtain data on weights of foods before and after processing, especially under industrial production conditions.

It is also very important to specify the processing and storage conditions (time, temperature, etc.) and to use paired samples (i.e., comparable raw and cooked samples). Using paired samples in retention studies would obviate disparities due to factors such as varietal differences, seasonal or climatic effects, degree of maturity, or non-uniform distribution of the analyte in the food or food lot, so that sampling errors would not pose a problem. Speek et al. (1988), for example, prepared samples of leafy vegetables in stalks by systematically picking off leaves from the top to the roots and alternately dividing them into two portions. One portion was analyzed raw and the other after processing. In the Food Science Laboratory of the Universidade Estadual de Campinas, Brazil, fruits or fruit vegetables are quartered longitudinally: two opposite sections are taken for analysis of raw samples and the other two opposite sections are submitted to processing before analysis.

It is also recommended that the data be analyzed statistically so that the real meaning of the results can be appreciated.

EFFECT OF HOME PROCESSING ON PROVITAMIN A CONTENT OF FOODS

Because of discrepancies in the results obtained and the different behavior of carotenoids in different foods, specific recommendations cannot be given in relation to the optimum conditions for home preparation/processing and storage for given foods. However, general guidelines can be made, such as keeping processing/heating time and temperature to a minimum, avoiding cutting into small pieces or maceration, reducing the time lag between cutting/chopping and processing, washing before cutting, cooking with the pot lid on, and keeping storage time to a minimum. In general, provitamin A losses increase from microwaving, steaming, boiling, and sautéing. Deep-frying, prolonged cooking, combination of several preparation/processing methods, baking, and pickling result in substantial losses of provitamins A.

Most of the data available on the provitamin A content of foods refer to the raw materials. It is evident, however, that data relating to the form in which the foods are consumed by the population are urgently needed and the influence of processing and storage on provitamin A levels has to be determined. This type of information will help consumers and processors choose the processing and storage conditions that favor provitamin A retention.

Results of retention studies are difficult to assess, as discussed in the preceding section. The principal obstacles to a meaningful interpretation of the results in many scientific papers are:

- processing and storage conditions are not or are only partially described
- different foods are prepared or cooked/processed differently, making comparisons of processing methods difficult
- different conditions are used for the same method of processing
- the procedure followed for the calculation of the retention or loss is not specified
- weight changes are not considered, and the percent loss or retention is calculated from the concentrations of the provitamins per unit of fresh and cooked weight.

Cooking

Dietz et al. (1988) compared water- and steam-blanching in four vegetables, retention being calculated according to Murphy et al. (1975) (Table 8). Boiling for 30 minutes retained 47 and 60 percent of the â-carotene in lettuce and carrots, respectively. Full retention of â-carotene occurred in boiled winged bean leaves and spinach; the greater than 100 percent retention was probably due to the more efficient extraction of heat-treated samples. Retention of á-carotene in boiled carrots was 64 percent. Steaming for 30 minutes resulted in very good retention of á- and â-carotene in all vegetables.

Table 8: Retention of â-carotene During Home Processing in Different Countries

Country/Reference	Calculation of Retention	Cooking conditions	Food product	Retention of â-carotene (%)
U.S.A.				
Dietz et al. 1988	Retention calculated according to Murphy et al. (1975)	**Water blanching:** 10 g sample cooked in 100 ml boiling water 30 minutes	Winged beans	119
			Lettuce	47
			Carrot	60[a]
			Spinach	112
		Steam blanching: 10 g sample steamed in household steamer 30 minutes at 100ºC.	Winged beans	83
			Lettuce	104
			Carrot	99[a]
			Spinach	132
PAKISTAN				
Nagra and Khan 1988	Retention calculated on the basis of original weight of uncooked vegetable.	**Cooking:** 5 g cut sample cooked in distilled water 60 minutes (simulating traditional method)	Bitter gourd	78
			Brinjal	62
			Cabbage	56
			Colocasia	67
			Carrot (red part)	41
			Carrot (white part)	67
			Cauliflower	67
			Colbash	66
			Gand godhi	50
			Green turnip	67
			Lady’s finger	50
			Large chillies	76
			Peas	61
			Spinach	75
			Squash	50
			Vegetable sponge	90
			Yellow turnip	81
THAILAND				

40

Country/Reference	Calculation of Retention	Cooking conditions	Food product	Retention of â-carotene (%)
Speek et al. 1988	Retention calculated as percentage of fresh	**Cooking:** 20 g sample submerged for 2-8 minutes in boiling tap water.	Bitter melon leaves	89
			Coriander	85
			Sweet basil	68
			Celery	84
			Vine spinach	89
			Lettuce, unheaded	77
			Hairy basil	76
			Cha-om	69
			Swamp cabbage	50
		Sautéing: 20 g sample cut into pieces, fried 3-5 minutes in 5 ml pre-heated oil at 200ºC, 3 ml water added during frying	Chinese swamp cabbage	76
			Chinese cabbage	84
			Chinese kale	24
			Chinese chive	19
			Cabbage	57
			Sugar peas	92
		Fermenting: 20 g sample crushed, salt and supernatant of already fermented vegetable were added, fermentation allowed for 1.5 days	Chinese chive	95
			Whole onion plant	78
			Pickled leaf mustard	67
			Eggplant, round shape	22
		Sun-drying: leaves were exposed to direct sunlight for 2 days	Neem tree	53
			Neem tree, duplicate	56
			Amaranth	50
			Sweet basil	39
Wasantwisut et al. 1995	Retention based on â-carotene content per fresh and cooked weight	**Blanching:** 98ºC, 5 minutes	Swamp cabbage	89
			Chinese cabbage	93
			Bai kaprao	95
		Boiling: 97ºC, 5 minutes	Water mimosa	96
		2 minutes	Bai kaprao	80
		Stir-frying: 178ºC, 3.5 minutes	Swamp cabbage	82
		2 minutes	Water mimosa	58
		3 minutes	Bai kaprao	72
INDIA				
Padmavati et al. 1992	Calculation of retention based on weight of raw vegetable	Processing conditions not specified Minimum processing (Salads)	Tomato salad (pieces)	94
			Carrot salad (pieces)	91
			Carrot salad (grated)	89
			Tomato raita	81

Country/Reference	Calculation of Retention	Cooking conditions	Food product	Retention of â-carotene (%)
		Short time cooking-chopping, sautéing	Carrot bhaji	82
			Methi chanadal bhaji	71
			Palak bhaji	71
			Palak paneer	70
			Mayalu bhaji	67
			Aloo methi	56
			Aloo palak	50
			Yellow pumpkin bhaji	39
			Amaranth curry	34
			Colocasia patal bhaji	36
			Methi dal	34
		Chopping + steaming + shallow frying	Coriander vadi	92
			Methi muthia	56
			Colocasia patra	38
		Chopping + deep-frying	Mayalu pakoda	39
			Palak bhaji	28
			Palak pakoda	26
			Coriander vadi (deep-fried)	29
			Methi muthia (deep-fried)	26
			Patra (deep-fried)	15
		Chopping and roasting	Methi thepla	90
		Maceration/grinding	Coriander chutney (blender)	87
			Curry leaves chutney	81
			Mint ratia	81
			Gongura chutney	74
			Mint chutney (stone grinder)	67
			Mint + coriander chutney (stone grinder)	60
		Prolonged cooking + grating/grinding	Pumpkin halwa	31
			Cream of carrot soup	30
			Palak soup	21
			Carrot halwa	20
			Tomato chutney	19
Reddy et al. 1995	Basis of retention calculation not specified	Processing conditions not specified		
		Cutting/sautéing	Amaranth dhal (with tamarind)	71
			Palak dhal (with tomato)	77
		Grinding	Coriander chutney	76
			Curry leaf powder	76

Country/Reference	Calculation of Retention	Cooking conditions	Food product	Retention of â-carotene (%)
		Grating/prolonged heating	Carrot halwa	48
			Pumpkin halwa	10
		Cooking without lid	Amaranth	17
			Koyyalkura	36
			Bacchali	52
			Palak	46
			Sweet potato	21
			Carrot	67
			Pumpkin	39
			Tomato	100
		Cooking with lid	Amaranth	31
			Koyyalkura	45
			Bacchali	62
			Palak	49
			Sweet potato	43
			Carrot	65
			Pumpkin	36
			Tomato	100
		Steaming	Amaranth	17
			Koyyalkura	60
			Bacchali	63
			Palak	70
			Sweet potato	36
			Carrot	72
			Pumpkin	36
			Tomato	100
		Sautéing	Amaranth	60
			Koyyalkura	62
			Bacchali	75
			Palak	71
			Sweet potato	46
			Carrot	74
			Pumpkin	53
			Tomato	100
BANGLADESH				
Rahman et al. 1990	Retention based on â-carotene content per fresh and cooked weight.	**Method I:** leafy vegetable chopped, washed, heated 7-9 minutes in water adhering to leaves, then transferred to another pot in which salt, onion, garlic, and chillies had been fried in oil. The mixture fried for 4-6 minutes	Lal sak	69
			Mula sak	60
			Palang sak	57
			Pui sak	67

Country/Reference	Calculation of Retention	Cooking conditions	Food product	Retention of â-carotene (%)
		Method II: chopped and washed vegetables, with same amount of salt, oil, and condiments as Method I, boiled for 8-10 min with pot lid on.	Lal sak Mula sak Palang sak Motor sak	88 86 89 89
BRAZIL				
Rodriguez-Amaya et al. 1995b	Retention calculated according to Murphy et al. (1975).	**Boiling:** cut vegetable cooked 5 minutes in boiling water.	Broccoli Okra Spinach	84 68 77
		Boiling: cut vegetable cooked 10 minutes in boiling water.	Carrot Nantes Carrot Imperador Green beans Indian eggplant	91[b] 94[b] 82 82
		Boiling: cut vegetable cooked 15 minutes in boiling water.	Squash	90[b]
		Sautéing: cut vegetable stir-fried 10 minutesin small amount of oil.	Green beans Squash	63 83
Almeida and Penteado 1987	Basis of retention calculation not specified.	**Boiling:** conditions not specified	Carrot	86[c]
Almeida-Muradian and Penteado 1992	Calculation of retention based on weight of raw root.	**Boiling:** Sweet potatoes peeled and cut into small pieces and cooked 10 minutes in boiling water (98ºC).	Sweet potato Acadian Centennial Heart Gold Vineland Bush	 96 87 94 82

[a] Boiling and steaming resulted in 64 and 139 percent retention, respectively, of á-carotene.

[b] á-carotene of Nantes carrots,Imperador carrots and squash was retained 90, 92 and 90 percent, respectively, on boiling. In the sautéed squash, 80 percent of á-carotene and 78 percent of á-cryptoxanthin were retained

[c] á-carotene was retained 88 percent

Boiling 18 Spanish vegetables for 10 to 38 minutes in a covered pot with a minimum of water resulted in much higher levels of â-carotene in the cooked vegetables than in the uncooked vegetables (Granado et al. 1992). This was apparently due to the greater extractability of the carotenoids in the boiled samples and not to true retention, because calculation on the dry weight basis yielded more than 100 percent retentions (101 to 344 percent for â-carotene, 129 to 313 percent for á-carotene and 107 to 326 percent for â-cryptoxanthin). Even with exhaustive extraction, the â-carotene levels in three vegetables expressed in terms of the raw weight were still higher in steamed and microwaved samples, giving retentions of 111 to 118 percent for steaming and 102 to 113 percent for microwaving (Khachik et al. 1992). Surprisingly, boiling green beans for one hour resulted in 112 percent retention.

On the other hand, traditional cooking of 17 Pakistani vegetables in water for 60 minutes led to a 41 to 90 percent retention (mean, 65 percent) in vitamin A activity (Nagra and Khan 1988) (Table 8). These lower retention levels were attributed to the time lag between preparation and cooking and the long cooking time. Frequent stirring during cooking might have also exposed â-carotene to atmospheric oxidation.

Low to good retentions of â-carotene during processing were reported by Speek et al. (1988) (Table 8). Cooking nine vegetables in boiling tap water resulted in a 50 to 89 percent retention in â-carotene (as percentage of fresh) with a mean of 76 percent. Sautéing six vegetables resulted in a 19 to 92 percent retention of â-carotene with an average of 59 percent. Fermenting four vegetables for 1.5 days gave a range of 22 to 95 percent retention with an average of 66 percent. Sun-drying four vegetables for two days retained only 39 to 56 percent (mean, 50 percent) of the â-carotene. In another Thai study (Wasantwisut et al. 1995), blanching, boiling, and stir-frying a few vegetables resulted in 89 to 95 percent, 80 to 96 percent, and 58 to 82 percent retention of â-carotene, respectively.

The influence of different preparation/processing procedures on the â-carotene content of commonly consumed Indian vegetables was investigated by Padvamati et al. (1992) (Table 8). Retention was higher when processing/heating was kept to a minimum. Destruction of â-carotene was least in the preparation of salads, the retention varying from 81 to 94 percent (mean, 89 percent), even when the vegetables were cut or grated. Retention ranged from 34 to 82 percent (mean, 55 percent) when foods were chopped and sautéed. After chopping, steaming, plus shallow frying, â-carotene retention was between 38 and 92 percent (mean, 62 percent). Chopping and deep-frying resulted in much lower retention, 15 to 39 percent (mean, 27 percent), which is understandable considering the severe heat treatment and the lipid-solubility of carotenes. Maceration and grinding led to a 60 to 87 percent (mean, 75 percent) retention of â-carotene. Using a blender/mixer reduced the â-carotene in chutneys less than the use of a traditional grindstone. This finding was attributed to the longer maceration in the stone grinder, which allowed for greater oxidation. Prolonged cooking combined with grating and grinding decreased â-carotene considerably, the retention amounting to 19 to 31 percent with a mean of 24 percent.

Losses of â-carotene were also verified in another Indian study (Reddy et al. 1995) where the same eight vegetables were used in several different processes, making comparisons easier (Table 8). Simple modifications of cooking practices led to appreciable differences in retention. Cooking in a pot without a lid retained 17 to 100 percent (mean, 47 percent) of the â-carotene. With the lid on, retention was higher, ranging from 31 to 100 percent (mean, 54 percent). Cutting vegetables after washing did not result in a significant loss of â-carotene. On the other hand, a 10 to 12 percent loss occurred when vegetables were cut and then washed, despite the water-insolubility of carotenoids. Steaming yielded 17 to 100 percent retentions (mean, 57 percent) and sautéing 46 to 100 percent (mean, 68 percent). Although a poor source of provitamin A, tomato demonstrated excellent retention of this nutrient (100 percent in all treatments). In fact, it was reported that the addition of tomato to a recipe enhanced the stability of â-carotene (Bhaskarachary et al. 1996). This agrees with an earlier finding that in lycopene-rich guava and papaya, the provitamin A carotenoids resisted processing and storage, probably because lycopene exerted a protective effect or it was preferentially oxidized (Padula and Rodriguez-Amaya 1987; Godoy and Rodriguez-Amaya 1991).

Notably, sautéing resulted in greater losses than boiling in other studies (Speek et al. 1988; Rodriguez-Amaya et al. 1995b; Apriyantano and Yumono 1995), but as Reddy et al. (1995) showed, boiling losses were greater than those of steaming (Dietz et al. 1988). Reddy et al. obtained 76 percent retention of â-

carotene in the preparation (grinding) of coriander chutney and curry leaf powder; retention in the preparation of carrot and pumpkin halwa (grating/prolonged heating) was only 48 and 10 percent, respectively. Although the percentages are not exactly the same, these latter findings agree with those of Padmavati et al. (1992).

Rahman et al. (1990) investigated three traditional food preparation practices in Bangladesh (Table 8).

- **Method I**: Tender green leafy vegetables were chopped into small pieces, washed, and heated for seven to nine minutes in the water adhering to the leaves (no extra water was added). The fluid that collected at the bottom of the cooking vessel was either discarded or allowed to evaporate. The vegetables were than transferred to another pot in which salt, onion, garlic, and fresh or dried chillies had been fried in a small quantity of oil; the mixture was fried for four to six minutes. This is the most commonly used cooking method in rural Bangladesh.
- **Method II**: Chopped and washed pieces of vegetables, with similar amounts of salt, oil, and condiments as in Method I, were boiled for eight to ten minutes with the pot lid on. The lid was opened two to four times to allow evaporation. This method is used by both rural and urban people.
- **Method III**: Vegetables and a few fresh chillies were placed on top of partially cooked rice. Once cooked, the vegetables were removed and mashed with raw onion, salt, and mustard oil to make a paste that would be eaten with rice. This method is used infrequently and in only two districts of Bangladesh.

Methods I and II involved four common vegetables. Both methods were carried out by 10 women who were accustomed to these cooking practices. Method III was performed with only one vegetable by eight women who were not accustomed to this cooking practice, but who were trained to cook this way. Retention of â-carotene was lowest with Method I (57 to 69 percent) and highest with Method III (89 to 98 percent). Method II yielded good retention (86 to 89 percent).

Boiling seven vegetables (five trials for each vegetable) to just-doneness (5 to 15 minutes) resulted in mean â-carotene retentions of 68 to 94 percent, while sautéing two of the vegetables yielded a rentention of 63 and 83 percent (Rodriguez-Amaya et al. 1995b) (Table 8). Boiling cut carrots retained 86 percent of the â-carotene (Almeida and Penteado 1987); retention in boiled, cut, sweet potatoes varied from 82 to 96 percent (Almeida-Muradian and Penteado 1992).

In Indonesia, boiling (2 to 15 minutes) and stewing (2 to 5 minutes in oil) green leaves under commonly used conditions resulted in â-carotene retentions of 14 to 61 percent and 20 to 38 percent, respectively (Hermana and Muhilal 1995) (Table 9). Simple changes in these practices that involved shorter cooking time, the addition of water, and use of a lid increased the retentions to 25 to 94 percent and 69 to 94 percent, respectively. It was not considered feasible to change the temperature because most families cook at normal atmospheric pressure.

Table 9: Comparison of â-carotene Retention in Laboratory Simulated Indonesian Community Cooking Methods and in Modified Methods

Vegetable/cooking method	Retention[d] (%)	
	Community method	Modified method[e]
Boiling[a]		
Cassava leaves	45	94
Papaya leaves	61	71
Sauropus leaves	14	25
Stewing[b]		
Amaranth	38	73
Chinese cabbage	20	94
Swamp cabbage	36	69
Chajota leaves	—	46
Steaming[c]		
Pumpkin leaves	—	69
Swamp cabbage	—	85
Chajota leaves	—	88

[a] 2-15 minutes at boiling temperature (95-100ºC), depending on the type/texture of vegetables

[b] 2-5 minutes in oil (150-180ºC)

[c] 2-20 minutes over boiling water (95-100ºC)

[d] Calculated on the dry weight basis

[e] Modification involved shorter cooking time, addition of water (stewing) and the use of lid during cooking

Source: Hermana and Muhilal (1995)

In sweet potato leaves subjected to microwave cooking at 2,450 MHz with an output power of 700W for up to eight minutes (Chen and Chen 1993), the â-carotene level decreased from 152 µg/g to 114 µg/g in two minutes, 72 µg/g in four minutes, and 44 µg/g in eight minutes. The major carotenoid lutein (nonprovitamin A) decreased from 210 µg/g to 92 µg/g after eight minutes of heating. Water convolvulus (Chen and Han 1990) and garland chrysanthemum (Chen 1992) were also cooked in a microwave oven, operated at 100ºC. The carotene content was reduced from 62 to 52 µg/g in water convolvulus and â-carotene from 16 to 7.3 µg/g in garland chrysanthemum after 16 minutes. Microwave cooking appeared to retain carotene and â-carotene better than conventional cooking (boiling) in these leafy vegetables. With water convolvulus, steam cooking (using a steam cooker) demonstrated a carotene retention intermediate between microwave and conventional cooking. In carrot, broccoli, and spinach, no difference in carotene content was observed between microwave (six minutes) and conventional cooking (12 to 20 minutes) (Park 1987).

A large loss of carotene (31 percent) occurred in baked sweet potato (Chandler and Schwartz 1988). Sweet potatoes were wrapped in aluminum foil, placed in a prewarmed conventional oven at 191ºC, and baked for 80 minutes until an internal temperature of 99ºC was reached. Microwaved potatoes showed a

smaller loss (23 percent), which could be explained by the shorter heat treatment. Sweet potatoes were microwaved at full power (6,000W) for seven minutes until reaching an internal temperature of 99ºC.

The effect of freezing and thawing, under ordinary home conditions, on the carotene content of carrot, broccoli, and spinach was evaluated by Park (1987). Not much carotene was lost in frozen vegetables left to thaw for four hours before cooking. However, degradation of carotene was severe after six hours of thawing. In analytical laboratories, it is recommended that frozen samples be thawed in the refrigerator to minimize enzymatic degradation of carotenoids (Schiedt and Liaaen-Jensen 1995). This may have implications at the household level.

Retention of â-carotene from crude palm oil used in four common Indian recipes was estimated to be 70 to 88 percent (Table 10) (Manorama and Rukmini 1991). Cake baked at 220ºC for 45 minutes retained 88 percent of the original â-carotene; this unexpected high retention was explained by the thorough blending of crude palm oil with the other ingredients, avoiding direct exposure of the â-carotene to heat. The good retention (77 percent) in deep-fried muruku (a chickpea snack), was attributed to the short exposure of the oil to high temperature (180ºC). It took five minutes for the oil to reach the temperature and another two to three minutes for the muruku to fry. The shallow-fried suji halwa (a sweet), and the upma (a semolina-based breakfast item), took 15 to 20 minutes to cook. Inexpensive and well accepted by children, suji halwa was considered an ideal food for vitamin A supplementation.

Crude palm oil, however, was found to be suitable only for a single frying. After four consecutive deep fryings, all the â-carotene in the oil was lost. In the first frying, incorporation of the carotenoids into the food material appeared to prevail over heat deterioration.

Many investigations have shown that carotenoids, including the provitamins A, are more concentrated in the peel than in the pulp of fruits (Gross 1987; Rodriguez-Amaya 1993). Thus, the simple peeling of fruits and vegetables can reduce the provitamin A content considerably. In paired samples of immature *C. pepo* and *C. moschata*, the whole fruits had â-carotene contents five times greater than the peeled samples (1.5 compared to 0.3 and 1.0 compared to 0.2 µg/g, respectively) (Arima and Rodriguez-Amaya 1988). The peel of the cucurbit hybrid Tetsukabuto contained 101 µg/g â-carotene while the pulp had only 16 µg/g â-carotene. In the Brazilian fruit cajá (*Spondias lutea*), peeling not only lowered the vitamin A value but also reduced the amount of available edible material drastically (8 g/fruit compared to 2 g/peeled fruit) (Rodriguez-Amaya and Kimura 1989).

Table 10: Retention of Crude Palm Oil â-carotene in Different Cooked Foods

Food	Recipe[a]	Retention (%)	â-carotene (µg) per 100 g serving
Cake	Oven-baked (220°C) batter of flour, eggs, and sugar	88	6,540
Muruku	Deep-fried extruded snack made of chick pea flour and rice flour (2:1); frying temperature, 180±3°C	77	4,400
Suji halwa	Shallow-fried, sweet preparation made with semolina; cooking temperature, 180±3°C	71	6,030
Upma	Common South Indian breakfast item, made essentially with semolina; cooking temperature, 180±3°C	70	1,850

[a] Crude palm oil is used as seasoning with different spices in Upma, as shortening medium in the cake, and as frying medium in muruku and suji halwa.

Source: Manorama and Rukmini (1991)

Drying

Because of the seasonal character of many fruits and vegetables, some affordable means of preserving farm or home garden produce is necessary. This will prevent waste during the harvest season and extend the time the food can be used, increasing the possibility of a year-round supply. Sun-drying, or simply laying the food under the sun is a popular traditional method of preserving fruits and vegetables in African and Asian countries. In areas where there is a shortage of water, it may be the only means of food preservation. However, excessive loss of carotenoids can occur.

Gomez (1981) investigated different drying conditions and treatment in four Kenyan green leafy vegetables (kale, amaranth, cowpea, and cassava). Drying leaves at ambient temperature and humidity took four to six days to reduce the moisture level to 6 to 8 percent. This slow drying was observed to result in the lowest retention of carotenes (calculated as percentage of carotene in fresh leaf controls), presumably due to continued enzymatic activity. A solar dryer of simple design and construction, part of which was covered with a black polyethylene sheet, was tested. Protected solar drying gave significantly higher carotene retention than light-exposed solar drying, except when used for cassava leaves. Steam-blanching as a pretreatment, though causing some initial loss of carotene through heat degradation, was shown to improve retention appreciably during drying and subsequent storage. Differences between light-exposed and protected solar drying became insignificant in all vegetables studied when steam-blanching was carried out. This study also highlighted the different characteristics of different vegetables. Cowpea and cassava leaves demonstrated excellent carotene retention while kale showed poor retention characteristics. This difference was attributed mainly to leaf texture, wilt stability, and photosensitivity.

Sun-drying is the cheapest and most accessible means of food preservation in developing countries, but considerable losses of provitamins A occur. Drying with a solar dryer and protecting the food from direct sunlight minimize destruction of the provitamins. Blanching to inactivate degrative enzymes also reduces losses during drying and storage.

Hermana and Muhilal (1995) reported a difference in the â-carotene retention of six different leafy vegetables dehydrated in a solar dryer (one and one-half to two drying days at 27 to 69°C) (Table 11). Amaranth had the highest (99 percent) and Chinese cabbage the lowest (73 percent) retention of â-carotene.

Table 11: Retention of â-carotene During Home Drying

Reference	Calculation of Retention	Drying Method	Food Product	Retention of â-carotene (%)
Hermana and Muhilal 1995	Retention calculated on the dry weight basis	Dehydrating with a solar dryer at 27-69°C for 1.5 to 2 days.	Amaranth	99
			Cassava leaves	98
			Chinese cabbage	73
			Papaya leaves	80
			Sauropus leaves	79
			Swamp cabbage	83
Rahman et al. 1995	Retention calculated on the dry weight basis	Oven-drying at 60°C, 12 hours	Pat sak	96
			Lal sak	98
		Sun-drying, covered, all day	Pat sak	82
			Lal sak	84
		Sun-drying, uncovered, all day	Pat sak	66
			Lal sak	64

Retention of â-carotene in two leafy vegetables during oven-drying and sun-drying was also evaluated by Rahman et al. (1995) (Table 11). Excellent retention (96 to 98 percent) was obtained with oven-drying; this method is not, however, feasible for use in rural communities where ovens are not readily available. The largest reduction in â-carotene was seen in vegetables sun-dried in a container without a cover (retention between 64 and 66 percent). Leafy vegetables sun-dried in a covered container retained 82 to 84 percent of their â-carotene.

The USAID Vitamin A Field Support Project (VITAL) conducted community-level solar drying activities in some countries where both the solar dryer and operating conditions had been optimized (Linehan 1994). Instructions on the construction of the dryer and the proper use of this drying technique are available (Linehan et al. 1993). A comparison of the vitamin A activity of solar-dried and sun-dried vegetables is given in Table 12; in most cases, values for solar-dried vegetables were higher than those for sun-dried

vegetables. Interestingly, traditional sun-drying seems to be well suited for cassava leaves; both in this study and in the earlier work of Gomez (1981), solar drying did not show better retention of provitamin A in cassava leaves. Retention of provitamin A in solar drying is estimated to be between 50 and 80 percent (data not shown).

Table 12: Comparison of the Vitamin A Activity of Selected Solar and Direct Sun-dried Foods

Food	**Solar-dried** RE[a]/100 g	**Sun-dried** RE[a]/100 g
Carrot	6,850	5,690
Amaranth	2,170	1,690
Pumpkin leaves	2,900	1,250
Cassava leaves	3,000	3,280
Sweet potato leaves	3,060	1,730

[a] 1RE = 6 µg â-carotene, 12 µg other provitamins A

Source: Linehan (1994)

EFFECT OF INDUSTRIAL PROCESSING ON PROVITAMIN A CONTENT OF FOODS

Despite their susceptibility to decomposition, carotenoids may be retained during industrial processing if good technological practices are followed. Processing at the lowest temperature and shortest time possible is recommended, but high temperature short time processing is a good alternative. Blanching may reduce the carotenoid content initially but will prevent further and greater losses during processing (especially slow processing) and storage. Peeling and juice extraction may cause greater reduction of provitamins A than thermal treatment. Using provitamin A-rich raw material will guarantee a product with high provitamin A content even when some losses occur during processing.

Because color is a decisive factor in consumers' preference for a given food, for a long time the major concern in industrial processing, in relation to carotenoids, was the maintenance of color; thus, measurement of total carotenoid content was sufficient. With the emphasis now on the nutritive value of foods, including vitamin A content, determining the levels of specific provitamin A carotenoids has became equally or more important. With the increased health-promoting role of carotenoids, there is more interest in the analysis of the nonprovitamin A carotenoids. As in the preceding sections, the focus of this discussion is on the effects of processing in an industrial or pilot-plant (simulating industrial conditions) scale on provitamins A.

Thermal processing of carrot (retorting conditions of 115.6ºC for 30 minutes) increased the concentrations of the *cis*-isomers of á- and â-carotene substantially while the *trans*-isomers decreased 26 and 35 percent, respectively (Ogunlesi and Lee 1979) (Table 13). Because the *cis*-á- and *cis*-â-carotenes have lower potencies compared with the *trans*-counterparts (Table 3), there was a 15 percent decrease in the vitamin A value. These results echoed an earlier warning by Sweeney and Marsh (1971) that processing vegetables by cooking or canning converts *trans*-carotenes to isomers with lower vitamin A activity. Such a conversion was found to lower the vitamin A values of green vegetables by 15 to 20 percent and those of yellow vegetables by 30 to 35 percent.

Table 13: Retention of Provitamins A in Pilot-plant or Industrial Processing

Reference	Processing condition	Food product	Carotenoid	Retention (%)
Ogunlesi and Lee 1979	Retorting conditions of 115.6EC, 30 minutes	Canned carrot	all-*trans*-á-carotene all-*trans*-â-carotene	74 65
Chandler and Schwartz 1988	Strips blanched at 100EC, two minutes	Sweet potato	all-*trans*-â-carotene	110
	Strips blanched at 100EC, 10 minutes		all-*trans*-â-carotene	93
	Puréed with Fitzmill comminutor		all-*trans*-â-carotene	110
	1st steam injection, 81EC, 30 minutes		all-*trans*-â-carotene	110
	2nd steam injection, 100EC		all-*trans*-â-carotene	100
	Canning in still retort, 116EC, 90 minutes		all-*trans*-â-carotene	77
	Dried in double drum dryer, 160EC, 25 rpm		all-*trans*-â-carotene	60
Godoy and Rodriguez-Amaya 1987	After hot-filling, sealed cans were immersed in boiling water, 20 minutes	Canned mango slices	â-carotene á-cryptoxanthin	109 87
	Mango purée heated in open steam-jacketed kettle to 80EC 10 minutes. After hot-filling, sealed cans were immersed in boiling water, 20 minutes	Canned or bottled mango purée	â-carotene á-cryptoxanthin	87 62
Godoy and Rodriguez-Amaya 1991	Processed as described above for mango purée	Canned or bottled papaya purée	â-carotene ã-carotene â-cryptoxanthin	88 100 74
Valadon and Mummery 1981	Puréed peeled oranges pasteurized at 99EC, 30 minutes	Canned orange purée (Spanish variety)	á-carotene â-carotene â-cryptoxanthin	22 100 104
		Canned orange purée (Turkish variety)	á-carotene â-carotene â-cryptoxanthin	100 125 87

Reference	Processing condition	Food product	Carotenoid	Retention (%)
Arima et al. 1992	Squash blanched 10 minutes, disintegrated, thermally processed in open steam-jacketed kettle 40 minutes with addition of sucrose and glucose, hot filled. Sealed cans immersed in boiling water 10 minutes	Canned sweetened pumpkin Menina Verde	á-carotene â-carotene	79 65
Bao and Chang 1994[a]	Unblanched: after juicing, salt was added and the juice heated 82EC, 5 minutes	Carrot juice	á-carotene â-carotene	57 59
	Blanched (water): carrot heated 5 minutes in boiling water.		á-carotene â-carotene	45 50
	Blanched (HOAc): carrot heated 5 minutes in boiling acetic acid solution.		á-carotene â-carotene	41 42
	Unblanched as described above and canned (115.6EC/25 minutes).		á-carotene â-carotene	48 53
	Blanched (water) as above and canned (115.6BC/25 minutes)		á-carotene â-carotene	33 29
	Blanched (HOAc) as above and canned (115.6EC/25 minutes).		á-carotene â-carotene	33 32
	Unblanched as described above and canned (121.1EC/10 minutes).		á-carotene â-carotene	36 41
	Blanched (water) as above and canned (121.1EC/10 minutes).		á-carotene â-carotene	34 30
	Blanched (HOAc) as above and canned (121.1EC/10 minutes).		á-carotene â-carotene	34 30
	Unblanched as above and concentrated in rotary evaporator 40 to 50EC until one-third of original weight		á-carotene â-carotene	57 49
	Blanched (water) as above and concentrated as described above.		á-carotene â-carotene	44 34

Reference	Processing condition	Food product	Carotenoid	Retention (%)
	Blanched (HOAc) as above and concentrated as described above.		á-carotene â-carotene	37 29
	Unblanched as above and freeze-dried until moisture fell below 10 percent.		á-carotene â-carotene	56 46
	Blanched (water) as above and freeze-dried as described above.		á-carotene â-carotene	41 30
	Blanched (HOAc) as above and freeze-dried as described above.		á-carotene â-carotene	36 26
Chen et al. 1995	Juice acidified to pH 4.0 with citric acid and heated at 105EC, 30 seconds, with lab. pasteurization system	Carrot juice	all-*trans*-á-carotene all-*trans*-â-carotene	92 96
	Juice (pH 6.1) heated at 110EC, 30 seconds, with UHT/HTST pasteurization system		all-*trans*-á-carotene all-*trans*-â-carotene	54 55
	Juice (pH 6.1) heated at 120EC, 30 seconds, with UHT/HTST pasteurization system		all-*trans*-á-carotene all-*trans*-â-carotene	46 52
	Juice pre-heated to 70EC before canning (still retort at 121EC, 30 minutes)		all-*trans*-á-carotene all-*trans*-â-carotene	39 45
Padula and Rodriguez-Amaya 1987	Fruits blanched 5 minutes, disintegrated, pulped, and heated to 87EC. Hot-filled and sealed cans pasteurized in boiling water 30 minutes	Guava juice	â-carotene	100
Dietz and Gould 1986	Extraction of juice (0.23” screen).	Tomato juice	â-carotene	80
	Pasteurization at 121EC, 42 seconds		â-carotene	79
	Canning (conditions not specified)		â-carotene	72

Reference	Processing condition	Food product	Carotenoid	Retention (%)
Howard et al. 1994	Peppers water-blanched 3 minutes at 100EC, packed and covered with brine solution. Sealed cans processed at 100EC 30 minutes	Canned Jalapeño-M		
		Green	á-carotene	73
			â-carotene	64
		Red	á-carotene	37
			â-carotene	69
		TAM Mild Jalapeño		
		Green	á-carotene	60
			â-carotene	91
		Red	á-carotene	80
			â-carotene	54
		TAM Veracruz		
		Green	á-carotene	50
			â-carotene	98
		Red	á-carotene	82
			â-carotene	83
Ramos and Rodriguez-Amaya 1993	Hot air drying at 65EC in a food industry.	Dehydrated spinach	â-carotene	88
	Frozen at -30EC and lyophilized at -10EC in a food industry	Lyophilized spinach	â-carotene	88
Ramos and Rodriguez-Amaya 1996[b]	Hot air drying at 70-80EC in a food industry.	Dehydrated carrot	â-carotene	104[b]
	Frozen at -30EC and lyophilized at -10EC in a food industry	Lyophilized carrot	â-carotene	84
Nyambaka and Ryley 1996	Sample blast frozen at -30EC, 1 hr., then dried with lab. freeze dryer (50 mm Hg).	Italian spinach	all-*trans*-â-carotene	67
	Sample dried 6 to 8 hr. in constructed solar dryer.	Italian spinach	all-*trans*-â-carotene	57
		Spring cabbage	all-*trans*-â-carotene	59
		Cowpea leaves	all-*trans*-â-carotene	62

[a] Retention calculated according to the formula:

$$\% \text{ Retention} = \frac{\% \text{ Yield (product)} \times \% \text{ Solid (product)} \times \text{Carotene content (product)}}{\% \text{ solid (fresh)} \times \text{Carotene (fresh)}}$$

[b] Retention calculated on the insoluble solid basis

Canned carrot and green peas were found to have higher carotenoid levels than fresh samples (Edwards and Lee 1986). This was not due to a true increase in the amount of carotenoids. In canned carrot, it was

mainly due to the leaching of soluble solids into the brine during processing, which increased the carotenoid concentration per unit weight of food. Loss of soluble solids during the thermal processing of carrot was earlier estimated to be 35 percent of the total solids (Ogunlesi and Lee 1979). On the other hand, the comparatively higher carotenoid content in canned peas was attributed to degradation of carotenoids by enzymatic activity in fresh green peas during the extraction step of the analysis. Fresh green peas, blended, and allowed to stand for two hours prior to extraction, showed a 68 percent decrease in total carotenoid content.

Isomerization and loss of *trans*-â-carotene during processing of sweet potato was monitored by Chandler and Schwartz (1988), on the basis of dry weight (Table 13). A significant increase in *trans*-â-carotene was observed after two-minute blanching, puréeing, and steam injection (81°C, 30 minutes) to gelatinize the starch; sweet potatoes blanched for 10 minutes had less *trans*-â-carotene than the two-minute blanched potatoes. The supposed increase was attributed to the enhanced extractability of heat-treated samples. A second steam injection (100°C) to inactivate enzymes maintained the initial raw product *trans*-â-carotene level. Canning (116°C, 90 minutes) reduced the *trans*-â-carotene level 23 percent and dehydration (drum drying, 160°C, 25 rpm) reduced it 40 percent. Heat processing induced the formation of *cis*-isomers, particularly 13-*cis*-â-carotene. Isomerization of sweet potato â-carotene had been studied earlier by Lee and Ammerman (1974), upon still and rotating retort canning. Agitating retorts usually require shorter processing time due to more rapid heat penetration and the use of higher retort temperatures. Sweet potato roots processed at 127°C (13 minutes) and 132°C (12.6 minutes) in an agitating retort, and at 116°C (34 minutes) in a still retort, had a significantly higher vitamin A value than those processed at 121°C (23 minutes still or 19.25 minutes agitating). It appeared that the shorter time at 127°C and 132°C and the lower 116°C temperature were favorable to the retention of all-*trans*-â-carotene.

Godoy and Rodriguez-Amaya (1987) found that â-carotene was maintained during processing (immersion of filled and sealed cans in boiling water for 20 minutes) of Tommy Atkins mango slices (Table 13). In Golden mango purée, â-carotene decreased from 18 to 16 µg/g (13 percent loss). Compared with sliced mango, mango purée had undergone physical disintegration and extra heat treatment at 80°C for 10 minutes, both of which favor carotenoid destruction. In both mango products á-cryptoxanthin decreased but, considering the very small amount present, the losses were not statistically significant. In the purée of Solo papaya, there was some albeit not statistically significant loss of â-carotene; ã-carotene was maintained; and â-cryptoxanthin decreased from 7.4 to 5.5 µg/g (26 percent loss) (Godoy and Rodriguez-Amaya 1991) (Table 13). Canning the purée (99°C for 30 minutes) of peeled oranges (Spanish and Turkish varieties) did not reduce the â-carotene level, but it did lower the á-carotene level substantially in the Spanish variety (Valadon and Mummery 1981) (Table 13). The principal provitamin A, â-cryptoxanthin, was maintained in the Spanish variety, but decreased 13 percent in the Turkish variety.

In canned sweetened Menina Verde pumpkin, which was submitted to drastic processing conditions (10-minute blanching, disintegrating, thermal processing in an open steam jacketed kettle for 40 minutes, and immersion of filled and sealed cans in boiling water for 10 minutes), the á-carotene content decreased 21 percent and the â-carotene content 35 percent (Arima et al. 1992) (Table 13). Nonetheless, the product would still be a good source of vitamin A because a provitamin A-rich variety was used as the raw material. In fact, with moisture loss, the vitamin A value of the processed squash was much greater than that of the raw squash (580 retinol equivalent per 100 g processed weight compared with 360 retinol

equivalent per 100 g fresh weight). Brazilian children prefer sweetened squash to boiled or sautéed squash..

The effect of processing on á- and â-carotene concentrations in Imperador carrot juice products was investigated by Bao and Chang (1994) (Table 13), using their own formula to calculate retention. The loss after juicing appeared considerable. Juice products from unblanched carrots were found to have higher levels of á- and â-carotene than those from blanched carrots. Blanching was carried out by heating trimmed carrots for five minutes in boiling water or in boiling acetic acid; this was done before juicing. The carrots designated as unblanched were also heated (82ºC for five minutes), but this was done after juicing. Retorting, concentrating, and freeze-drying the juice partly reduced the carotene levels. In most cases, reduction due to processing was greater for â-carotene than for á-carotene.

Chen et al. (1995) studied the effect of various processing methods on the á- and â-carotene content of carrot juice. Destruction of the carotenes was greatest with still retorting at 121ºC for 30 minutes and lowest with pasteurization of acidified juice at 105ºC for 25 seconds using a laboratory system (Table 13). Retentions in high temperature short time heating at 120ºC or 110ºC for 30 seconds were between those observed in the above two processes. Chen et al. found that á-carotene tended to be retained less than â-carotene.

In pasteurized guava juice, the small amount of â-carotene was retained, although immersion of the bottled juice in boiling water was purposely extended to 30 minutes, which was double the usual time needed for this type of product (Padula and Rodriguez-Amaya 1987) (Table 13).

Retention of â-carotene in tomato juice manufactured from 12 tomato cultivars was evaluated by Dietz and Gould (1986). Significant loss was seen only in the juice extraction step (20 percent loss); slight losses occurred on pasteurization (1 percent), and canning (8 percent). Because a two-fold difference was seen between cultivars with the highest and lowest â-carotene content, the choice of cultivar was considered the most important factor in obtaining tomato juice with the highest vitamin A activity.

Lotha and Khurdiya (1994) compared five different methods of extracting juice from Kinnow mandarin — hydraulic pressing, hand reaming, using a screw type extractor, crushing without peel, and crushing with peel. The juice yield was in the range of 36 to 48 percent. Crushing with or without peel resulted in juices with the highest â-carotene levels, but juice obtained by crushing with peel was not acceptable because of its intense bitterness. The â-carotene content was lowest in juice obtained by hydraulic pressing.

Liu and Luh (1977) verified the influence of stage of maturity of the raw tomato on the carotenoid composition of canned tomato paste. Tomatoes at pink, medium red, and full red maturities were transformed into pastes of 26.5 percent total solids by the hot-break process at 104.5ºC for 20 seconds, finishing (removal of skin and seeds), cooling, and vacuum evaporation. The tomato pastes, after filling and sealing of cans, were hot processed for 30 minutes in boiling water. *Trans*-â-carotene was highest in pastes obtained from fully ripe tomatoes (14.8 µg/g) and lowest in pastes from pink tomatoes (8.5 µg/g).

In processed Jalapeño peppers, on the other hand, the influence of the maturity stage was not as clear, with the retention of á-carotene and â-carotene randomly higher or lower in peppers processed at the green or red stage (Howard et al. 1994) (Table 13).

Baloch et al. (1977a) assessed enzymatic oxidation of carotenoids in unblanched carrots, incomplete extraction of pigments from raw carrots, and leaching of soluble solids during processing of carrots, to find possible explanations for apparent increases in carotenoid content during processing. The leaching of soluble solids was found to be the major cause of these increases in carrots, when retentions were calculated on the dry weight basis. When the results were calculated on a water insoluble solid basis, no such increase was observed.

The effects of blanching and predrying treatments on the stability of carotenoids in papaya and pineapple were examined by Sian and Ishak (1991). Carotenoids decreased progressively in both fruits as blanching temperature and time increased. After drying, the unblanched papaya and pineapple retained the highest carotenoid content. Pigment retention after blanching or drying was lower in the pineapple than in the papaya. Sulfur dioxide prevented carotenoids from oxidation. Carotenoids were also more protected when more moisture was retained by adding glycerol and sugar.

Carotene levels in vacuum dried (16 hours at 55ºC, 15 inch Hg) carrot, broccoli, and spinach were significantly greater than those of the microwave dried (high heat setting, 750 watts) vegetables (Park 1987). Park concluded, nonetheless, that dehydration, regardless of drying method, significantly reduced the carotene content of these vegetables. In contrast, in industrial dehydration (hot air drying at 65ºC) and lyophilization (freezing at -30EC and lyophilization at -10EC) of spinach previously immersed in salt and bicarbonate solutions, only a 12 percent loss of â-carotene occurred in both drying methods (Ramos and Rodriguez-Amaya 1993). No loss in â-carotene was observed in the industrial dehydration (hot air drying at 70-80ºC) of steam blanched carrots, but lyophilization brought about a 16 percent decrease (Ramos and Rodriguez-Amaya 1996). These losses are small, considering the drastic processing treatment involved in dehydration and the greater exposure to oxygen. Calculation of losses was done on the dry weight basis for spinach and on a water insoluble solid basis for carrot, because the high soluble solid leaching in carrot resulted in more than 100 percent retention of â-carotene when dry weight was used.

In a recent study on reversed-phase HPLC separation of the geometric isomers of á- and â-carotene in dark green leafy vegetables, 67 percent of all-*trans*-â-carotene was retained after freeze-drying Italian spinach and 57 to 62 percent after solar-drying Italian spinach, spring cabbage, and cowpea leaves (Nyambaka and Ryley 1996) (Table 13). However, the â-carotene concentrations of freeze-dried spring cabbage and cowpea leaves were so high that retentions were not presented.

Carotene was completely retained during home dehydration of green pepper (Desrosiers et al. 1985). In peach, 73 percent of the carotene was retained after the predrying treatment (blanching, peeling, and dipping in ascorbic acid). The level retained fell to 37 percent after dehydration. The high retention of carotene in green pepper was attributed to the presence of natural antioxidants.

In a series of papers, Mínguez-Mosquera et al. reported on the influence of industrial processing of paprika on carotenoid composition. The drying and milling stages did not affect all of the pigments equally (Mínguez-Mosquera et al. 1993). The yellow pigments, particularly â-carotene, were the most unstable; the red pigments were highly stable. In drying the Bola pepper variety at 35EC, a period of carotenoid biosynthesis, including â-carotene and â-cryptoxanthin, occurred after harvesting (Mínguez-Mosquera et al. 1994a), which was strongly favored by light. At the final stages of drying, light had a strong degradative effect. It was suggested that in order to obtain dry peppers for paprika with a 20 to 40 percent increase in carotenoid concentration, the drying process should consist of a first phase of illumination and a second phase of darkness. Two industrial drying processes were compared: slow drying

by wood combustion and fast drying using hot air (Mínguez-Mosquera et al. 1994b). The concentration of some pigments increased in Bola peppers dried with wood combustion, which was interpreted as a reflection of biosynthesis. During fast drying, degradative losses were evident.

Carotenoids in two varieties of pepper, Bola and Agridulce, behaved differently during drying (Mínguez-Mosquera and Hornero-Méndez 1994b). The major carotenoid capsanthin increased in the Bola variety and decreased in the Agridulce variety. In contrast, â-cryptoxanthin went down in the Bola variety but increased in the Agridulce pepper. The â-carotene level was reduced in both varieties. All the carotenoids quantified decreased during milling. The combined loss of â-cryptoxanthin due to drying and milling was 79 percent in the Bola variety and 65 percent in the Agridulce pepper. The corresponding losses for â-carotene were 82 and 67 percent. The Agridulce variety was found to be more suitable for paprika production, giving a final product with a more intense color and higher provitamin A content.

During slow industrial drying (30 to 35EC) of the Agridulce variety, three phases were discerned (Mínguez-Mosquera et al. 1994c). In the first phase, there was a decrease of the pigment content of the fruits. The second phase indicated an increase in carotenoid concentration, although the previous loss was not compensated. In the third phase, degradation prevailed. This pattern was shown by both â-carotene and â-cryptoxanthin under illumination and in darkness.

While other carotenoids underwent transformations, â-carotene and lutein in olives resisted fermentation and the curing process (210 or 89 days) (Mínguez-Mosquera et al. 1989; Mínguez-Mosquera and Gandul-Rojas 1994). In mustard, â-carotene and lutein were reportedly reduced to one-third of their original contents after 50 days of curing (Fan et al. 1993).

The traditional method of palm oil production retained more â-carotene (80 percent) than the mechanized process (23 percent) (Jideani 1992). The explanation was that the palm fruits processed in the traditional method were not exposed to very high temperatures during processing. When palm oil was heated to 160 to 200EC, the destruction rate of â-carotene doubled for every 20EC rise in temperature.

STABILITY OF PROVITAMINS A DURING STORAGE OF PROCESSED FOODS

Retention of provitamins A during storage of processed foods is favored by low storage temperature, protection from light, exclusion of oxygen — by vacuum or hot-filling, modified atmosphere packaging, or oxygen-impermeable packaging — and the presence of a natural or added antioxidant. In general, salt treatment and sulfiting also increase retention. Provitamins A in canned or bottled products are generally well retained for at least a year. Carotenoids in dehydrated products are more likely to undergo degradation during storage because the greater surface area and porosity increase their exposure to oxygen and light. Blanched products generally resist carotenoid decomposition during storage better than unblanched foods.

The susceptibility or resistance of carotenoids to degradation during food storage was recently reviewed (Rodriguez-Amaya 1993b). The effects of factors such as carotenoid structure, nature of the matrix, available oxygen, moisture content/water activity, light, temperature, antioxidants, pro-oxidants, fatty acids, sulfites, and sodium chloride in model systems and in food were discussed in detail. In this section, only the changes in provitamin A carotenoids during storage will be discussed.

Retention of provitamins A in canned or bottled fruits and vegetables is generally good for about a year; thereafter, substantial losses can occur (Table 14). During 10 month's storage at room temperature and simulation of conditions in supermarkets in terms of light exposure, the â-carotene content of bottled guava juice remained practically unchanged (Padula and Rodriguez-Amaya 1987). Ambient storage of mango slices in lacquered (epoxy) or plain tin-plate cans did not provoke significant change in the â-carotene level during 10 months (Godoy and Rodriguez-Amaya 1987). However, the â-carotene content decreased about 50 percent after 14 months and 84 percent after 24 months, regardless of the type of can used. This provitamin A had a greater tendency to degrade in bottled (18 percent loss after 10 months) than in canned mango purée. As in the case of mango slices, however, both bottled and canned purée suffered about a 50 percent loss of â-carotene after 14 months, the loss reaching 83 percent after 24 months. In bottled papaya purée, the small amount of â-carotene fell by a small but not statistically significant level after 14 months of storage (Godoy and Rodriguez-Amaya 1991). During the first 10 months, â-cryptoxanthin did not change significantly but decreased 27 percent after 14 months of storage.

In purée made from Spanish variety orange, the á-carotene, â-carotene, and â-cryptoxanthin (principal provitamin) concentrations decreased 50, 44, and 38 percent after 27 months at 10°C and 50, 11, and 30 percent during the same period at 21°C (Valadon and Mummery 1981). In the purée of Turkish variety

orange, â-carotene and â-cryptoxanthin levels were both reduced 60 percent at 10ºC and 80 and 79 percent, respectively, at 21ºC after 27 months. Canned tomato juice lost 12 percent of its â-carotene after seven months at ambient storage (Dietz and Gould 1986).

Table 14: Retention of Provitamin A Carotenoids During Storage of Processed Foods

Reference	Storage condition	Food product	Carotenoid	Retention (%)
Padula and Rodriguez-Amaya 1987	Ambient temperature, 10 months	Bottled guava juice	â-carotene	93
Godoy and Rodriguez-Amaya 1987	Ambient temperature, 14 months	Canned (lacquered epoxy) mango slices	â-carotene	52
		Canned (plain tin-plate) mango slices	â-carotene	46
		Canned (lacquered epoxy) mango purée	â-carotene	51
		Bottled mango purée	â-carotene	48
Godoy and Rodriguez-Amaya 1991	Ambient temperature, 14 months	Bottled papaya purée	â-carotene	88
			â-cryptoxanthin	73
Valadon and Mummery 1981	10EC, 27 months	Canned orange (Spanish variety) purée	á-carotene	50
			â-carotene	56
			â-cryptoxanthin	62
	21EC, 27 months		á-carotene	50
			â-carotene	89
			â-cryptoxanthin	70
	10EC, 27 months	Canned orange (Turkish variety) purée	â-carotene	40
			â-cryptoxanthin	40
	21EC, 27 months		â-carotene	20
			â-cryptoxanthin	21
Dietz and Gould 1986	Ambient temperature, 7 months	Canned tomato juice	â-carotene	88
Kon and Shimba 1989	0EC, 3 months	Freeze-dried, blanched squash	â-carotene	91
			á-cryptoxanthin	25
	30EC, 3 months		â-carotene	47
			á-cryptoxanthin	0

Reference	Storage condition	Food product	Carotenoid	Retention (%)
Gee 1979	Stored in air, 6 months	Dried unblanched tomato	â-carotene	45
		Dried unblanched carrot	â-carotene	24
		Dried unblanched spinach	â-carotene	85
	Stored in sealed pouches, 5 months			
	with air	Dried unblanched tomato	â-carotene	41
		Dried unblanched carrot	â-carotene	22
		Dried unblanched spinach	â-carotene	67
	with CO_2	Dried unblanched tomato	â-carotene	58
		Dried unblanched carrot	â-carotene	89
		Dried unblanched spinach	â-carotene	93
	with vacuum	Dried unblanched tomato	â-carotene	77
		Dried unblanched carrot	â-carotene	78
		Dried unblanched spinach	â-carotene	90
	with N_2	Dried unblanched tomato	â-carotene	77
		Dried unblanched carrot	â-carotene	92
Urbányi and Horti 1989	Stored at -20EC, for 174-176 days	Quick-frozen Jubileum tomato of:		
		1st degree ripeness	á-carotene	57
			â-carotene	41
			ã-carotene	38
		2nd degree ripeness	á-carotene	51
			â-carotene	36
			ã-carotene	42
		3rd degree ripeness	á-carotene	43
			â-carotene	30
			ã-carotene	49
	Stored at -20EC, for 370-371 days	Quick-frozen tomato:		
		Kecskeméti 407	á-carotene	101
			â-carotene	104
			ã-carotene	78
		Kecskeméti Jubileum	á-carotene	79
			â-carotene	61
			ã-carotene	36

Reference	Storage condition	Food product	Carotenoid	Retention (%)
		Kecskeméti 555 (K3)	á-carotene	104
			â-carotene	111
			ã-carotene	75
Wu et al. 1992	Frozen at -20EC, 16 weeks	Unblanched green beans	â-carotene	93
		Unblanched broccoli	â-carotene	93
Cavalcante and Rodriguez-Amaya 1995	Frozen at -18EC, 90 days	Unblanched pitanga (*Eugenia uniflora*) pulp	â-carotene	34
			â-cryptoxanthin	62
			ã-carotene	31
Reddy et al. 1995	Ambient temperature, 60 days	Pickled gogu	â-carotene	9
		Pickled carrot	â-carotene	26

Considering the use of light-permeable packaging and additional light-exposure situations during storage of carotenoid-containing products, Pesek and Warthesen (1987) studied carotenoid photodegradation in vegetable juice containing mainly tomato and carrot juice, which had been exposed to 230 ft-c of light at 4ºC. After four days of light exposure, only 25 percent of the initial á- and â-carotene remained, while 75 percent of the lycopene was still present. Structural differences were thought to be responsible for the difference in the degradation rates. Carotene loss was extensive after eight days. The control samples (held in darkness) showed no or negligible destruction of carotenoids.

The effects of various components, additives, and different storage conditions on the stability of carotenoids in sulfited mango pulp were investigated by Sudhakar and Maini (1994). The mango pulps were packed in different containers: glass, polyvinyl chloride, and high density bottles; polyethylene and polypropylene pouches. Storage was at low (2ºC$\pm$1), room (14.5 to 33.9ºC), and high temperatures (40ºC$\pm$1) or in a cool chamber (10.6 to 27.5ºC). Carotenoids were more stable in higher levels of sulfur dioxide. Ascorbic acid and antioxidants also helped to protect the carotenoids from degradation. Retention of carotenoids was higher in the pulp packed in glass containers, with the least surface area exposed to air, and stored at low temperatures. Carotenoids were retained better in pulps of Neelum mango, followed by Totapuri and Chausa varieties. However, the Deshehari and Rataul varieties had higher total carotene contents at the end of the four-month storage period because these mangoes had higher initial carotene levels.

Among the various forms of processed foods, dried or dehydrated products are considered more likely to undergo carotenoid degradation during storage because of the increase in surface area and porosity, the latter being associated with lyophilized foods. Dried carrot has been studied the most.

Blanching before conventional hot air drying was reported to enhance the stability of carotenoids during storage. In dehydrated unblanched carrot, packed in paper-aluminum foil-polyethylene laminate pouches

stored at room temperature (16 to 35°C), only 8.7 percent of the initial carotenoid content remained after eight months (Arya et al. 1982). In carrot blanched in boiling water for six minutes before drying, 21 percent of the carotenoids remained. In another study (Baloch et al.1987) where dried carrots were vacuum-packed in tin-plate cans and kept at 37°C, only 33 percent of the original carotenoids were retained in the unblanched carrot after 440 days of storage. During the same storage period, 48 percent of carotenoids were maintained in the dehydrated carrot blanched for five minutes. The beneficial effect of blanching on the stability of carotenoids during storage is generally attributed to the inactivation of enzymes (peroxidase and lipoxidase) that catalyze carotenoid destruction.

On the other hand, it was observed earlier that higher losses of carotenoids occurred in blanched than in unblanched freeze-dried carrots (Arya et al. 1979). It was also noted that increased leaching of soluble solids during the pre-dehydration steps increased carotenoid degradation during the dehydration and storage of carrots (Baloch et al. 1977a). It was suggested in both studies that some substances capable of stabilizing carotenoids were leached out.

Freezing before hot air drying considerably improved the stability of carotenoids in dehydrated carrot (Arya et al. 1982). About 90 percent of initial carotenoids were retained in pre-frozen dehydrated carrot compared with 20 percent in unblanched and 40 percent in blanched dried carrots after three months of ambient storage.

A combination of sulfiting and blanching, to a level sufficient to inactivate enzyme activity, was considered to be the most effective method for enhancing the storage life of dehydrated carrot (Baloch et al. 1987). Optimization of the blanching process, however, would be highly desirable to gain the maximum benefit from the sulfur dioxide treatment. Sulfiting was found to have a marked positive effect on the stability of carotenoids in both unblanched and blanched carrots during dehydration and storage at 37°C. The effectiveness of sulfur dioxide was reduced by increasing the blanching time to over one minute, the period in which carrot was just adequately blanched. Sodium metabisulfite was also shown to reduce carotenoid destruction (Arya et al. 1982).

Salt treatment (soaking in 10 percent sodium chloride solution for 30 minutes at 20°C) and blanching in the salt solution (2.25 minutes at 96°C) before air drying carrot were found to significantly improve carotenoid stability (Speck et al. 1977). This agreed with the finding of Arya et al. (1979) that soaking blanched carrot in a 5 percent salt solution prior to drying significantly reduced carotenoid degradation. The use of antioxidants also impeded carotenoid destruction.

Carotenoids of dehydrated carrot appeared to be more stable at a water activity (a_w) of 0.43 (Arya et al. 1979). Both below and above this level, the rate of carotenoid destruction increased significantly; this increase was greater at lower than at higher a_w. In freeze-dried papaya, the carotenoids were more stable at 0.33 a_w and, as in carrot, both below and above this optimum a_w, the rate of destruction was higher (Arya et al. 1983). However, contrary to what was seen in carrot, there was a greater increase in carotenoid destruction at higher a_w than lower a_w. The dependence of carotenoid retention on the storage temperature was also demonstrated. After 36 weeks of storage, about 80 percent of the total papaya carotenoid was retained at 0°C compared with 22 and 12 percent at room temperature and 37°C, respectively.

During a six month storage period, the carotene content of home dehydrated pepper decreased slowly in a linear manner, amounting to 14 percent after six months (Desrosiers et al. 1985). In dehydrated peach, the

carotene level was significantly reduced (44 percent) during the first two months but stabilized thereafter. Exposure to light during the six month storage period had an adverse effect on carotene retention in green pepper but not in peach.

Loss of â-carotene in freeze-dried squash during storage at 30°C reached 15, 20, and 53 percent after one, two, and three months, respectively (Kon and Shimba 1989) (Table 14). However, at 0°C the â-carotene loss was only 9 percent after three months. The small amount of á-cryptoxanthin was reduced 75 percent at 0°C and disappeared at 30°C.

Carrot, spinach, and tomato dried without blanching or chemical treatment, with a_w of 0.3 to 0.5, were stored in air, vacuum, nitrogen, or carbon dioxide at room temperature in the dark (Gee 1979) (Table 14). The stability of the â-carotene improved when it was protected from oxygen. For tomato, nitrogen and vacuum storage were more protective than carbon dioxide storage; â-carotene loss was lowered from 59 percent in air to 42 percent in carbon dioxide, and 23 percent in nitrogen and under vacuum after five months. For carrot and spinach, protection was better in all three oxygen-excluding conditions.

Carotenoid stability in spray-dried, foam-mat-dried, and freeze-dried egg powders packed in cans, paper-aluminum foil-polyethylene laminate pouches, under air or nitrogen, and high density polyethylene gauge bags during storage at 4E, 19 to 27E, 37E, 42E and 55°C up to 365 days was appraised by Rao (1992). Air packed and polyethylene packed samples showed greater loss of carotene. Carotenoid retention was better at lower temperatures.

Quick-frozen (-30°C) tomato cubes made from the same cultivar at three stages of ripeness were stored in polyethylene bags at -20°C for 174 to 176 days (Urbányi and Horti 1989) (Table 14). The á-carotene and â-carotene content, as well as the ã-carotene content — which was unusually high in the tomatoes used — all decreased progressively and considerably, regardless of the stage of maturity of the raw tomatoes. In frozen tomato cubes prepared from three cultivars, all at the fully ripe stage, the levels of these three provitamins A fluctuated throughout the storage period, although the level of ã-carotene had a more defined downward trend.

The â-carotene content of green beans and broccoli hardly changed during frozen storage at -20°C for 16 weeks (Wu et al. 1992) (Table 14). There was apparently no significant difference in the â-carotene concentration between raw and unblanched vegetables.

On the other hand, considerable losses of carotenoids (66 percent for â-carotene, 38 percent for â-cryptoxanthin, and 67 percent for ã-carotene) occurred in unblanched pitanga pulp stored for 90 days at !18°C (Cavalcante and Rodriguez-Amaya 1995) (Table 14), indicating enzymatic oxidation. Bocaiúva fruits stored intact at -20°C for five months did not show any reduction in vitamin A activity (Hiani and Penteado 1989b).

Blanched and unblanched pulp of three commercial varieties of mango were packed in polyethylene, polypropylene, paper-aluminum foil-polyethylene laminate pouches, frozen and stored at -12°C (Thakur and Arya 1988). Loss in total carotenoid was considerably higher in unblanched than in blanched mango pulp. After 12 months of storage, carotenoid retention in unblanched samples was 68 to 87 percent in foil laminate, 28 to 57 percent in polypropylene, and 18 to 46 percent in polyethylene pouches compared with 80 to 95 percent, 57 to 82 percent, and 51 to 72 percent, respectively, in blanched samples. Among the three packaging materials tested, the foil laminate pouch provided the best protection for the carotenoids.

Pickling is a common food storage practice in India. Gogu (hibiscus), which is a rich source of carotene, is commonly used for preparing pickles; carrot pickles are also common. The carotene content of both types of pickles, however, decreased steadily and substantially during storage (Reddy et al. 1995). After 60 days of storage, gogu pickles retained only 9 percent and carrot pickles 26 percent of their original â-carotene level.

Several investigations into the stability of carotenoids in paprika have been carried out. The drying temperature and the type of dryer used were found to have an effect on the stability of carotenoids during the storage of red pepper powder (Malchev et al. 1989). Degradation of carotenoids occurred at a higher rate in samples dried at a more elevated temperature. Under equal temperature conditions, the carotenoids in pepper dried in a spray dryer degraded more rapidly than those in pepper dried in an air-fountain dryer. Carotenoid destruction in red pepper was also affected by water activity, package atmosphere, storage temperature, and treatment of the pepper (Lee et al. 1992). Nitrogen flushing or high water activity improved carotenoid retention. To maintain good color quality, it was suggested that red pepper be kept in the form of coarse powder with seeds, at a_w below 0.3, and in a nitrogen atmosphere. Reducing the package free space volume and lowering storage temperature also favored retention of carotenoids.

In comparing ground paprika with and without seeds, no difference in total carotenoid loss was seen after five months of storage (Okos et al. 1990). At the end of the subsequent seven months, the paprika containing seeds had less carotenoid loss than the paprika without seeds. Among different paprika cultivars, the seeds of F-03 (hot) showed the highest level of the antioxidant tocopherol, and the powder of this cultivar showed the lowest degradation of carotenoids during storage (Biacs et al. 1992). The addition of tocopherol and ascorbic acid to the ground product reduced color loss during storage, the latter being more effective.

EFFECT OF COOKING/PROCESSING ON THE BIOAVAILABILITY OF PROVITAMIN A

Insufficient and somewhat conflicting data do not allow an appraisal of the effect of processing and storage on the bioavailability of provitamins A in foods at this time. Urgent, concerted, and intense research is needed on this very important but poorly investigated subject.

Having data on the provitamin A content of food alone is obviously not sufficient. Knowledge of their bioavailability — the proportion of ingested nutrient that becomes available to the body for metabolic processes — is necessary. It is, however, very difficult to assess how much of the provitamin A in a food is actually absorbed by the human body and converted to retinol. Despite many attempts through the years, current information on this issue is meager, fragmentary, and often conflicting. The complexity and differing nature of the matrix in which the provitamins A are embedded in food, the well-known variation in individual human response, the many factors that influence absorption and bioconversion, and the lack of adequate indicators of bioavailability in humans all contribute to the difficulty in establishing the bioavailability of provitamin A in food.

It is especially important to pursue investigation of the bioavailability of â-carotene in dark green leafy vegetables because they are the most widely available and affordable sources of provitamin A worldwide, with â-carotene contents much higher in these vegetables than those in most fruits and non-leafy vegetables.

Dark-green leafy vegetables (Pereira and Begum 1968; Lala and Reddy 1970; Devadas et al. 1978; Jayarajan et al. 1980; Devadas et al. 1980; Charoenkiatul et al. 1985; Hussein and El-Tohamy 1989), grated carrot (Roels et al. 1958; Hussein and El-Tohamy 1989), papaya (Devadas et al. 1980), palm oil (Roels et al. 1963; Lian et al. 1976; Rukmini 1994) and combinations of sweet potato and dark green leafy vegetables (Jalal 1991), amaranth, leaf protein, and â-carotene (Devadas and Murthy 1978), carrot, papaya, and coriander-mint chutney (Wadhwa et al. 1994), were found to increase serum retinol concentration in children of regions where the prevalence of vitamin A deficiency was high. A sweet from the palm fruit buriti was also shown to improve the vitamin A status of children (Mariath et al. 1989). Except for one study (Jalal 1991), one or more weaknesses in the experimental designs of the above studies, such as the lack of negative and/or positive control groups, high rate of drop out, large variation in response within the treatment group, and small number of subjects were pointed out by de Pee and West (1996). On the other hand, two recent studies did not find an improvement in the vitamin A

status of vitamin A-sufficient children (Bulux et al. 1994) and breast-feeding women (de Pee et al. 1995) who were given cooked carrot and stir-fried dark green leafy vegetables, respectively.

Only two studies in humans (Van Zeben 1946; Hussein and El-Tohamy 1990) compare the bioavailability of the same food in raw and processed form. It is reasonable to suspect that cooking or processing may enhance the bioavailability of vitamin A-active carotenoids. As mentioned previously, carotenoids in nature may be complexed with proteins, bound to other components, or physically protected in some other way. Although this physical protective system prevents carotenoid degradation, it may limit its bioavailability when the food is ingested uncooked. Cooking would denature protein and soften the cell walls. The greater ease with which carotenoids in thermally treated foods can be extracted during analysis may imply that they are also biologically more available. On the other hand, provitamins A in sautéed foods may suffer losses during cooking, but may be more bioavailable because of the presence of oil, which is known to enhance absorption of provitamins A. Thus, the choice of processing conditions for foods should be a compromise between increasing bioavailability and keeping degradation losses to a minimum.

The few studies that have some bearing on this subject, however, do not support the above supposition. An early study claimed that absorption of carotene from cooked carrot was considerably less than from grated carrot (Van Zeben 1946). Grated carrot at 30, 50, or 75 g/day and carrot juice at 30 or 45 ml/day did not change the serum concentration of retinol in children (Hussein and El-Tohamy 1990). An increase in retinol level was seen when grated carrot was administered at 150 g/day. Hussein and El-Tohamy (1989) and Roels et al. (1958) reported increased serum retinol concentration when raw grated carrot was used as a supplement. The studies that did not find any effect on serum retinol concentration involved cooked carrot (Bulux et al. 1994) and stir-fried leafy vegetables (de Pee et al. 1995).

Some interesting results have been obtained at the Asian Vegetable Research and Development Center in Taiwan in studies with rats. The bioavailability of â-carotene in raw sweet potato was greater than that of fried sweet potato, which in turn was about two times higher than in cooked or baked sweet potato according to Tsou and Yang (personal communication). The bioavailability of â-carotene in cooked sweet potato leaves appeared to be higher than that in raw sweet potato leaves, while the bioavailability of â-carotene from raw and cooked carrot is similar. Previously, vegetables rich in chlorophylls and nonprovitamin A carotenoids were found to have lower provitamin A bioavailability (AVRDC 1986). The contention that chlorophyll and the other carotenoids have inhibitory effects was corroborated by experiments with purified pigments. A possible inhibitory effect of fiber was also indicated. Results from studies with chicks also suggested that various types of dietary fiber reduce the bioavailability of â-carotene (Erdman et al. 1986).

In rat studies, provitamin A in boiled peach palm was observed to be much more bioavailable than the provitamin A in mango (Yuyama et al. 1991). Because different fruits were used, it is not possible to ascertain whether the difference was due to cooking, the higher fat content of peach palm, or other factors and the laboratory animal experiments will have to be confirmed in human studies.

A study with nonsmoking female volunteers found that plasma â-carotene concentrations fell when pectin was added to the meal (Rock and Sevendseid 1992). It has been suggested that the inhibitory effect of pectin may explain the reduced plasma â-carotene response after ingesting carotenoid-rich food compared with an equivalent dose of synthetic â-carotene supplement. Clearly, much more work is needed on this very important but poorly investigated subject.

FINAL CONSIDERATIONS AND RECOMMENDATIONS TO PROGRAM MANAGERS

Despite the experimental and presentation inadequacies found in many papers and some discrepancies in the results, some conclusions can be drawn:

1. Dark green leafy vegetables, palm oil, palm fruit, carrot, orange sweet potato, mature squashes and pumpkins, and some yellow or orange tropical fruits appear to be the most promising sources in terms of the provitamin A content.
2. The provitamin A content varies considerably from one food to another. Significant variations also exist between samples of the same food because of factors such as stage of maturity, varietal or cultivar differences, climatic or geographical effects, portion of the plant used, post-harvest handling, and storage.
3. The tropical climate of many poor areas of the world enhances biosynthesis of carotenoids, increasing their concentrations during ripening/maturing of fruits and vegetables. On the other hand, this same ambient condition may hasten destruction of carotenoids during post-harvest handling and storage.
4. Carotenoid biosynthesis may continue in fruits, fruit vegetables, and root crops, even after harvest, provided these plant materials are kept intact and not treated in any way that would inactivate the enzymes responsible for carotenogenesis. In leaves and other vegetables, post-harvest degradation of carotenoids appears to prevail, especially at high storage temperature and under conditions that favor wilting.
5. Carotenoids are naturally protected in plant tissues; cutting of fruits and vegetables into small pieces or maceration increases exposure to oxygen and brings together carotenoids and enzymes, which catalyze carotenoid oxidation.
6. The stability of carotenoids differs in different foods, even when the same processing and storage conditions are used. Carotenoids *per se* have different susceptibilities to degradation. For example, although results are somewhat conflicting, á-carotene seems to be less stable than â-carotene. Optimum conditions for provitamins A retention during preparation/processing differ from one food to another.
7. The major cause of carotenoid destruction during processing and storage of foods is enzymatic or non-enzymatic oxidation. Isomerization of *trans*-provitamins A to the *cis*-isomers, particularly during heat treatment, also lowers the vitamin A value of foods, but not to the same extent as oxidation. Enzymatic degradation of carotenoids may be a more serious problem than thermal decomposition in many foods.
8. Reported increases in carotenoid content during thermal processing are not likely to be true increases but are consequences of the analytical process, such as loss of carotenoids in fresh samples due to

enzymatic activity, greater extractability of carotenoids from processed samples, and unaccounted loss of water and leaching of soluble solids.

9. In general, retention of provitamins A decrease in the following order: microwaving is less harmful than steaming, steaming is less harmful than boiling, and boiling is less harmful than sautéing. Deep-frying, prolonged cooking, combination of several preparation and processing methods, baking, and pickling (with the possible exception of the pickling of olives) all result in substantial losses of provitamins A. Whatever the processing method chosen, retention of provitamins A decreases with longer processing time, higher processing temperatures and cutting or maceration of the food. Simple modifications, such as cooking with the lid on; reducing the time lag between peeling/cutting and cooking/processing; shorter cooking/processing time; and minimum storage time improve retention significantly.
10. The heat treatment in blanching may provoke some losses of provitamins A, but the inactivation of oxidative enzymes will prevent further and greater losses during slow processing (as in drying) and storage.
11. Freezing (especially quick-freezing) and frozen storage generally preserve the provitamins, but long time thawing is detrimental.
12. Peeling and juicing result in substantial losses of provitamins A, often surpassing those of heat treatment.
13. Traditional sun-drying, although the cheapest and most accessible means of food preservation in poor regions, causes considerable provitamin A destruction. Drying in a solar dryer, even of simple and inexpensive design, can appreciably reduce losses. Protecting the food from direct sunlight also has a positive effect.
14. Natural or added antioxidant, sulfiting, and salt treatment may reduce carotenoid degradation during storage of processed foods.
15. Exclusion of oxygen, such as through vacuum or hot filling, oxygen-impermeable packaging, or inert atmosphere; protection from light; and storage at low temperatures all protect carotenoids from decomposition.
16. There are insufficient data to appraise the effect of home/industrial preparation and processing on the bioavailability of provitamins A.

In programs designed to promote production and consumption of provitamin A-rich foods, including nutrition education, planners and executors must consider or advocate that the consumer consider the following recommendations:

1. Identify locally available or potentially available, acceptable rich sources of provitamins A. In countries where provitamin A data are not sufficient or not available, data from other countries may serve as a guide. However, locally relevant data must eventually be obtained. The food color may serve as the first clue — dark green leaves or dark orange sweet potato, squash, pumpkins or fruits — to finding important local sources.

2. Process provitamin A-rich food cultivars at optimum maturity/ripeness, the stage at which the food is both appropriate for processing and when it contains a high level of provitamins A.
3. Avoid peeling foods when the peel is edible and its presence will not adversely affect the acceptability of the food, such as increased bitterness. Losses during juicing should also be minimized.
4. Consume or thermally process foods immediately after chopping or macerating. Otherwise, blanching should be considered to inactivate the enzymes.
5. Advocate simple measures such as cooking to just doneness, cooking with the lid on, adding tomato, washing before peeling and cutting, avoiding macerating or cutting into very small pieces, keeping the food intact during storage, and keeping storage time at a minimum. To minimize problems with acceptability of foods, modifications of traditional cooking practices should be tried first.
6. Establish, optimize, and adapt conditions for blanching, processing, and storage for the food concerned. To save time and resources, information on conditions already tested for the food concerned must be sought.
7. Minimize processing time and temperature. High-temperature, short-time processing is a good alternative.
8. Use solar-drying, in which the food is protected from direct sunlight, instead of traditional sun-drying. This appears to be a promising, feasible, and inexpensive means of preserving foods in developing countries. Blanching before solar-drying should be tested, although it may not be practical in areas where there is water shortage.

RESEARCH NEEDS

It is evident that several research areas urgently need to be addressed. Better and more data on the provitamin A content of foods are required. It is essential that differences in results reflect true, natural compositional variations and not analytical errors. The best means of evaluating and improving laboratory and assay method performance is through interlaboratory collaborative studies, especially when a complicated analysis is involved. This can be done in two ways:

- The same homogenous samples can be distributed to different laboratories to be analyzed by methods chosen by the respective laboratories. This type of study will show how well the laboratories are conducting the analyses and which methods are likely to produce accurate results.
- Samples can be analyzed by the different laboratories, using one selected method. The results will demonstrate the performance of the chosen method in an interlaboratory setting.

Guidelines and procedures for adequate sampling and subsampling are prerequisites for the acquisition of reliable data and should be established by a competent international committee. Laboratories experienced in carotenoid analyses can serve as training centers and coordinators of laboratory proficiency testing and method validation. Because vitamin A deficiency is a problem in poor areas of the world, aside from accuracy and precision, sustainability must be considered in choosing the method(s) to be recommended.

Retention studies will also have to be continued to clarify conflicting results; test other processing/storage conditions and other foods; and to evaluate other means of improving retention. Because of discrepancies in the results obtained and the different behavior of carotenoids in different foods, more research is needed before definite recommendations can be made on optimum processing and storage conditions for specific foods. This should be done using reliable analytical methods that separate and individually quantify the different provitamins A, paired samples, well-defined and described processing/storage conditions, and calculations that truly represent retention or loss. The results should be submitted to statistical analysis. Modern home preparation methods such as microwaving or steam cooking; industrial processing such as quick freezing, freeze-drying, and HTST processing; and storage conditions such as refrigeration and modified atmosphere packaging as practiced in developed countries are conducive to excellent retention of provitamins A. However, they are impracticable in many areas where vitamin A deficiency is a public health problem. Solar-drying, for example, may be the only currently possible means of food preservation in some countries. Simple canning, under good technological practices, may be an option in some regions. In any case, the search for affordable, feasible food preservation techniques with maximum retention of provitamins A must go on.

There is a dearth of information on the effect of preparation and processing on the bioavailability of provitamins A, and this aspect needs urgent, concerted, and intense research. The good experimental design needed for this type of work must include accurate determination of the provitamin A content of the food being tested, rather than using data obtained from previous analyses or values taken from food

composition tables, given the compositional variations amply demonstrated in this review. Moreover, there must be some means of compensating for possible significant day-to-day variation, or certifying compositional reproducibility of the food, throughout the experimental period. If the provitamin A concentration of the food is overestimated, the bioavailability will consequently be underestimated and vice versa. In the two studies (both of which were considered to be of strong expermental designs) where vegetables were shown to be ineffective in improving vitamin A status, very little description of the vegetable supplements and their handling was given, in contrast to the detailed accounts in other parts of the studies. It is relatively easy to determine and maintain the provitamin A content of a capsule or a fortified simple food throughout an experiment; the same cannot be said of fruits and vegetables.

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