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Review

Structure, health benefits, antioxidant property and processing and storage of carotenoids

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The conjugated system in which the π electrons are delocalised over the entire length of the polyene chain is responsible for the molecular shape, chemical and physical reactivity and the antioxidant properties of carotenoids. It is sensitive to heat, light and oxygen. Enzymatic and non -enzymatic oxidation is the major cause of carotenoid destruction during processing and storage of food. Bio-availability of carotenoids from different food requires disruption of the food matrices. Thermal treatment and freezing increases the extractability of β-carotene from the food matrices. Ripening is accompanied by increased carotenoid biosynthesis.

Key words: Carotenoids, Health benefits, Free radicals, Vitamin A deficiency, conjugated double bond, Bioavailability, Processing and Storage.

INTRODUCTION

Carotenoids have attracted the interest of researchers from diverse fields including chemistry, biochemistry, food science and technology, medicine, pharmacy and nutrition for more than a century and these fascinating compounds continue to be intensely investigated. In nature, carotenoids are mainly responsible for the red, yellow and orange colors. However, in green plant tissues, the color of carotenoids is masked by the more dominant pigment, chlorophyll and becomes evident only during the degradation of chlorophyll. This phenomenon can be seen during the ripening of fruits as well as in autumn leaves. In food, in addition to their function as the natural pigments and pro -vitamin A precursor role of certain carotenoids, these compounds can be used as food additives for coloring (European Parliament and Council Directive, 1994). The natural characteristic of carotenoid color is significantly present in fruits and vegetables.

Carotenoids are naturally occurring colored compounds that are abundant as pigments in plants. Between 500 and 600 specific carotenoids have been identified, of which only about twenty-four commonly occur in human foodstuffs. The principal carotenoids of foods are β-carotene, β-cryptoxanthin, lycopene, lutein and violaxanthin. Except for violaxanthin, these are also the principal carotenoids found in the human plasma and together with zeaxanthin, are the carotenoids most studied in terms of human health.

Carotenoid pigments, which are abundant in many fruits and vegetables have been studied for a number of years because of their diverse roles in photobiology, photochemistry and photomedicine.

STRUCTURE

Carotenoids are isoprenoid compounds, biosynthesized by tail to tail linkage of two C_{20} geranylgeranyl diphosphate molecules. This produces the parent C_{40} carbon skeleton from which all individual variations are derived. This skeleton can be modified by:

Cyclization at one end or both ends of the molecule to give different end groups.
Changes in hydrogenation levels.
Addition of oxygen containing functional groups.

Carotenoids that contain one or more oxygen atoms are known as xanthophylls, the parent hydrocarbon as

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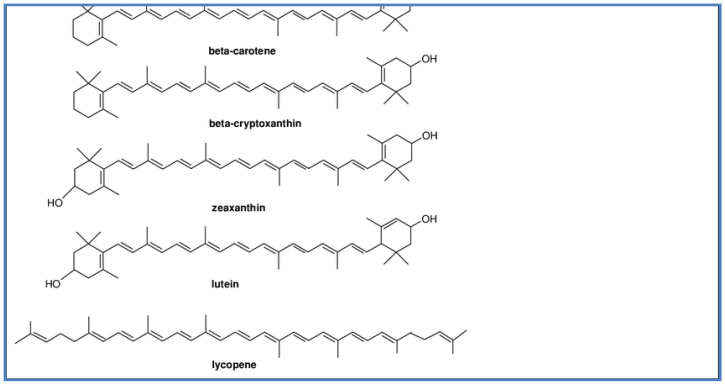

Figure 1. Structure of β-carotene.

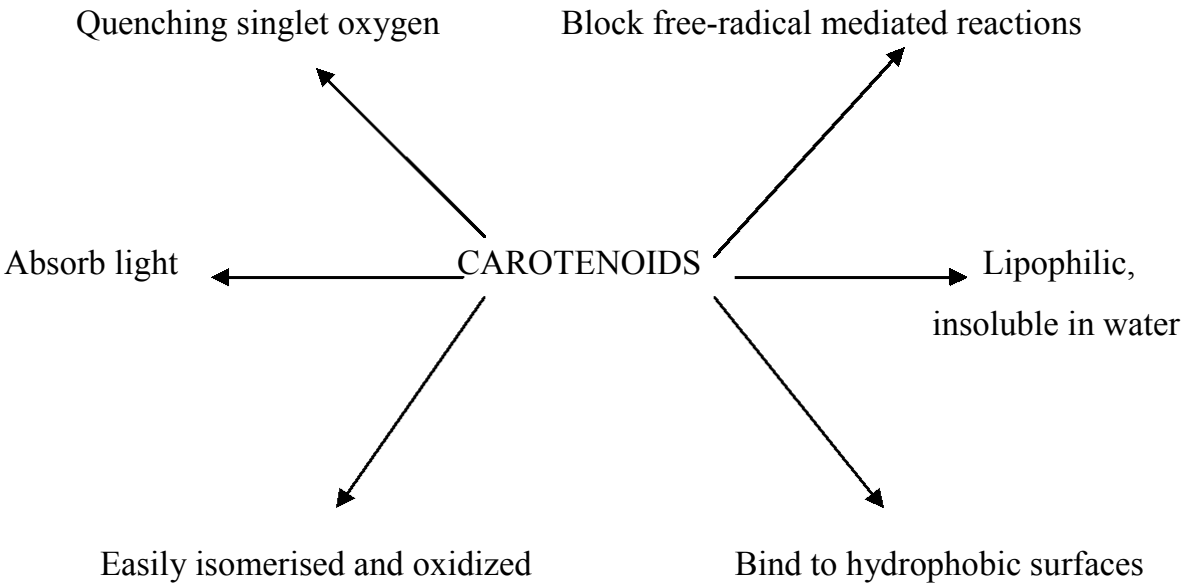

Figure 2. Important physical and chemical properties of carotenoids.

carotene. The long system of alternating double and single bonds constitutes a conjugated system in which the π electrons are effectively delocalised over the entire length of the polyene chain (Figure 1). This feature is responsible for the molecular shape, chemical reactivity and light absorbing properties, and hence color of carotenoids (Britton, 1995). At least seven conjugated double bonds are needed for the carotenoid to impart color. Each double bond in the polyene chain of a carotenoid can exist in two configurations, *trans* or *cis* geometrical isomers. Most carotenoids occur in nature predominantly or entirely in the all *trans* form (Britton, 1995). Important physical and chemical properties of carotenoids are summarized in Figure 2.

DISTRIBUTION AND ABSORPTION OF CAROTENOIDS

The carotenoids of fruit, vegetables and animal products are usually fat-soluble and are associated with lipid fractions. Due to the hydrophobic character, carotenoids are associated with lipid portions of human tissues, cells and membranes. They may also be esterified or complexed with protein (Simpson et al., 1981). During proteolytic digestion, carotenoids are released from associated proteins and aggregate with other lipids. In humans, it has been reported that between 5 and 50% of carotenoids are absorbed (Blomhoff, 1991). Absorption efficiency of carotenoids is known to be affected by the presence or absence of other carotenoids in the diet such

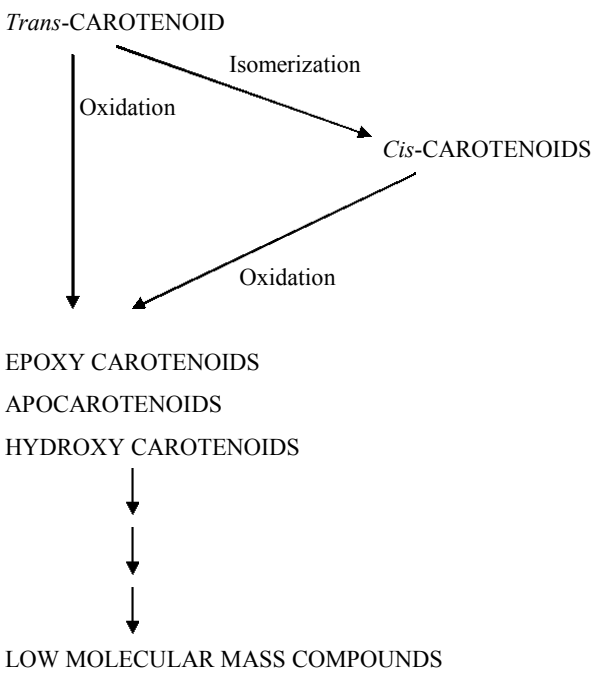

Figure 3. Possible scheme for carotenoid degradation.

as dietary fat and proteins (Hollander, 1981; Shiau et al., 1990) and by bile salts (Olson, 1990). The formation of micelles is a necessary precondition implicating the importance of dietary fat intake for the absorption of carotenoids (Furr and Clark, 1997; Kayden and Traher, 1993). As the amount of carotenoids in the diet increases, the absorption efficacy decreases (Tang et al., 1999). Many factors influence the absorption and utilization of pro-vitamin A such as the amount, type , 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 (Rodriguez-Amaya, 1997) . After absorption, pro-vitamin A carotenoids are cleaved in mucosal cells to form retinal, which is then reduced to retinol. Some unconverted carotenoids are directly absorbed and pass into blood where their composition reflects the diet.

Carotenoids are thus absorbed in the intestinal mucosa with the aid of dietary fat, the uptake of which appears to be by passive diffusion along a concentration gradient between the mixed micelle and the mucosal cell and incorporated into chylomicrons for transport in the serum (Linder, 1991). During normal dietary intake, the hydrocarbon carotenoids (mainly β-carotene and lycopene) and the oxy-carotenoids (mainly leutin) each account for about half of the total plasma carotenoids, with a total serum carotenoid concentration of about 1-2 μmol/L. This apparent difference in carotenoid profile between food products and biological matrices (such as plasma and tissues) is suggestive of selective uptake and/or differences in metabolism between carotenoids (Van den Berg, 1999). The different structural features possessed by carotenoids account for selective distribution in organ tissue, biological activity and provitamin A potency, or *in vivo* conversion to vitamin A. Adipose tissue is the largest body pool for carotenoid (about 80-85% of carotenoids are distributed here) (Bendich et al., 1989) whereas the serum concentrations are fairly constant and slow to change during periods of low intake. The estimated half life was found to be 11-14 days for lycopene, α-carotene, β-carotene, leutin and zeaxanthin (Rock et al., 1992; Miccozzi et al., 1992).

Scientists have long suspected that individuals differ in their ability to absorb β-carotene and convert it to vitamin A. Pro-vitamin A gets converted to vitamin A only when needed by the body, thus avoiding potential toxicity from an overdose of vitamin A. This variation in the body's response to β-carotene is gene-based.

FACTORS AND MECHANISM OF CAROTENOID DEGRADATION

Degradation of carotenoids in foods is complex in nature as various factors such as nature and composition of foods, processing treatments, packaging and storage conditions, activity of lipooxygenase and other enzymes, and coupled oxidation with lipids are considered to play a vital role. Knowledge of carotenoid degradation is fragmentary. The possible scheme for carotenoid degradation is shown in Figure 3.

The polyene chain is the cause of instability of carotenoids including their susceptibility to oxidation and geometric isomerization. Heat, light and acids promote isomerization of *trans*- carotenoids to the *cis*- form. Oxidation depends on the available oxygen, the carotenoids involved, and their physical conditions. It is generally accepted that the initial stage of oxidation involves epoxidation and formation of apocarotenals (Hunter and Krakenberger, 1947; El-Tinay and Chichester, 1970; Ramakrishnan and Francis, 1979; Marty and Berset, 1988). Subsequent fragmentation results in a series of low molecular weight compounds similar to those obtained in fatty acid oxidation, which contribute to the desirable flavor of wine and tea but can be responsible for the off flavor of dehydrated carrots and sweet potato flakes (Falconer et al., 1964).

CAROTENOIDS AND HEALTH BENEFITS

The importance of carotenoids in foods goes beyond their role as natural pigments. Biological functions and actions have been increasingly attributed to these compounds. β-Carotene has an important nutritional role as the principal precursor of vitamin A, which is involved in vision, cell

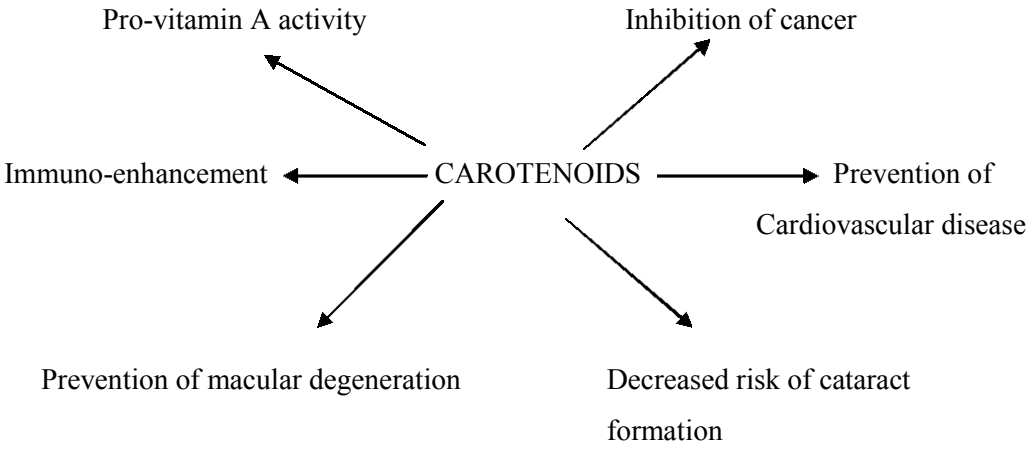

Figure 4. Health promoting functions attributed to carotenoids.

differentiation, synthesis of glycoprotein, mucus secretions from epithelial tissues, reproduction, overall growth and development of bones (Wolf, 1980). On a worldwide basis, about 60% of dietary vitamin A is estimated to come from pro-vitamin A. (Simpson, 1983). According to Simon (1990) world vitamin A deficiency is the most common dietary deficiency. Furthermore, one million children (6 months to six years old) suffer a severe enough level of vitamin A deficiency to cause eye disease, 250,000 of them lose their sight and 150,000 of them die within several months of becoming blind. Diets that are deficient in vitamin A have precipitated the death of children from measles, diarrhea and other disease because of impaired immunity (Sommer et al., 1983). As a result, dietary sources and adequacy of pro-vitamin A continues to be the major concern. The daily pro-vitamin A intake recommended by the FAO is 250 to 400 retinol equivalents (RE) for children, 575 to 725 RE for adolescent and 750 RE for adults. On the other hand, the focus has also shifted to the other health promoting effects of carotenoids. Evidence is accumulating that most of the degenerative diseases that afflict humanity have their origin in deleterious free radical reactions. Carotenoids have been linked with the enhancement of the immune system and decreased risk of degenerative disease such as cancer, cardiovascular disease, age related muscular 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 4). Carotenoids have also been identified as a potential inhibitor of Alzheimer's disease (Zaman et al., 1992).

There are also claims that β-carotene acts as a suppressor of the Human Immunodeficiency Virus (Garewal et al., 1982) and helps in protective immunity against infectious disease such as measles (West et al., 1989; Underwood, 1990; Ross, 1992) . Carotenoids are also present intracellularly and may be involved in the regulation of gene expression or effect cell functions like inhibition of monocyte adhesion and platelet activation (Devraj et al., 1996; Parker, 1997; Rock, 1997). These biological effects are independent of the pro-vitamin A activity and have been attributed to the antioxidant property of carotenoids, through deactivation of free radicals and singlet oxygen quenching (Burton, 1989; Krinsky, 1989; Palozza and Krinsky, 1992).

Despite the large number of publications concerning the health benefits of β-carotene, Davison et al. (1993) pointed out that the characteristics that make a given carotenoid effective and the relative efficacy of the individual carotenoids are not known. Moreover, dose response and pharmacokinetics relationships remain unexplored. This needs to be done because of the possible negative effect of β-carotene such as the potential hemorrhagic toxicity of a large dose of β-carotene (Takahasi, 1995).

ANTIOXIDANT PROPERTY OF CAROTENOIDS

Britton (1995) defined that for carotenoids to be an effective antioxidant, it would have to remove the free radicals from the system either by reacting with them to yield harmless products or by disrupting free radical chain reactions. The electron rich conjugated double bond structure is primarily responsible for the excellent ability of β-carotene to physically quench singlet oxygen without degradation and for the chemical reactivity of β -carotene with free radicals and for its instability toward oxidation (Britton, 1995; Krinsky, 1994). The maximum protection to quench singlet oxygen is given by those carotenoids

having nine or more double bonds (Foote et al., 1970). Handelman (1996) suggested that the following structural properties could contribute to antioxidant functions of carotenoids:

A multiplicity of closely spaced energy levels between the excited state and ground state of the carotenoids, such that the carotenoid can dissipate excited state energy via small collisional exchanges with the solvent.
Minimal tendency for the excited state carotenoid to sensitize other molecules.

Resonance states in the excited state carotenoid allowing delocalization and stabilization of the excited state.

Multiple potential sites on the carotenoid for attack by active oxygen.

Quenching of singlet molecular oxygen

A considerable amount of work has been carried out on the quenching of singlet molecular oxygen ($^1O_2^\bullet$) by carotenoids and how this reaction protects against $^1O_2^\bullet$ mediated photo-oxidation reactions. Singlet oxygen can be carried by electronic transfer from the excited state (normally triplet state) of a sensitizer (SENS) to oxygen (reactions 1 and 2) in biological systems.

$$SENS \rightarrow {}^1SENS^\bullet \rightarrow {}^3SENS^\bullet \quad (1)$$

$$^3SENS^\bullet + {}^3O_2 \rightarrow SENS + {}^1O_2^\bullet \quad (2)$$

Sensitizers such as porphyrins, chlorophyll and riboflavin can sensitize $^1O_2^\bullet$ production and this can lead to deleterious effects including DNA damage and lipid peroxidation (Piette, 1991; Girotti, 1990). Foote and Denny (1968) first demonstrated that β-carotene could inhibit photo-sensitised oxidation and therefore was efficient quencher of $^1O_2^\bullet$. Farmillo and Wilkinson (1973) showed that electron exchange energy transfer quenching is the principal mechanism of carotenoid photo-protection against $^1O_2^\bullet$ although, chemical quenching can also occur leading to destruction of the carotenoid (Liebler, 1993). The carotenoid triplet state ($^3CAR^\bullet$) is produced via electronic energy transfer (reaction 3).

$$^1O_2^\bullet + CAR \rightarrow {}^3O_2 + {}^3CAR^\bullet \quad (3)$$

Once produced $^3CAR^\bullet$ can easily return to the ground state dissipating the energy as heat or it can be quenched physically via enhanced intersystem crossing by $^3O_2$. Thus, the carotenoids act as catalysts deactivating $^1O_2^\bullet$.

As the number of conjugated double bond increases the energies of the excited states decreases (Murrell and Harget, 1972) and this is reflected in the dependence of the $^1O_2^\bullet$ quenching rate constant on carotenoid chain length. Whilst showing that carotenoids are extremely good quenchers of $^1O_2^\bullet$ *in vitro*, they are not directly relevant to biological environment and little work has been carried out to test how effectively carotenoids protect cells against $^1O_2^\bullet$ related damage.

Interactions of carotenoids with free radicals

Carotenoids can also react with free radicals. However, unlike the quenching of singlet oxygen which mainly leads to energy dissipation as heat, the reactions of a carotenoid with a free radical will lead to electron transfer or possibly addition reactions. The major fact to note is the 'odd' electron which characterizes a free radical is not lost. Thus for a free radical ($R^\bullet$) reacting with a carotenoid (CAR), reactions such as

$$R^\bullet + CAR(H) \rightleftharpoons RH + CAR^* \quad (4)$$

and

$$R^\bullet + CAR \rightleftharpoons R^- + CAR^+ \quad (5)$$

are expected, depending on the redox potential of the species involved. Furthermore, carbon-centered radicals are known to react readily (by addition) with oxygen, for example,

$$R^\bullet + O_2 \rightarrow RO_2^\bullet \quad (6)$$

giving peroxyl radicals. Thus, it is expected that the antioxidant property of carotenoid may depend on the oxygen concentrations.
The experimental data on chemical parameters of carotenoid-free radical interactions is limited. Presently available data have proposed electron transfer, addition reaction and hydrogen abstraction as mechanisms for reactions between carotenoids and a range of free radicals.

Electron transfer forming carotenoid radical cation. Oxidizing radicals with high redox potential can remove one electron from the carotenoid molecule to give the radical cation ($CAR^{\bullet +}$) (4)

$$R^\bullet + CAR \leftrightarrow R^- + CAR^{\bullet +} \quad (7)$$

Addition reactions forming a carotenoid-adduct radical, which reacts further to form a nonradical product. As noted by Edge et al. (1997), the radical cation forming process may often arise via an additional complex, such as $CAR + CCl_3OO^\bullet \rightarrow [CAR\text{-}CCl_3OO]^\bullet \rightarrow CAR^{\bullet+} + O_2CCl_3^-$ (8,9)

$$ROO^\bullet + CAR \rightarrow ROO\text{-}CAR^\bullet \quad (8)$$

$ROO\text{-}CAR^{\bullet} + ROO^{\bullet} \rightarrow ROO\text{-}CAR\text{-}$ (9)

Hydrogen abstraction forming the neutral carotenoid radical $CAR^{\bullet}$ (7) For example,

$CAR + CCl_4 \rightarrow CAR^{\bullet} + HCL + CCl_3^{\bullet}$

$R^{\bullet} + CAR(H) \leftrightarrow RH + CAR^{\bullet}$ (10)

the intestinal tract and thus result in increased levels of uptake of the protected carotenoids.

The opening of the β-ionone rings increases the scavenging ability. However, the addition of polar groups onto the rings (e.g. carbonyl and hydroxyl groups) decreases the scavenging ability, possibly due to their electron withdrawing effects as did the lowering the number of conjugated double bonds. Hence an increase in scavenging properties is dependant on extending the chromophore, the better overlap of the C=C π orbitals and increasing the electron density in the conjugated chain.

DIETARY FACTORS AFFECTING THE BIO-AVAILABILITY OF CAROTENOIDS

To increase our understanding of the potential benefit of carotenoids, it is important to obtain more insight into their bioavailability from foods and the factors that determine the bioavailability. The absorption of carotenoids includes several steps (Van hot Hoff et al., 1998). The mnemonic "SLAMENGHI" describes these factors as Species of carotenoids, Linkage at molecular level, Amount of carotenoids, Matrix, Effectors, Nutrient Status, Genetics, Host related factors and Interactions among these variables (Castenmiller et al., 1998). Factors that may interfere with the rate of each of these steps will affect the overall bioavailability of the carotenoids ingested (Van het Hof et al., 2000).

Bio-availability of carotenoids from different food matrices: Disruption of the food matrices and release of carotenoids constitute the first step in carotenoid absorption. The relative bio- availability of β-carotene from vegetables compared with purified β-carotene ranges between 3 and 6% for green leafy vegetables, 19 and 34% for carrots and 22 and 24% for broccoli (Brown et al., 1989; Micozzi et al., 1992; De Pee et al., 1995; Torronen et al., 1996; Castenmiller et al., 1999; van het Hof et al., 1999). These differences may be the result of differences in intracellular location of carotenoids. In leaves, they are present in chloroplasts, whereas in fruits and possibly also other parts of the plant, carotenoids are located in chromoplasts. This has led to the speculation that the chloroplasts may be less efficiently disrupted in the intestinal tract than chromoplasts (De Pee et al., 1998). The presence of dietary fiber in vegetables and fruits usually lowers the bio-availability of carotenoids from plant foods (Erdmann et al., 1986). It has been suggested that fiber interferes with micelle formation by partitioning bile salts and fat in the gel phase of dietary fiber.

Disruption of the food matrix: Not only the intracellular location, but also the intactness of the cellular matrix may be a determinant of carotenoid bio- availability from vegetables and fruits. As early as 1948, Van Zeben and Hendriks reported that homogenization improved the bio-availability of β-carotene from carrots in humans. Some studies have found that cooking enhances the carotenoid content measured in vegetables, possibly due to increased extractability of carotenoids from vegetable matrix (Dietz et al., 1988; Khachik et al., 1992). The increased extractability due to heat treatment may be associated with improved bio-availability of carotenoids from vegetable matrix. Rock et al. (1998) reported recently that the plasma response of β-carotene was enhanced after consumption of pureed, cooked carrots and spinach compared with their raw, unhomogenized form (two fold higher increase in plasma β-carotene) after consumption of the vegetables.

Amount of dietary fat present: An important step in the absorption process of carotenoids that may affect their bio-availability involves the incorporation of released carotenoids into mixed micelles. Among other factors, formation of these micelles is dependant on the presence of fat in the intestine. Therefore, ingestion of fat along with carotenoids is thought to be crucial. Several ivestigators have studied the importance of dietary fat for the absorption of β-carotene (Jayarajan et al., 1980; Dimitrov et al., 1988; Prince et al., 1991). From the findings of Jayarajan et al. (1980) it appears that 5 g of fat in a meal is sufficient to ensure carotenoid uptake.

Type of fat and digestibility of fat soluble components present in the diet: Borel et al. (1998) reported that the type of fat present in the diet also influences carotenoid bio-availability. They showed that if β-carotene was added to a meal with short or medium chain triglycerides, the incorporation of β-carotene into chylomicrons was low compared to the addition of β-carotene to a meal containing long chain triglycerides. Medium chain triglycerides are absorbed primarily via the portal vein; thus the chylomicron formation is low after a meal containing only these types of triglycerides.

Interactions between carotenoids: Interactions at the intestinal level may reduce the absorption of either of the carotenoids. Competition for absorption may occur at the level of micellar incorporation, intestinal uptake, and in lymphatic transport or at more than one level. On the

Table1. Cultivar and geographic effects on the principal carotenoids (μg/g) of papaya (*Carica papaya*)

carotenoid	Comman SP	Solo BA	Formosa SP	Formosa BA	Tailandia BA
β-carotene	1.2	2.5	1.4	6.1	2.3
β-cryptoxanthin	8.1	9.1	5.3	8.6	9.7
Lycopene	-	21	19	26	40

SP- Sao Paulo, BA – Bahia.
Reference : Kimura et al., (1991).

Table 2. Principal carotenoids (μg/g) of Tommy Atkins mangoes (*Mangifera indica*) at three stages of maturity*.

Carotenoid	Stage of maturity		
	Mature green	Partially ripe	Ripe
β-carotene	2.0 ± 0.8	4.0 ± 0.8	5.8 ± 2.5
violaxanthin	6.9 ± 3.0	18±7	22±9

*Mercadante and Rodriguez-Amaya (1998).

Table 3. Principal carotenoids (μg/g) of *Curcurbita moschata* c.v Menina Verde at two stages of maturity*.

Carotenoid	Stage of maturity	
	Immature	Mature
β-carotene	1.5	39
α-carotene	0.1	23

* Armia and Rodriguez-Amaya (1988).

other hand, simultaneous ingestion of various carotenoids may induce an antioxidant sparing effect in the intestinal tract and thus result in increased levels of uptake of the protected carotenoids.

VARIATION IN THE CAROTENOID COMPOSITION

In a given food, qualitative differences occur due to factors such as cultivar or variety, stage of maturity, climate or geographic site of production, part of the plant utilized, farming practices, processing and storage of food.

Differences among cultivars of the same food are well documented. An example is shown in Table 1. The red fleshed papayas Solo, Formosa and Tailandia produced in the Brazilian state of Bahia differed particularly in the lycopene content with the Formosa papayas twice as much as compared to other two cultivars (Kimura et al., 1991).

In most carotenoid containing fruits and vegetables, ripening is accompanied by enhanced carotenoid biosynthesis such as in apricot (Katayama et al., 1971), mango (John et al., 1970; Mercandate and Rodriguez-Amaya, 1993), orange (Rotstein et al., 1972), muskmelon (Reid et al., 1970), papaya (Wilberg and Rodriguez-Amaya, 1995) and pepper (Rahman and Buckle, 1980; Howard et al., 1994; Moya et al., 1994; Minguez-Mosquera and Hornero-Mendez, 1994). As the chloroplasts are converted to chromoplasts, the chromoplasts carotenoid pattern is transformed into a complex composition and the carotenoids increase significantly both in number and in quantity, especially in the principal pigments. An example of this is shown in Table 2 for Tommy Atkins mangoes, in which the β-carotene content increased from the mature green to the ripe stage (Mercadante and Rodriguez-Amaya, 1998). Greater differences would be expected when comparison is made from immature stage. This was indeed the case with *Curcurbita moschata* cultivar Menina Verde (Table 3). β-Carotene content increased dramatically during maturation (Arima and Rodriguez-Amaya, 1988). The β-carotene content of mature leaves of lettuce (12 μg/g) and endive (14 μg/g) were about three times those of the young leaves (3.5 and 4.2 μg/g, respectively) (Ramos and Rodriguez-Amaya, 1987). Carotenogenesis may continue even after harvest, provided the fruit or vegetable remain 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).

Climatic effects are seen in fruits of the same cultivars produced in regions of differing climates, elevated temperature and greater exposure to sunlight, thus increasing carotenogenesis. Comparing papayas of the cultivar Formosa which are produced in the hot Northeastern state of Bahia with those produced in the temperate South eastern state of Sao Paulo, the former fruits had a higher β-carotene content thus signifying greater carotenogenesis (Table 1). The tropical climate enhances carotenoid biosynthesis.

The presence of certain chemicals might inhibit carotenogenesis. Kale leaves at the same stage of maturity, produced in neighboring farms were compared. All constituent carotenoids were significantly higher in samples collected from an organic farm than in those taken from a conventional farm using agrochemical (Mercadante and Rodriguez-Amaya, 1991). This indicated that one of the chemicals used in the latter farm, probably herbicide, inhibited carotenoid biosynthesis in the leaves.

Table 4. Effect of processing on the β-carotene content of some food preparation of Brazil*.

Cooking conditions	Food Product	Retention of β-carotene
Cut vegetables cooked 5 minutes in boiling water	Broccoli	84
	Okra	68
	Spinach	77

*Rodriguez-Amaya et al. (1995).

Table 5. Influence of different processing procedures of β-carotene content of some vegetables of Thailand*.

Cooking conditions	Food Product	Retention of β-carotene
Blanching : 98°C, 5 min	Swamp Cabbage	89
	Chinese Cabbage	93
	Bai kaprao	95
Boiling : 97°C, 5 min	Water mimosa	96
2 min	Bai kaprao	80
Stir-frying: 178°C, 3.5 min	Swamp cabbage	82
2 min	Water mimosa	58
3 min	Bai kaprao	72

*Wasantwisut et al. (1992).

Carotenoids are found at higher levels in the peel than in the pulp of most carotenogenic fruits (Gross, 1987; Rodriguez-Amaya, 1993). In *Spondia lutea*, for example, β-carotene appeared slightly higher in the peeled fruit. (Rodriguez-Amaya and Kimura, 1989).

PROCESSING AND STORAGE OF CAROTENOIDS IN FOODS

Processing and storage of foods have become integral parts of the modern day food chain. More emphasis should be given to increase the production and consumption of locally available carotenoid rich foods. Food processing, storage and preparation also have substantial effects on the content and bio-availability of carotenoids in foods. Seasonal produce are processed during peak harvest, thus diminishing losses and making the products available all year around.

Processing, however, can cause degradation of labile nutrients, biologically active compounds and substances important to food quality. Carotenoids, being highly unsaturated compounds, are prone to isomerization and oxidation, resulting in loss of color and biological activity. Rodriguez-Amaya (1999) has drawn certain conclusion regarding the processing and storage of carotenoids in food. They are:

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. Optimum conditions for pro-vitamin A retention during preparation/processing differ from one food to another. Rodriguez-Amaya et al. (1995) investigated the processing effect through some food preparation of Brazil (Table 4).

The major cause of carotenoid destruction during processing and storage of foods is enzymatic or non-enzymatic oxidation. Isomerization of trans pro-vitamin 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. Occurrence of *trans* to *cis* isomerization as a consequence of thermal processing has been shown by several authors. In a recent work, 10-39% increases in the percentage of total *cis* isomers of pro-vitamin A carotenoid in several fruits and vegetables was observed by Lessin et al. (1997). Canning of sweet potato caused the largest increase, followed by processing of carrots, tomato juice, collard, tomato, spinach, peach and orange juice.

Whatever the processing method chosen, retention of carotenoids decreases with longer processing time, higher processing temperature and cutting or pureeing of the food. Reducing processing time and temperature, and the time lag between peeling, cutting and pureeing and processing improves retention significantly. High temperature/short processing time is a good alternative. The influence of different processing procedures on the β-carotene content of commonly consumed vegetables of

Thailand was investigated by Wasantwisut et al. (1992) (Table 5).

The heat treatment in blanching may provoke some losses of carotenoids, but the inactivation of oxidative enzyme will prevent greater losses during holding before thermal processing, slow processing and storage. Published reports range from substantial losses (Sweeney and Marsh, 1971; Khachik et al., 1986; Chandler and Schwartz, 1988; Speek et al., 1988; Nagra and Khan, 1988; Dikshit et al., 1988; Rahman et al., 1990) to little or no change (Gody and Rodriguez- Amaya, 1987; Khachik et al., 1992) and an increase in the amount of β-carotene levels in the food as a result of boiling (Gomez, 1981).

Freezing and frozen storage generally preserves the carotenoids but slow thawing can be detrimental, particularly when the product has not been properly blanched. Wu et al. (1992) found no change in the β-carotene content of green beans and Broccoli during U.S. retail market simulation and frozen storage (blanched) at –20°C for 16 weeks. No appreciable changes in the β-carotene content of blanched Guku leaves was observed when stored for 16 weeks at –10°C (Benhurra and Chitsiku, 1997).

Peeling and juicing results in substantial losses of carotenoids, often surpassing those of heat treatment. Drying generally causes considerable carotenoid destruction. 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 lyophillized foods. Dried carrots were vacuum packed in tin plate cans and kept at 37°C, 33% of the original carotenoids were retained in the unblanched carrots after 440 days of storage. During the same period, 48% of carotenoids were maintained in the dehydrated carrots blanched for five minutes (Baloch et al., 1987).

Natural or added antioxidant, salt treatment and sulphiting may reduce carotenoid degradation. A combination of sulphiting 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 carrots (Baloch et al., 1987). Sodium metabisulphite was also shown to reduce carotenoid destruction (Arya et al., 1982), while salt treatment (soaking in 10% NaCl solution for 30 min at 20°C) and blanching in the salt solution (2.25 min at 96°C) before air drying carrot were found to significantly improve carotenoid stability (Speck et al., 1977). Sudhakar and Maini (1994) studied the stability of carotenoids in mango pulp with various additives and found that ascorbic acid and antioxidants help protect the carotenoids from degradation.

Exclusion of oxygen, protection from light and low temperature diminishes carotenoid decomposition during storage.

CONCLUSION

The conjugated system of alternating double and single bond in which the π electrons are effectively delocalised over the entire length of polyene chain is responsible for the physical and chemical properties of carotenoids and is primarily responsible for the excellent ability of carotenoids to physically quench singlet oxygen without degradation. Carotenoids are absorbed in the intestinal mucosa with the help of dietary fat and are cleaved in the mucosal cells to form retinal which is reduced to form retinol. The biological properties of the carotenoids have been attributed to the antioxidant property of the carotenoid and is independent of the pro-vitamin A activity.

Qualitative changes of β- carotene in foods occur due to several factors such as cultivar, stage of maturity, climate, part of the plant utilized, farming practices, processing and storage of food. The major cause of carotenoid destruction during processing and storage of food is enzymatic and non-enzymatic oxidation. Thermal processing, freezing and addition of antioxidant generally preserves the carotenoids, but drying, juicing and peeling results in substantial losses. Bio-availability of carotenoids from different food matrices depends not only on the intracellular location but also on the intactness of the cellular matrices. Blanched, cooked and pureed vegetables results in increased extractability of β-carotene compared to the raw homogenized form.

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