
1 Nomenclature, structures, and physical and chemical properties

1.1 INTRODUCTION

Carotenoids are naturally occurring yellow, orange, or red pigments, notable for their wide distribution, structural diversity, and multiple functions and actions. It is estimated that about 100 million tons of these compounds are produced annually in nature (Isler et al., 1967). According to the last compilation, approximately 750 naturally occurring carotenoids have been reported, of which about 500 have been properly characterized (Britton et al., 2004). Currently, the total number of reported carotenoids is probably about 800, of which between 520 and 550 have been fully characterized (Britton, personal communication). This number includes the enormous variety of carotenoids in algae, bacteria, and fungi. In foods, they are not as numerous, but the composition can still be complex and variable.

1.2 NOMENCLATURE

A semisystematic nomenclature for carotenoids (Table 1.1) that conveys structural information, including the stereochemistry (three-dimensional structure), was devised by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC/IUB, 1975; Weedon and Moss, 1995). The names are based on the stem name “carotene,” preceded by Greek-letter prefixes (β , ϵ , ψ , κ), that denotes the two end groups, together with the numbering system for the carbon atoms. The numbering of the carotenoid skeleton is shown for lycopene and β -carotene in Figure 1.1. Changes in hydrogenation and the presence of oxygen-containing substituents are indicated by standard prefixes and suffixes used in organic chemistry. The absolute stereochemistry of chiral, optically active carotenoids is indicated by the R/S designation.

Carotenoids have trivial names, usually derived from the biological sources from which they were first isolated. For the sake of simplicity, these short and familiar trivial names will be used throughout this book. The *E/Z* designation is now preferred to indicate the configuration of the double bonds and will be used in this book instead of the still widely used *trans/cis* terminology.

Table 1.1 Trivial and semisystematic names of food carotenoids.

Trivial Name	Semisystematic Name
Antheraxanthin	(3S,5R,6S,3'R)-5,6-epoxy-5,6-dihydro- β,β -carotene-3,3'-diol
β -Apo-8'-carotenal	8'-apo- β -caroten-8'-al
β -Apo-10'-carotenal	10'-apo- β -caroten-10'-al
Astaxanthin	(3S,3'S)-3,3'-dihydroxy- β,β -carotene-4,4'-dione (3R,3'R)-3,3'-dihydroxy- β,β -carotene-4,4'-dione (3R,3'S)-3,3'-dihydroxy- β,β -carotene-4,4'-dione
Aurochrome	5,8,5',8'-diepoxy-5,8,5',8'-tetrahydro- β,β -carotene
Auroxanthin	(3S,5R,8RS,3'S,5'R,8'RS)-5,8,5',8'-diepoxy-5,8,5',8'-tetrahydro- β,β -carotene-3,3'-diol
Bixin	methyl hydrogen (9'Z)-6,6'-diapocarotene-6,6'-dioate
Canthaxanthin	β,β -carotene-4,4'-dione
Capsanthin	(3R,3'S,5'R)-3,3'-dihydroxy- β,β -caroten-6'-one
Capsorubin	(3R,5R,3'S,5'R)-3,3'-dihydroxy- κ,κ -carotene-6,6'-dione
α -Carotene	(6'R)- β,ϵ -carotene
β -Carotene	β,β -carotene
β -Carotene-5,6-epoxide	(5R,6S)-5,6-epoxy-5,6-dihydro- β,β -carotene
β -Carotene-5,6,5',6'-diepoxy	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β,β -carotene
δ -Carotene	(6R)- ϵ,ψ -carotene
ϵ -Carotene	(6R,6'R)- ϵ,ϵ -carotene
γ -Carotene	β,ψ -carotene
ζ -Carotene	7,8,7',8'-tetrahydro- ψ,ψ -carotene
Citranaxanthin	5',6'-dihydro-5'-apo-18'-nor- β -caroten-6'-one
Crocetin	8,8'-diapocarotene-8,8'-dioic acid
β -Citaurin	(3R)-3-hydroxy-8'-apo- β -caroten-8'-al
Cryptoflavin	5,8-epoxy-5,8-dihydro- β,β -caroten-3-ol
α -Cryptoxanthin	(3'R,6'R)- β,ϵ -caroten-3'-ol
β -Cryptoxanthin	(3R)- β,β -caroten-3-ol
β -Cryptoxanthin-5,6-epoxide	5,6-epoxy-5,6-dihydro- β,β -caroten-3-ol
Echinenone	β,β -caroten-4-one
Fucoxanthin	(3S,5R,6S,3'S,5'R,6'R)-5,6-epoxy-3'-ethanoyloxy-3,5'-dihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro- β,β -caroten-8-one
Isocryptoxanthin	β,β -caroten-4-ol
Isozeaxanthin	β,β -carotene-4,4'-diol
Lactucaxanthin	(3R,6R,3'R,6'R)- ϵ,ϵ -carotene-3,3'-diol
Lutein	(3R,3'R,6'R)- β,ϵ -carotene-3,3'-diol
Lutein-5,6-epoxide	(3S,5R,6S,3'R,6'R)-5,6-epoxy-5,6-dihydro- β,ϵ -carotene-3,3'-diol
Luteochrome	5,6,5',8'-diepoxy-5,6,5',8'-tetrahydro- β,β -carotene
Luteoxanthin	5,6,5',8'-diepoxy-5,6,5',8'-tetrahydro- β,β -carotene-3,3'-diol
Lycopene	ψ,ψ -carotene
Lycophyll	ψ,ψ -carotene-16,16'-diol
Lycoxanthin	ψ,ψ -caroten-16-diol
Mutatochrome	5,8-epoxy-5,8-dihydro- β,β -carotene
Mutatoxanthin	(3S,5R,8RS,3'R)-5,8-epoxy-5,8-dihydro- β,β -carotene-3,3'-diol
Neochrome	5',8'-epoxy-6,7-didehydro-5,6,5',8'-tetrahydro- β,β -carotene-3,5,3'-triol
Neoxanthin	(3S,5R,6R,3'S,5'R,6'S)-5',6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro- β,β -carotene-3,5,3'-triol
Neurosporene	7,8-dihydro- ψ,ψ -carotene
Phytoene	7,8,11,12,7',8',11',12'-octahydro- ψ,ψ -carotene
Phytofluene	7,8,11,12,7',8'-hexahydro- ψ,ψ -carotene
Rubixanthin	(3R)- β,ψ -caroten-3-ol
Violaxanthin	(3S,5R,6S,3'S,5'R,6'S)-5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β,β -carotene-3,3'-diol
α -Zeaxarotene	(6R)-7',8'-dihydro- ϵ,ψ -carotene
β -Zeaxarotene	7',8'-dihydro- β,ψ -carotene
Zeaxanthin	(3R,3'R)- β,β -carotene-3,3'-diol
Zeinoxanthin	(3R,6'R)- β,ϵ -carotene-3-ol

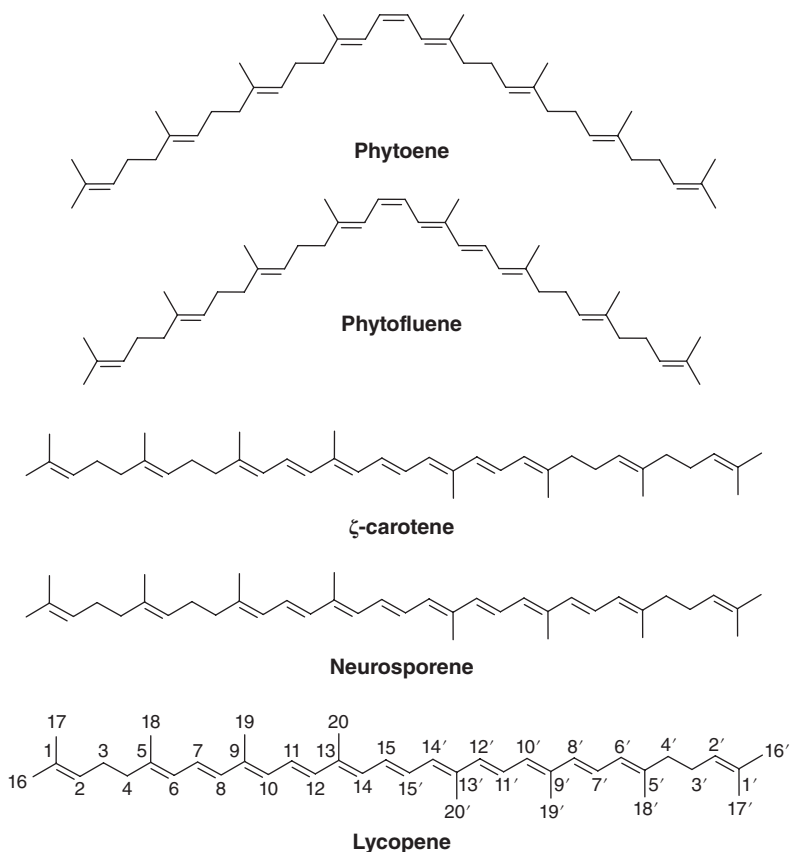


Figure 1.1 Structures of food carotenoids.

1.3 NATURE OF CAROTENOIDS IN FOODS

Carotenoids in foods are generally C_{40} tetraterpenes/tetraterpenoids formed from eight C_5 isoprenoid units joined head to tail, except at the center where a tail-to-tail linkage reverses the order, resulting in a symmetrical molecule (Figure 1.1). The most distinctive structural feature is a centrally located, long system of alternating double and single bonds, in which the π -electrons are effectively delocalized throughout the entire polyene chain. This conjugated double-bond system constitutes the light-absorbing chromophore that gives carotenoids their attractive color and is mainly responsible for their special properties and many functions. However, it also renders the molecule susceptible to geometric isomerization and oxidative degradation.

The basic linear and symmetrical skeleton has lateral methyl groups separated by six C-atoms at the center and the others by five C-atoms. Modification occurs in many ways, including cyclization, hydrogenation, dehydrogenation, introduction of oxygen-containing groups, migration of the double bonds, rearrangement, chain shortening or extension, or combinations thereof, resulting in a wide array of structures.

Carotenoids may be acyclic (e.g., lycopene, ζ -carotene) or may have a six-membered ring at one (e.g., γ -carotene, δ -carotene) or both ends (e.g., β -carotene, α -carotene) of the molecule. Exceptionally, capsanthin and capsorubin have five-membered rings.

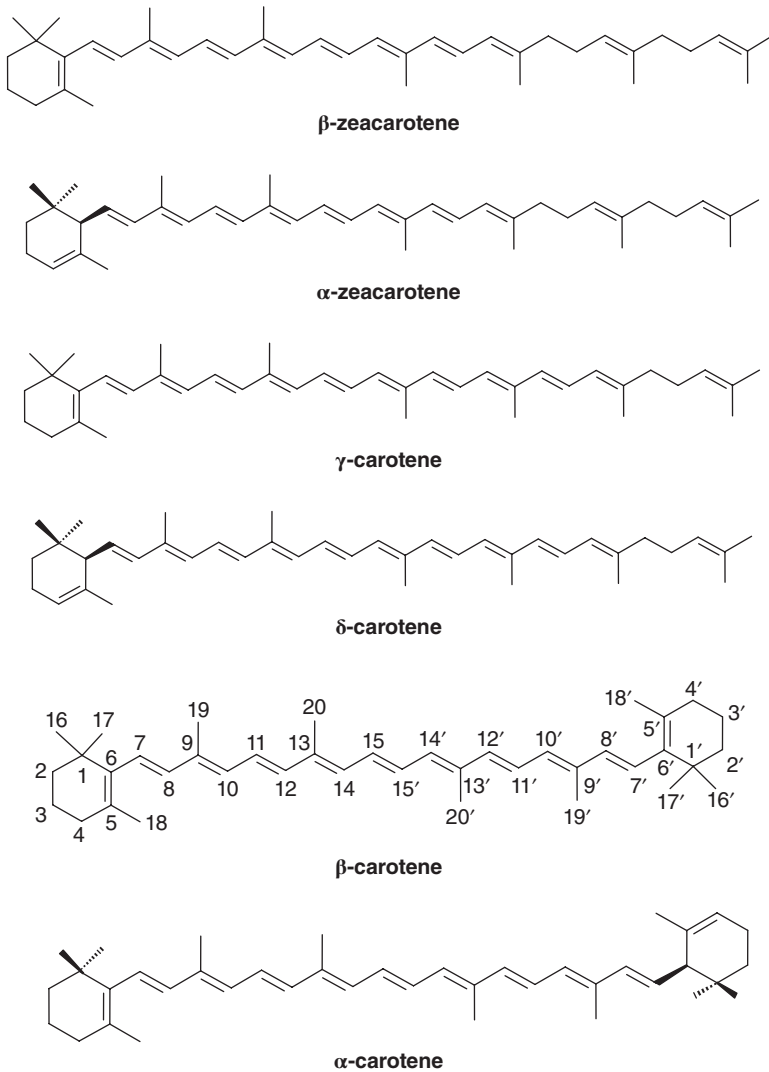


Figure 1.1 (Continued)

Hydrocarbon carotenoids (e.g., β -carotene, lycopene) are known as carotenes, and the oxygenated derivatives are called xanthophylls. Common oxygen-containing groups are hydroxyl (as in β -cryptoxanthin), keto (as in canthaxanthin), epoxy (as in violaxanthin), and aldehyde (as in β -citraurin) substituents. These functional groups are mainly responsible for the degree of polarity, solubility, and chemical behavior of the xanthophylls.

In foods, about a hundred carotenoids have been found. Typically a plant food would have one to five major carotenoids with a series of carotenoids in trace or very small amounts (Rodriguez-Amaya, 1999). Because plants are able to synthesize carotenoids *de novo*, along with the principal carotenoids, low levels of their biosynthetic precursors

and derivatives are also found. The carotenoid composition is variable and usually complex. These carotenoids are located in subcellular organelles (plastids), mainly associated with proteins in the chloroplasts and deposited in crystalline form or as oily droplets in chromoplasts (Bartley and Scolnik, 1995).

Unable to biosynthesize carotenoids, animals are limited to absorbing dietary carotenoids, which are accumulated unchanged or slightly altered to form carotenoids typical of animal species. Consequently, carotenoids are not as widely distributed in foods of animal origin and the composition is simpler.

1.3.1 Carotenes

Of the acyclic carotenes (Figure 1.1), lycopene and ζ -carotene are the most common. Lycopene is not as widely encountered as ζ -carotene, but when found in fruits and fruit vegetables, it is usually the predominating pigment. Examples of lycopene food sources are red- or pink-fleshed tomato, watermelon, papaya, guava, grapefruit, and the Brazilian fruit pitanga (Rodríguez-Amaya et al., 2008).

ζ -carotene is ubiquitous, but it is usually present at low levels except in Brazilian passion fruit and in carambola, in which it occurs as a major pigment. Phytoene and phytofluene are probably more widely distributed than reported; because they are both colorless, their presence may often be overlooked. Neurosporene, when found in foods, is usually in small amounts.

The bicyclic β -carotene (Figure 1.1) is the most widespread of all food carotenoids, found in virtually all foods analyzed, as a minor or as the major pigment (Rodríguez-Amaya et al., 2008). Examples of foods where β -carotene is the main carotenoid are acerola, apricot, carrot, loquat, melons, orange-fleshed sweet potato, and palm fruits. The bicyclic α -carotene and the monocyclic γ -carotene sometimes accompany β -carotene, generally at much lower concentrations. Appreciable amounts of α -carotene are found in carrot, red palm oil, and some varieties of squash and pumpkin. High levels of γ -carotene are found in rose hips and pitanga. Less frequently encountered is δ -carotene, although it is the principal carotenoid of the high delta strain of tomato and the Brazilian peach palm fruit.

1.3.2 Xanthophylls

A wide variety of xanthophylls are found in foods (Figure 1.2). The hydroxylated lycopenes, lycoxanthin and lycophyll, are rarely encountered; they are sometimes found in trace amounts in tomato. Rubixanthin, a derivative of γ -carotene, is the main pigment of rose hips (Hornero-Méndez and Mínguez-Mosquera, 2000) and also occurs in appreciable amount in pitanga. β -Cryptoxanthin is the main pigment of many orange-fleshed fruits, such as peach, nectarine, orange-fleshed papaya, persimmon, fruit of the tree tomato, and the Brazilian fruit *Spondias lutea* (Rodríguez-Amaya et al., 2008). It often appears as a secondary pigment.

Interestingly, in contrast to the relative abundance of the parent carotenes, with β -carotene predominating over α -carotene, lutein (dihydroxy derivative of α -carotene) is normally present in plant tissues at considerably higher levels than zeaxanthin (dihydroxy derivative of β -carotene). Lutein is the predominant carotenoid in yellow edible flowers,

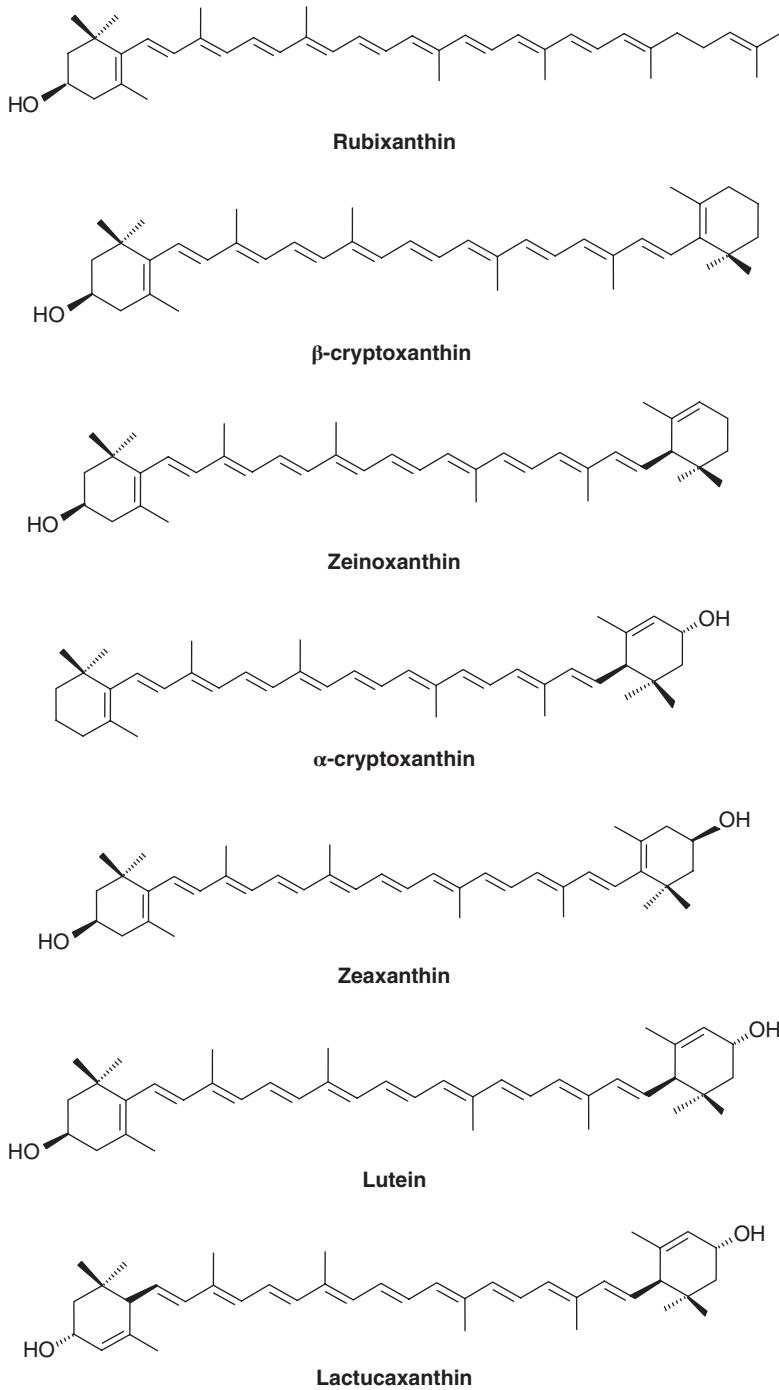


Figure 1.2 Structures of food xanthophylls.

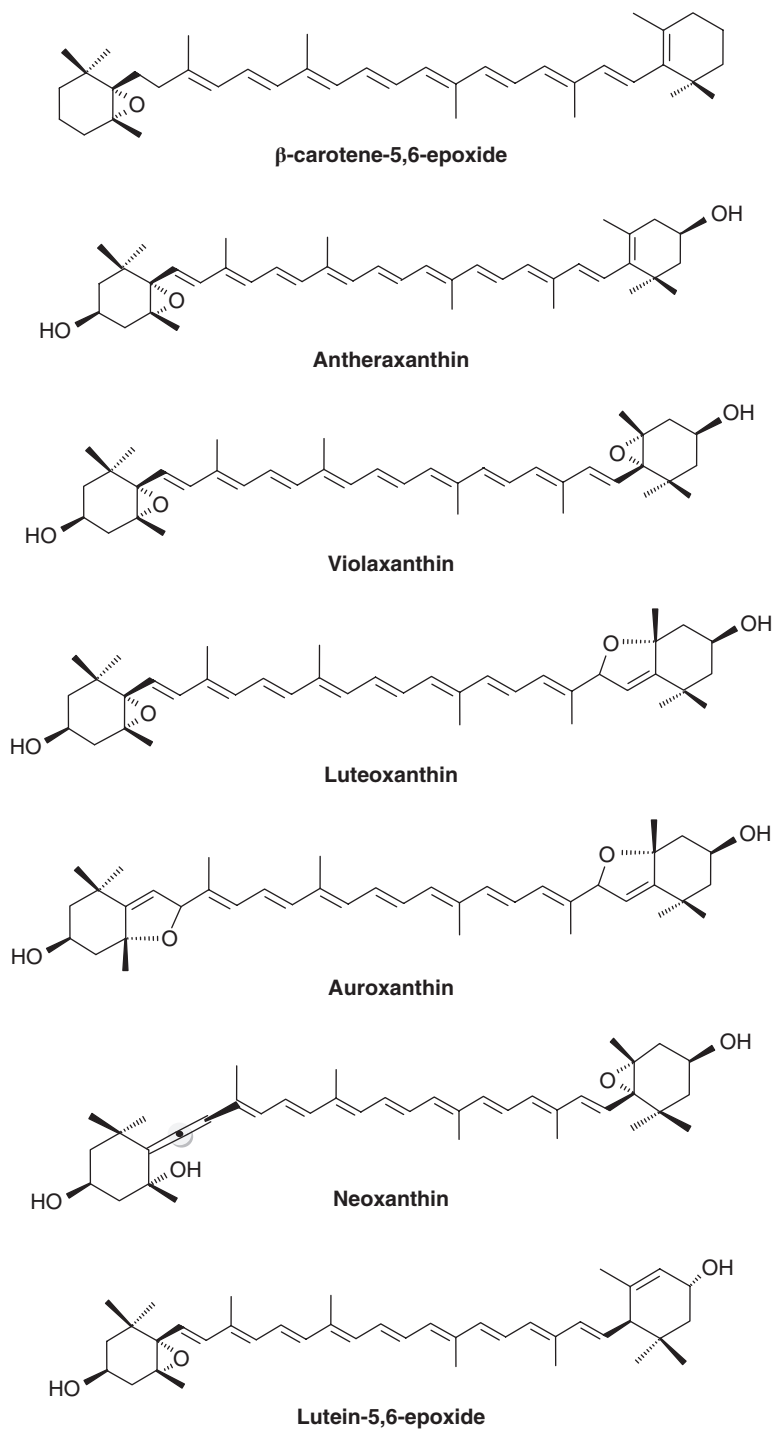


Figure 1.2 (Continued)

green leaves and other green vegetables, and some varieties of *Cucurbita maxima* (Rodriguez-Amaya et al., 2008). Except for yellow corn, the Brazilian fruit *Cariocar villosium*, and the East Asian fruit goji (*Lycium barbarium*) (Peng et al., 2005), in which it is the major pigment, zeaxanthin is a minor food carotenoid. It does not usually reach high levels because biosynthesis often stops at the precursor β -carotene, which is the preponderant pigment of many foods. Moreover, when formed, zeaxanthin is easily transformed to violaxanthin. Lutein appears to undergo limited epoxidation. The monohydroxy derivatives of α -carotene, α -cryptoxanthin, and zeinoxanthin, are minor carotenoids of some foods.

Carotenols in green leaves (Kobori and Rodriguez-Amaya, 2008) are unesterified, and those of corn (Rodriguez-Amaya and Kimura, 2004; Oliveira and Rodriguez-Amaya, 2007) are mostly unesterified. Carotenols in ripe fruits are generally esterified with fatty acids. However, the carotenols of a few fruits, particularly those that remain green when ripe, such as kiwi (Gross, 1987), undergo limited or no esterification. The principal carotenoid, lutein, occurs free or esterified in one (monoester) or both hydroxyl groups (diester) in the edible nasturtium (Niizu and Rodriguez-Amaya, 2005) and marigold (Breithaupt et al., 2002) flowers, with the esters predominating. Esterification, which occurs progressively during maturation, appears to be important physiologically. Acylation increases the lipophilic character of the xanthophylls, facilitating their accumulation in the chromoplasts (Gross, 1987).

Epoxy carotenoids comprise a large group of xanthophylls in foods. The zeaxanthin epoxide derivatives, antheraxanthin, mutatoxanthin, violaxanthin, luteoxanthin, auroxanthin, and neoxanthin, are widely encountered. The 5,8-epoxide of neoxanthin, neochrome, is occasionally detected.

The epoxides derived from β -carotene, β -carotene-5,6-epoxide, β -carotene-5,8-epoxide (mutatochrome), β -carotene-5,6,5',6'-diepoxide, β -carotene-5,6,5',8'-diepoxide (luteochrome), and β -carotene-5,8,5',8'-diepoxide (aurochrome), and those of β -cryptoxanthin, especially β -cryptoxanthin-5,6-epoxide and β -cryptoxanthin-5,8-epoxide (cryptoflavin), are also frequently found.

Except for violaxanthin and neoxanthin, carotenoid epoxides are usually detected in trace levels. Because they can be generated during analysis, in spite of their wide distribution, their natural occurrence is often questioned. Easily degraded, violaxanthin may be underestimated in foods, as was shown in mango (Mercadante and Rodriguez-Amaya, 1998). In commercially processed mango juice, violaxanthin, the main carotenoid of the unprocessed fruit, was not detected. Instead the 5,8,5',8'-diepoxy derivative, auroxanthin, appeared in appreciable amounts.

The existence of species-specific carotenoids (Figure 1.3) has also been demonstrated. The most prominent examples are capsanthin and capsorubin, the predominant pigments of red pepper. Carotenoids with two ϵ -rings are rare, and of the many leafy vegetables and fruits already analyzed, lactucaxanthin (Figure 1.2) has been found only in lettuce.

Although not as widely distributed and not as structurally diverse as in plants, carotenoids also occur in animal products. Astaxanthin (Figure 1.4) is the main carotenoid of some fish, such as salmon and trout, as well as crustaceans (e.g., shrimp, lobster, and crab). In salmon, it occurs in three optical forms: 75%–85% (3S,3'S), 12%–17%

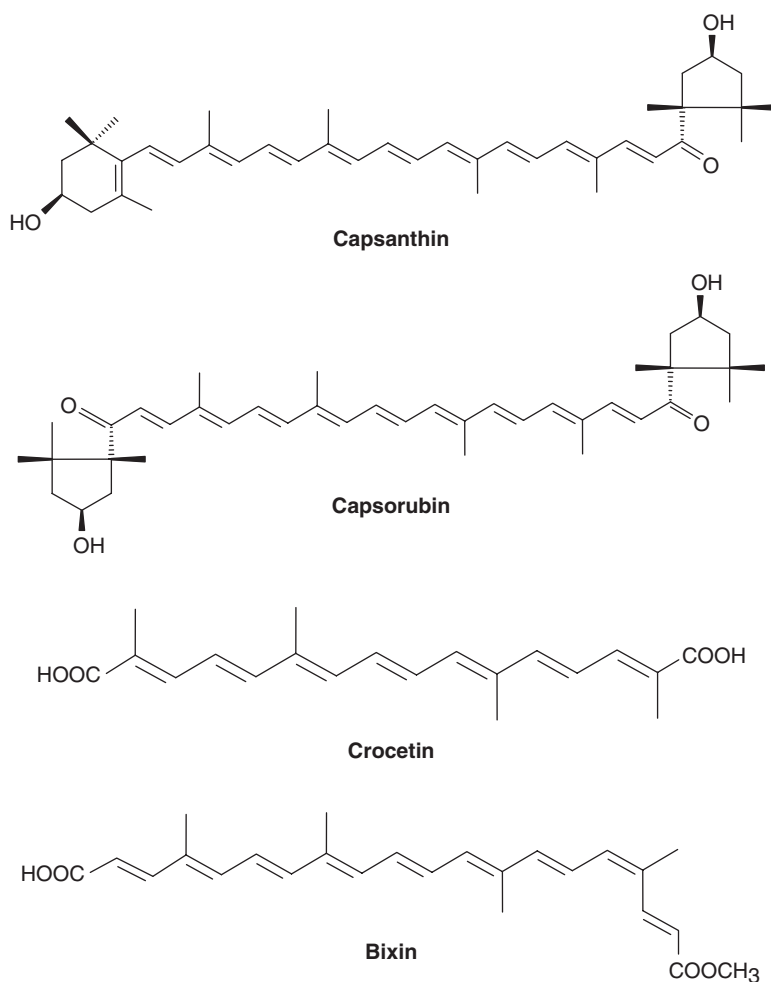


Figure 1.3 Structures of major carotenoids in food colorants.

(3R,3'R), and 2%–6% (3R,3'S, *meso*) (Schiedt, 1998). The three isomers are also found in shrimp as a 1:2 (meso form):1 mixture. In lobster all three isomers are equally well bound in the blue crustacyanin astaxanthin complex (Renstrom et al., 1982). The marketed synthetic astaxanthin is also a 1:2 (meso form):1 mixture, whereas that produced by the alga *Haematococcus* sp. is the optically pure (3S,3'S) isomer. The intermediates in the transformation of dietary carotenoids to astaxanthin, such as canthaxanthin, echinenone, isocryptoxanthin, and isozeaxanthin, are often detected as accompanying minor carotenoids. Tunaxanthin in its various stereoisomeric forms is also a major carotenoid of fish.

Astaxanthin may be found free, esterified in one or both hydroxyl groups with fatty acids, or as a complex with proteins (carotenoproteins) or lipoproteins (carotenolipoproteins) (Shahidi and Brown, 1998). Crustacean astaxanthin is a mixture of the three

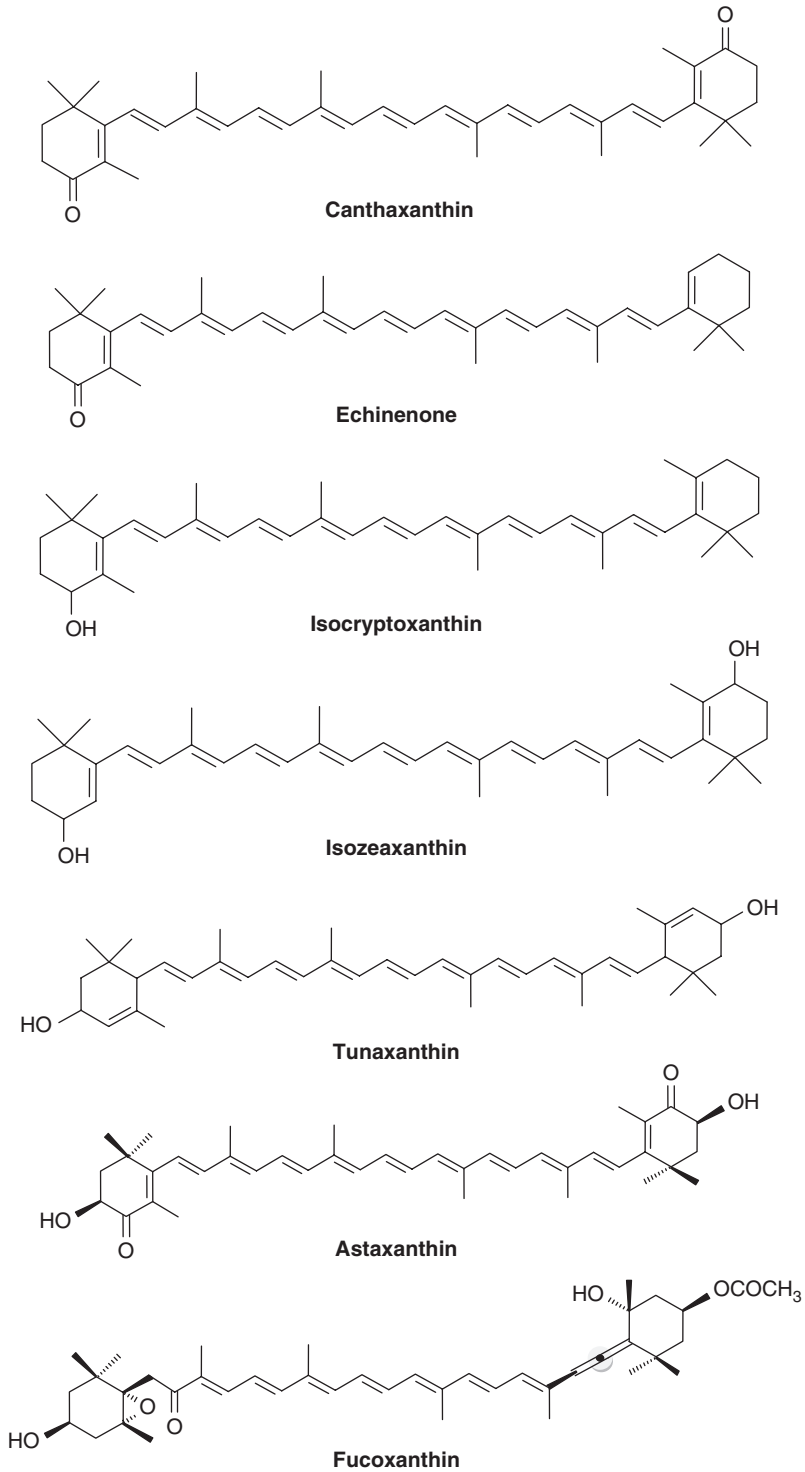


Figure 1.4 Structures of typical animal and algal carotenoids.

forms. Found in algae, it is always esterified; synthetic astaxanthin is not esterified (Johnson and An, 1991). In the red crab langostilla (*Pleuroncodes planipes*), astaxanthin diesters comprised approximately 70%, monoesterified astaxanthin approximately 12%, and unesterified astaxanthin approximately 8% of the total carotenoids (Coral-Hinostroza and Bjerkgeng, 2002).

Although of limited occurrence in foods, fucoxanthin (Figure 1.4) is among the most abundant carotenoids in nature. It is found in edible brown algae (Yan et al., 1999; Miyashita et al., 2011; Christaki et al., 2012), largely consumed in Asian countries. It has a more complicated structure than most food carotenoids, having an allenic bond, several functional substituents, including secondary and tertiary hydroxyl, epoxy, and keto, and is a natural acetate.

1.3.3 Z-isomers

In nature, carotenoids exist primarily in the generally more thermodynamically stable all-*E* configuration. The structures in Figures 1.1 to 1.4 are generally in this form. The first two carotenoids formed biosynthetically, phytoene and phytoflene, have the 15-*Z* configuration in most natural sources. Another exception is bixin, which occurs naturally in the *Z*-form. As the improved efficiency of chromatographic methods permitted their separation, small amounts of *Z*-isomers have been increasingly reported in raw foods. On the other hand, it is well known that thermal processing enhances geometric isomerization, increasing the levels of *Z*-isomers in processed foods.

Theoretically, each carbon-carbon double bond in the polyene chain of carotenoids may exhibit *E-Z* isomerization. However, some double bonds are prevented from undergoing this isomerization because the *Z*-configuration is sterically hindered (Figure 1.5) (Zechmeister et al., 1941). This is the case with C-7,8, C-11,12, C-7',8' and C-11',12' double bonds, in which steric hindrance between a hydrogen atom and a methyl group prevents a *Z*-configuration (Weedon and Moss, 1995). Thus, the *Z*-isomers of symmetrical β -carotene (Figure 1.6) (Lessin et al., 1997; Marx et al., 2000; Dachtler et al., 2001) and zeaxanthin (Dachtler et al., 2001; Humphries and Khachik, 2003; Updike and Schwartz, 2003; Aman et al., 2005) commonly found in foods are the 9-*Z*-, 13-*Z*-, and the 15-*Z*-isomers, the formation of which has relatively little hindrance as it comes from two hydrogen atoms.

Carbon-carbon double bonds located in the cyclic part of the carotenoid structure, as the C-5,6 double bond in β -carotene, are also sterically hindered and are not isomerized. However, this double bond in the acyclic lycopene is unhindered and 5-*Z*-lycopene is

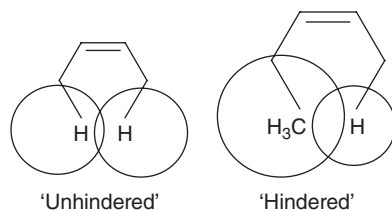


Figure 1.5 Sterically unhindered and hindered *Z*-configurations.

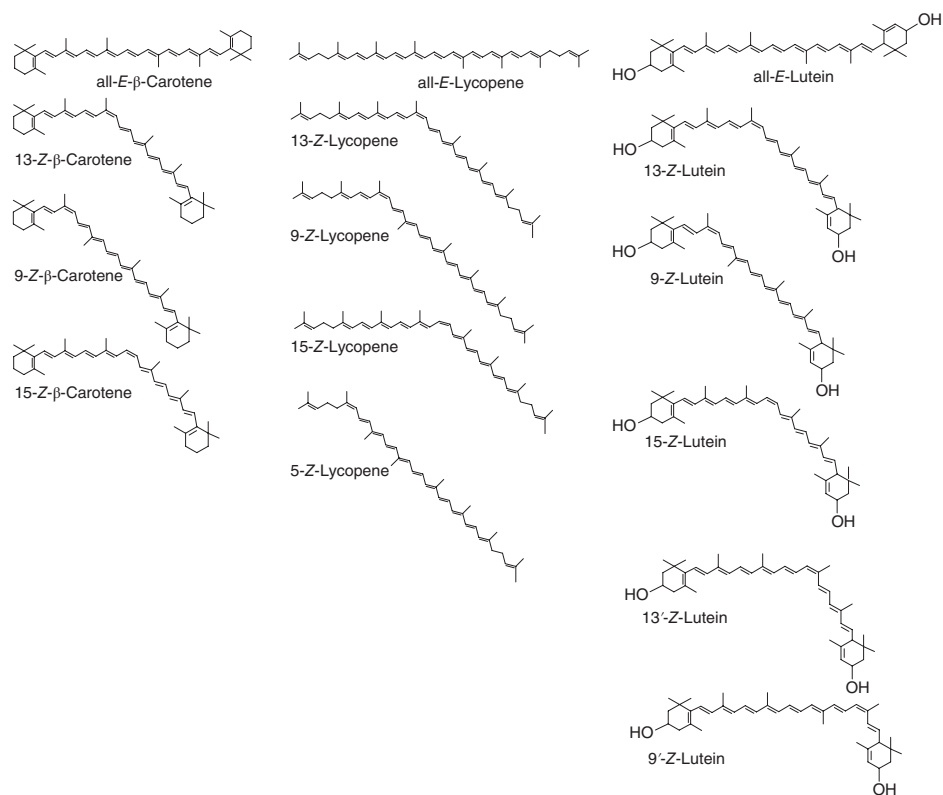


Figure 1.6 Geometric isomers of β -carotene, lycopene, and lutein commonly found in foods.

increasingly detected in tomato and tomato products, along with the 9-*Z*-, 13-*Z*-, and the 15-*Z*-isomers (Figure 1.6) (Tiziani et al., 2006; Li et al., 2012; Stinco et al., 2013).

Being unsymmetrical, all-*E*- α -carotene (Lessin et al., 1997), all-*E*- β -cryptoxanthin (Lessin et al., 1997), and all-*E*-lutein (Figure 1.6) (Dachtler et al., 2001; Humphries and Khachik, 2003; Updike and Schwartz, 2003; Aman et al., 2005; Achir et al., 2010) give rise to 13'-*Z*- and 9'-*Z*-isomers in addition to 13-*Z*-, 9-*Z*-, 15-*Z*-isomers in foods.

1.3.4 Apocarotenoids

Carotenoids in which the carbon skeleton has been shortened by removal of fragments from one or both ends of the usual C_{40} structure are called apocarotenoids. Natural examples are bixin, the major pigment of the food colorant annatto, and crocetin, the main coloring component of saffron (Figure 1.3). They are believed to be produced by Glover-Redfearn degradation of C_{40} carotenoids.

As with the epoxy carotenoids, apocarotenoids are initial products of the oxidative degradation of carotenoids. Thus, traces of these compounds are often found in foods.

β -Apo-8'-carotenal, β -Apo-10'-carotenal, and β -citraurin (Table 1.1), for example, are common minor pigments of citrus fruits.

1.4 PHYSICOCHEMICAL PROPERTIES

The major structural features that account for variations in properties and biological activities of carotenoids are length and rigidity of the molecule, length of the conjugated double-bond system, cyclized or acyclic end groups, and presence of polar substituents in the predominantly hydrocarbon molecule.

The physical and chemical properties of carotenoids *in vitro* provide valuable insights, but these properties *in vivo* are affected by a wide range of factors other than their basic chemical characteristics, including interactions with other molecules in their microenvironment, and may be significantly different from those of free carotenoids in organic solvent solution. Thus, extrapolations of *in vitro* results to *in vivo* situations should be done with caution.

1.4.1 Size and shape

The overall molecular geometry (size, shape, presence of functional groups) is essential in ensuring that carotenoids fit into cellular and subcellular structures in the correct location and orientation, so that they can function efficiently (Britton, 1995).

Acyclic carotenoids such as lycopene are long, linear molecules. Cyclization shortens the overall length of the molecule and increases the effective bulk of the end groups and the space they occupy.

The size and shape of *Z*- and all-*E* isomers are substantially different, with marked influence on their properties and biological actions and functions. The all-*E* isomers are linear and rigid molecules, whereas the *Z*-isomers are bent—thus their ability to assemble in supramolecular structure, fit into subcellular structures, and interact with enzymes differs. The tendency of *Z*-isomers to crystallize or aggregate is usually much less; thus, *Z*-isomers have lower melting point and may be more readily solubilized, absorbed, and transported than their all-*E* counterparts (Britton, 1995; Schieber and Carle, 2005).

1.4.2 Solubility

Carotenoids are highly lipophilic. They are insoluble in water and soluble in varying degrees in organic solvents such as acetone, alcohol, ethyl ether, chloroform, and ethyl acetate. Carotenes are readily soluble in petroleum ether, hexane, and toluene; xanthophylls dissolve better in methanol and ethanol. Crystalline carotenoids may be difficult to dissolve in the above solvents but do dissolve in benzene and dichloromethane (Schiedt and Liaaen-Jensen, 1995). Both β -carotene and the xanthophyll lutein have excellent solubility in tetrahydrofuran (Craft and Soares, 1992).

In the cell, carotenoids are expected to be restricted to hydrophobic areas, such as the inner core of membranes, but association with protein allows them access to aqueous environments (Britton, 1995).

1.4.3 Light absorption and color

Because the π electrons of the extended conjugated double bond system are highly delocalized and the excited state is of comparatively low energy, the excitation energy required is relatively small, corresponding to light in the visible region in the wavelength

range of 400–500 nm. Carotenoids are therefore brightly colored. The transition involved is $\pi \rightarrow \pi^*$, in which one of the bonding π electrons of the conjugated double bond system is raised to a previously unoccupied π^* antibonding orbital.

The conjugated double bond system is the chromophore that gives carotenoids their color and provides the visible absorption spectra that serve as basis for their identification and quantification. At least 7 conjugated double bonds are needed for a carotenoid to have perceptible color, as in ζ -carotene, which is light yellow. Phytoene (3 conjugated double bonds) and phytofluene (5 conjugated double bonds) are colorless. Lycopene, with 11 conjugated double bonds in an acyclic structure, is red. Cyclization takes the π electrons of the ring double bond out of plane with those of the chain because of steric hindrance between the ring methyl group at C-5 and the hydrogen at C-8 of the polyene chain. Consequently, the monocyclic γ -carotene and the bicyclic β -carotene, although possessing the same number of conjugated double bonds as lycopene, are red-orange and yellow-orange, respectively. Hydroxy substituents do not affect the chromophore; thus, both α -carotene and its dihydroxy derivative, lutein, are pale yellow. Similarly, the monohydroxy and dihydroxy derivatives of β -carotene, β -cryptoxanthin and zeaxanthin, have the same color as β -carotene.

Capsanthin, with its conjugated double bond system consisting of nine double bonds in the polyene chain, one in the β -ring, and the carbonyl group double bond, and capsorubin, with its nine conjugated double bonds in the polyene chain extended by the double bonds of two carbonyl groups, give the intense color of red pepper. Astaxanthin, having nine conjugated double bond in the polyene chain extended by two double bonds in β -rings and two carbonyl group double bonds, is responsible for the vivid red color of cooked shrimp, lobster, and crabs. In the raw crustaceans, astaxanthin is complexed with protein, transforming the color to blue, black, or grey. Heating denatures the protein and the red color of free astaxanthin appears. The reddish color of salmon and trout flesh also comes from astaxanthin.

1.5 ANTIOXIDANT PROPERTIES

An antioxidant is defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation. Carotenoids are natural antioxidants of importance in foods and in humans. They may enhance the stability and extend the shelf life of foods. On the other hand, the antioxidant activity is the most cited mode of action of carotenoids in the reduction of the risk of chronic degenerative diseases. Most researchers in the last two to three decades have focused attention on the antioxidant role of food carotenoids in human health rather than in the food itself.

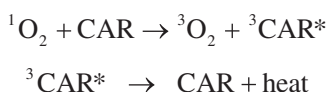
The antioxidant properties of carotenoids are due to their exceptional singlet oxygen ($^1\text{O}_2$) quenching ability and their free-radical scavenging activity.

1.5.1 Quenching of singlet oxygen

Photosensitizers in biological systems (e.g., chlorophyll, riboflavin, myoglobin) can absorb energy from light and, in its excited triplet state, transfer energy to atmospheric triplet-state oxygen ($^3\text{O}_2$), forming the highly reactive and destructive singlet oxygen ($^1\text{O}_2$).

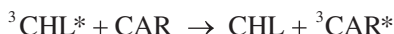
Carotenoids can interfere in this process in two ways: (1) by deactivating the triplet state photosensitizer, thus preventing the formation of singlet oxygen, and (2) converting singlet-state oxygen back to its ground triplet state. In both cases, transferred energy is released as heat.

It is well documented that carotenoids quench $^1\text{O}_2$ (Krinsky, 1989, 2001; Edge et al., 1997). This occurs through physical or chemical quenching, the efficacy of the former greatly exceeding that of the latter. Physical quenching involves the transfer of excitation energy from $^1\text{O}_2$ to the carotenoid; oxygen then returns to its ground state and the carotenoid is elevated to its excited triplet state. The energy is dissipated through rotational and vibrational interactions between the excited carotenoid and the surrounding solvent, yielding ground state carotenoid and thermal energy. The carotenoid remains intact and can undergo further cycles of singlet oxygen quenching.



In chemical quenching, the carotenoid combines with oxygen or is oxidized, leading to its destruction (bleaching) and yielding a variety of oxidized products.

Carotenoids can also quench the excited triplet-state chlorophyll or other excited sensitizers, thereby preventing the formation of $^1\text{O}_2$.



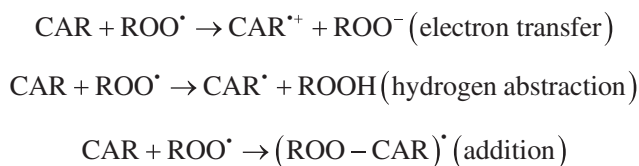
The quenching ability of carotenoids increases with increasing number of conjugated double bonds, maximum protection being given by those having nine or more double bonds (Foote et al., 1970). To a lesser extent, it is also affected by the carotenoid end groups (cyclic or acyclic) and the nature of the substituents in cyclic end groups.

Oxygen quenching depends on the environment. Efficient $^1\text{O}_2$ quenchers in solvents may be inefficient in the cell membrane (Edge and Truscott, 2009). The aggregation and the orientation of a carotenoid in the lipid bilayer may be major factors in determining the $^1\text{O}_2$ quenching efficiency.

Singlet oxygen is involved in the photooxidation of vegetable oils and oil-containing foods. Addition of various carotenoids to foods containing unsaturated fatty acids improves the shelf life, mainly because of $^1\text{O}_2$ quenching (Kiokias and Gordon, 2004).

1.5.2 Free radical scavenging

Although not as efficient as in quenching $^1\text{O}_2$, carotenoids can also scavenge free radicals, thereby breaking chain propagation. Interaction with free radicals can occur in three main ways (Young and Lowe, 2001; El-Agamey et al., 2004; Skibsted, 2012):



The factors that can affect the rates and mechanisms of free-radical reactions include the nature of the free radical and its environment (aqueous or lipid regions) and structural features of the carotenoid (number of conjugated double bonds, cyclic or acyclic, polar or apolar end groups, redox properties) (Everett et al., 1996; Mortensen and Skibsted, 1997; Rice-Evans et al., 1997; El-Agamey et al., 2004). The nature of the radical species appears to have greater effect than the carotenoid structure (Mortensen et al., 1997; Martinez et al., 2009).

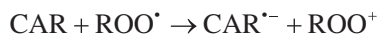
Carotenoids react with a wide range of radicals, such as $\text{CCl}_3\text{O}_2^\bullet$, RSO_2^\bullet , NO_2^\bullet , and various arylperoxyl radicals through electron transfer, producing the radical cation $\text{CAR}^{+\bullet}$ (Everett et al., 1996; Mortensen and Skibsted, 1997; Mortensen et al., 2001). Glutathione and 2-mercaptoethanol thiyl radicals react via radical addition to generate carotenoid-thiyl radical adducts $(\text{RS-CAR})^\bullet$ (Everett et al., 1996; Mortensen and Skibsted, 1997). The RSO_2^\bullet radical (generated by conjugation of the RS^\bullet radical with molecular oxygen) undergoes both radical addition, producing $(\text{RSO}_2\text{-CAR})^\bullet$, and electron abstraction, forming $\text{CAR}^{+\bullet}$ (Everett et al., 1996; Mortensen and Skibsted, 1997). With less strongly oxidizing radicals, such as alkylperoxyl radicals, hydrogen atom transfer can occur, leading to the neutral carotenoid radical (Mortensen et al., 2001). Hydrogen abstraction, characteristic of lipid oxidation, has often been suggested as a mechanism of carotenoid antioxidant activity (Woodall et al., 1997a; El-Agamey and McGarvey, 2003).

Steady-state photolysis experiments showed that alkyl, alkoxy, and alkylperoxyl radicals all reacted with β -carotene (Mortensen and Skibsted, 1998). However, laser flash photolysis experiments indicated that the reaction with peroxyl radicals was slower than with alkyl and alkoxy radicals, indicating that β -carotene was a poor direct scavenger of peroxyl radicals. The authors suggested that scavenging of peroxyl radicals by this carotenoid proceeded not by electron transfer but by adduct formation and/or hydrogen abstraction.

El-Agamey and McGarvey (2003) observed that the carotenoid radical formed depends on the polarity of the solvent medium. Only addition radicals are formed in apolar solvents, whereas these adducts decay to carotenoid radical cations in polar solvents.

The β -carotene radical cation and adduct radicals are highly resonance stabilized and undergo slow bimolecular decay to nonradical products (Everett et al., 1996). In the scavenging of lipid-derived peroxyl radicals (LOO^\bullet), the carotenoid radicals are less reactive than the LOO^\bullet ; carotenoids thus act as chain-breaking antioxidants in lipid peroxidation.

Carotenoids may also be reduced, forming radical anions:



The balance between electron donation and electron acceptance forming radical cations and radical anions, respectively, varies among carotenoids and is important in their role in antioxidant networks (Skibsted, 2012).

Structural features of the carotenoid govern not only their reactivity but also their location and orientation within the lipid bilayer (Britton, 1995) and even their tendency to self-aggregate under polar conditions (Ruban et al., 1993). β -Carotene and other carotenes lie parallel with the membrane surface, deep within the hydrophobic core

(Johansson et al., 1981; van de Ven et al., 1984). The dihydroxy zeaxanthin spans the membrane entirely.

1.5.3 Relative efficacy of individual carotenoids

Comparison of the effectiveness of carotenoids as antioxidant has been carried out in homogenous solution and liposomes (cell membrane models). The results are somewhat inconsistent but can be better understood when the different influencing factors are considered. Noting that the efficiency of carotenoids as antioxidants did not follow their capability as radical scavengers or the order of their oxidation potential, J. Liang et al. (2009) suggested that the long-standing controversy about the function of carotenoids as antioxidant might be related to the extrapolation of properties determined in solution to more complex biological systems, where other factors (e.g., spatial organization and interaction between antioxidants) become important.

Having an extended conjugated double bond system in an acyclic structure, lycopene has been shown in different systems to be one of the most potent antioxidants (Mascio et al., 1989; Mortensen et al., 1997; Woodall et al., 1997b; Cantrell et al., 2003; Stahl and Sies, 2003). In a mixed solvent system, its singlet oxygen quenching ability was twice as high as β -carotene and 10 times higher than that of α -tocopherol (Mascio et al., 1989). It was twice as efficient as β -carotene in scavenging nitrogen dioxide radical (Tinkler et al., 1994; Böhm et al., 1995). According to real-time kinetic studies, it was also more efficient than zeaxanthin, lutein, echinenone, canthaxanthin, and astaxanthin as reductive scavengers of radicals (Mortensen and Skibsted, 1997).

Astaxanthin quenched $^1\text{O}_2$ in a mixed solvent system (Mascio et al., 1989), model membrane (Cantrell et al., 2003), and chlorophyll-sensitized and photooxidized soybean oil (Lee and Min, 1990). Its quenching capacity in a mixed solvent system was lower than lycopene, almost equivalent to γ -carotene, slightly higher than canthaxanthin, and greater than α - and β -carotene (Mascio et al., 1989).

As with other carotenoids, astaxanthin's free radical scavenging efficiency depends on the nature of the free radical, the scavenging mechanism, and the environment. In a free radical-initiated system, Terão (1989) found canthaxanthin and astaxanthin better antioxidants than β -carotene and zeaxanthin. Astaxanthin and canthaxanthin and other carotenoids containing keto-groups in the 4 position of the β -ionone ring were found more effective than β -carotene and zeaxanthin in preventing free-radical oxidation of methyl linoleate in solution (Jorgensen and Skibsted, 1993). In organic solvent and liposomal media, astaxanthin showed higher activity toward peroxy radical generated by the lipophilic generator AMVN (2,2'-azobis-2,4-dimethylvaleronitrile) than lutein, lycopene, α - and β -carotene, and α -tocopherol (Naguib, 2000). Goto et al. (2001) reported that astaxanthin is twice as effective as β -carotene in inhibiting the production of peroxides induced by ADP and Fe^{2+} in liposomes. This potent antiperoxidative activity of astaxanthin was attributed to efficient radical trapping at the surface and inside the phospholipid membrane.

On the other hand, astaxanthin and canthaxanthin ranked among the least efficient reductive scavengers of radicals according to both theoretical calculations and real-time kinetic studies (Skibsted, 2012). Real-time detection following laser flash photolysis of transient carotenoid radical cations formed in chloroform with phenoxy radicals

established the antioxidant hierarchy as follows: lycopene > β -carotene > zeaxanthin > lutein > echinenone > canthaxanthin ~ β -apo-8'-carotenal > astaxanthin (Mortensen and Skibsted, 1997).

Differences in the reactivity of carotenoids had been related to differences in their electron density. For example, the presence at C-4 and C-4' of hydroxy (as in isozeaxanthin) and especially keto groups (as in astaxanthin and canthaxanthin) decreased reactivity by preventing hydrogen abstraction from these positions (Britton, 1995; Woodall et al., 1997a, b).

β -cryptoxanthin and zeaxanthin were found to be more protective than β -carotene against peroxy radicals in liposomal membranes (Woodall et al., 1997a). Since all three carotenoids have 11 conjugated double bonds, and β -carotene and zeaxanthin behaved similarly when tested against peroxy radicals in solution, the presence of one and two hydroxyl groups in β -cryptoxanthin and zeaxanthin, respectively, accounts only partially for the difference. Chemical reactivity of the carotenoid toward peroxy radicals, as demonstrated in homogenous solution, is not the only factor that determines its ability to protect membranes against oxidation; the position and orientation of the carotenoid in the lipid bilayer are additional important factors (Woodall et al., 1997a; Young and Lowe, 2001). The hydrocarbons lycopene and β -carotene are located in the hydrophobic inner core of the bilayer and are not in a position to readily intercept free radicals entering the membrane from the aqueous phase. Carotenoids with polar end groups span the bilayer with their end groups located near the hydrophobic-hydrophilic interface where free radicals attack first.

Sujak et al. (1999) investigated the effect of lutein and zeaxanthin on oxidative damage of egg yolk lecithin liposomal membranes induced by exposure to UV radiation and incubation with the water-soluble peroxy radicals generator AAPH (2,2'-azobis (2-methylpropionamidine)dihydrochloride). Lutein and zeaxanthin protected lipid membranes against free radical attack with almost the same efficacy. Both carotenoids also slowed down UV-induced lipid oxidation at a very similar rate at the initial stage of the experiments, but zeaxanthin appeared to be a better photoprotector during prolonged UV exposure. The difference in protective efficacy was attributed to a different organization of zeaxanthin-lipid and lutein-lipid membranes.

1.6 PROOXIDANT EFFECT

A possible prooxidant property of carotenoids came into focus as an aftermath of intervention studies that showed an increase rather than a decrease in the incidence of lung cancer with β -carotene supplementation. According to Palozza (1998), there is evidence for a prooxidant activity of carotenoids *in vitro* and *in vivo*. The carotenoids shift from being antioxidants to prooxidants, depending on their redox potential and the biological environment. Both Krinsky (2001) and Young and Lowe (2001), however, considered it unlikely that carotenoids would act as prooxidants in biological systems, although they may lose their effectiveness as antioxidants at high concentrations or at high partial pressures of oxygen.

The concept that carotenoids can be prooxidants arose from the findings of Burton and Ingold (1984) that at high, nonphysiologic, oxygen tensions (760 torr, 100%

oxygen) and high carotenoid concentrations (>500 μM), β -carotene behaves as a prooxidant. A close look at the data, however, strongly suggested that it was actually a decrease in antioxidant activity under those conditions and not necessarily a prooxidant effect (Krinsky, 2001). Moreover, the oxygen tension in the lung would only be 150 torr for inspired air, dropping rapidly to 15 torr or less in the tissues; at this level there was no indication of a decrease in antioxidant capacity. Evidence of prooxidant action has also been obtained at high, nonphysiologic concentrations (50 μM). Even in the major intervention trials where individuals received between 20 and 25 mg/d of β -carotene for extended periods, the plasma β -carotene concentration ranged from 0.4 to 5 μM , well below the levels where it had been reported to act as prooxidant.

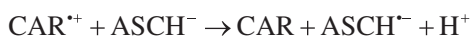
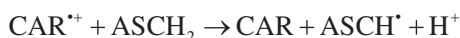
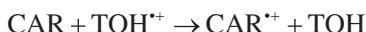
It is also important to consider that studies on the antioxidant-prooxidant potential are carried out with the carotenoids dissolved in organic solvents. In biological systems, these compounds are predominantly found associated with protein or lipoprotein structures and, in this condition, behave differently from those in solution (Young and Lowe, 2001).

The factors that may influence the antioxidant or prooxidant activities of carotenoids in biological systems are: (1) the structure (i.e., size, shape, and the nature, position, and number of substituent groups) and physical form (aggregated or monomeric, *Z* or *E* configuration, etc.) of the carotenoid molecule, (2) the location or site of action of the carotenoid molecule within the cell, (3) the potential for interaction with other carotenoids or antioxidants (especially vitamins C and E), (4) the concentration of the carotenoid, and (5) the partial pressure of oxygen (Britton, 1995; Young and Lowe, 2001).

1.7 INTERACTION WITH OTHER ANTIOXIDANTS

In the human organism, carotenoids are part of the antioxidant defense system, interacting synergistically with other antioxidants (Stahl and Sies, 2003). Combinations of carotenoids or carotenoids with other antioxidants are more effective than single compounds.

Truscott (1996) proposed a mechanism for the interaction of vitamins C and E with β -carotene whereby the carotenoid molecule repairs the vitamin E radical and the resulting carotenoid cation radical is, in turn, repaired by vitamin C. An additive response was observed with β -carotene and vitamin E; a synergistic response was seen only in the presence of vitamin C.



Synergistic protection of human cells both in vivo and in vitro by β -carotene with vitamins E and C against lethal damage by both $\text{NO}_2\cdot$ and $\text{OONO}^-/\text{HOONO}$ (formed from $\text{NO}\cdot$ and the superoxide radical $\text{O}_2^{\cdot-}$) was reported by Böhm et al. (1998).

On the other hand, in red palm oil, the carotenes appeared to be the primary substrate for lipid-derived radicals and tocopherols/tocotrienols regenerated the carotenes

(Schroeder et al., 2006). Regeneration of the carotenes by tocopherols or tocotrienols also seemed to explain the antioxidant synergism observed for liposomes.

The synergistic effect was shown to be dependent on the type of antioxidant and its concentration (Shi et al., 2007). In a homogenous solution of linoleic acid methyl ester and in linoleic acid methyl ester oxidation induced by an azo-initiator, lycopene appeared to interact synergistically with vitamin E at a specific concentration and ratio, but β -carotene showed no synergistic effect with vitamin E at the levels used in the study.

A synergistic response among carotenoids had been also reported (Stahl et al., 1998). A combination of lutein and lycopene was found to be most effective against AMVN-induced oxidation in multilamellar liposomes. In a phosphatidyl choline liposome, a combination of astaxanthin with β -carotene or astaxanthin with lycopene showed significant antioxidant synergism (J. Liang et al., 2009). It was suggested that astaxanthin, anchored in the water/lipid interface, acted as a radical transfer bridge, scavenging radicals at the interface and transferring an electron from a nonpolar and more reducing carotenoid in the membrane interior.

Skibsted (2012) envisioned carotenoids forming antioxidant networks based on one-electron transfer with other carotenoids, depending on the balance between ionization energy and electron affinity of the individual carotenoids, as demonstrated by real-time kinetic studies and quantum mechanical calculations. The more hydrophilic xanthophylls serve as molecular wiring across membranes in these networks, anchoring in water/lipid interfaces, resulting in synergism with more lipophilic carotenoids.

Synergism had also been observed between isoflavonoids and β -carotene (Han et al., 2007, 2010; R. Liang et al., 2010). This seems to involve the regeneration of the carotenoid radical cation formed in the lipid phase by the isoflavonoid in the lipid/water interfaces. The monoanionic and dianionic forms of the isoflavonoids, not the neutral forms, regenerated the oxidized carotenoid, and electron transfer from the isoflavonoids was faster for astaxanthin than for the other carotenoids, in agreement with its highest electron accepting index (Han et al., 2010).

The coexistence of carotenoids and polyphenols in foods is advantageous because carotenoids are efficient in $^1\text{O}_2$ quenching but not as effective in free radical scavenging, whereas polyphenols are very good free radical quenchers but not as efficient in $^1\text{O}_2$ quenching. Together, they have at least an additive effect.

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