2. Occurrence, Commercial Sources, Use and Application, Analysis and Human Use and Exposure

The most abundant source of carotenoids is plant tissues, although they are also found in microorganisms, insects, birds, fish and other animals. Only plants and microorganisms, however, can synthesize carotenoids *de novo*. Nature produces about 100 million tonnes of carotenoid pigments per year (Isler *et al.*, 1967); most of this output is in the form of fucoxanthin and peridinin (characteristic pigments in marine algae and diatoms, respectively) and β-carotene, lutein, violaxanthin and neoxanthin (in green leaves) (Ong & Tee, 1992). Zeaxanthin also occurs widely but in smaller amounts in green leafy vegetables, while others like lycopene, capsanthin and bixin constitute the pigments in specific plants.

The distribution of carotenoids in various plant materials has been reviewed (Borenstein & Bunnell, 1966; Bauernfeind, 1972; Goodwin, 1976, 1980; Speek *et al.*, 1988). Most of the data reported before 1980 were obtained by open-column chromatography, which did not permit adequate separation of carotenoids. More accurate quantification of carotenoids in foods has been possible since the introduction of HPLC. Information on the carotenoid composition of biological materials reported below is thus derived from recent references, when possible. Although modern methods allow improved qualitative and quantitative descriptions of the carotenoid composition of foods, assessing dietary intake will always be fraught with difficulties, which include the variable composition of vegetables through the year (Heinonen *et al.*, 1989), differences

between what is eaten and what might be sampled for analysis, difficulties in assessing what is eaten, influence of the fat content of the diet on absorption and the influence of cooking methods on bioavailability (Micozzi *et al.*, 1990; Khachik *et al.*, 1992a). It is generally considered that cooking vegetables improves the bioavailability of carotenoids, since it breaks down the cellulose structure of the plant cell (Khachik *et al.*, 1992a; de Pee & West, 1996). One report (Micozzi *et al.*, 1990) suggested that microwave cooking has a destructive effect and that much more xanthophylls were destroyed than hydrocarbons; however, later studies indicated that carotenoids were generally (with the exception of epoxycarotenoids) resistant to heating by microwaving, boiling and stewing (Khachik *et al.*, 1992a).

Ever since the elucidation of the structure of β-carotene and other carotenoids, much effort has been devoted to the synthesis of carotenoids and other polyenes. The first synthesis of β-carotene was reported independently by Karrer and Eugster (1950), Inhoffen *et al.* (1950) and Milas *et al.* (1950). The method of Inhoffen *et al.* was later developed into an industrial process, and synthetic β-carotene has been produced commercially since 1954. Today, six synthetic carotenoids (Fig. 6) have become commercially important as colourants for food and feed: 8′-apo-β′-caroten-8′-al (β-apo-8′-carotenal) (C_{30}), β-apo-8′-carotenoic acid ethyl ester (ethyl 8′-apo-β-caroten-8′-oate) (C_{30} skeleton), citranaxanthin (5′,6′-dihydro-5′-apo-18′-nor-β-caroten-6′-one (C_{33}), β-carotene (C_{40}), canthaxanthin (C_{40}) and a mixture of the stereoisomers of astaxanthin ((3*RS*,3′*RS*)-3,3′-dihydroxy-β,β-carotene-4,4′-dione) (C_{40}).

Natural extracts and carotenoids isolated from them are also produced commercially, including β-carotene from the alga *Dunaliella* and from red palm oil, lycopene from tomatoes and lutein from marigold flowers. There may be considerable variation in the geometrical isomer composition of a carotenoid from different natural sources and between natural and synthetic products.

The commercial products are available as crystalline materials or in formulations such as solutions or suspensions in natural oils or stabilized, water-soluble beadlets. The physical state of the carotenoid may differ substantially in different preparations and from that in the natural microenvironment within a food matrix.

It is not intended in this review to describe all of the more than 100 carotenoids that occur in foods eaten by humans: only the specific carotenes, α-carotene, β-carotene and lycopene, and the xanthophylls, lutein, zeaxanthin, β-cryptoxanthin and canthaxanthin, are considered. α- and β-carotene, β-cryptoxanthin, lycopene and lutein are the main carotenoids in serum or plasma, and a relatively limited number of foods are the major contributors to these carotenoids in the diet. Table 1 shows both the uniformity and diversity of diets in western countries, with marked differences in the contributions made by different carotenoids. For example, carrots, citrus products and tomato products make major contributions to the dietary supplies of α-carotene, β-cryptoxanthin and lycopene, respectively; carrots and spinach are major dietary sources of β-carotene and lutein, respectively, but the greater diversity of foods that supply the latter components increases the differences between countries and reduces the contribution made by specific foods. This is especially so in the case of lutein. It must also be realized that specific food types affect the bioavaibility of carotenoids in blood, and caution should be exercised in using specific foods as proxies for the intake of specific carotenoids. Dietary preferences, cooking methods and many other factors (de Pee & West, 1996) have a major influence on the true contribution of specific foods to the carotenoid composition of plasma.

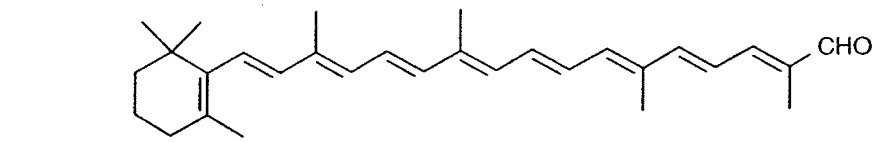
2.1 Carotenes

2.1.1 β-Carotene

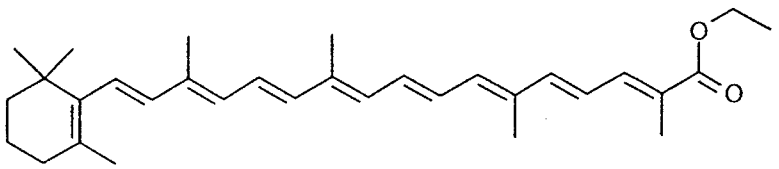
2.1.1.1 Occurrence

In temperate and tropical countries, important food sources of β-carotene are green leafy vegetables, carrots, peas and beans; carrots are a major source of both β- and α-carotene in many countries in the world. Dairy products and margarines contribute to less than 1% of daily

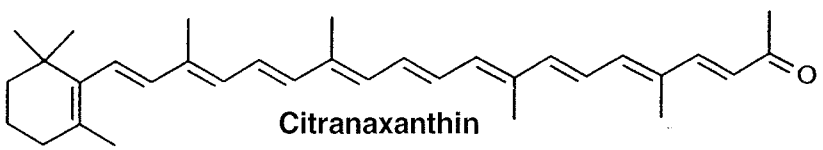
Figure 6. Carotenoids produced by industrial synthesis



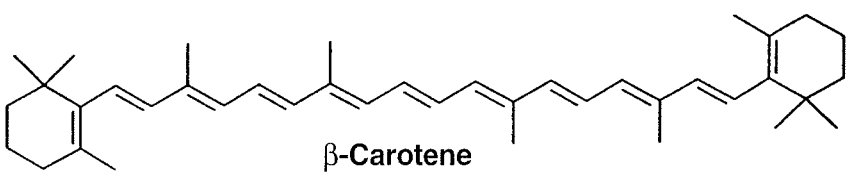
β-Apo-8'-carotenal (8'-apo-β-caroten-8'-al)



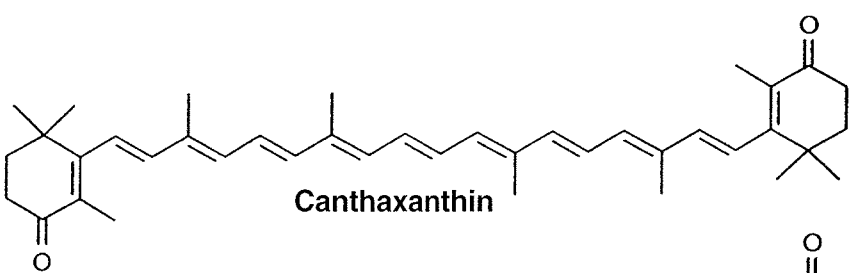
β-Apo-8'-carotenoic acid ethyl ester (ethyl 8'-apo-β-caroten-8'-oate)



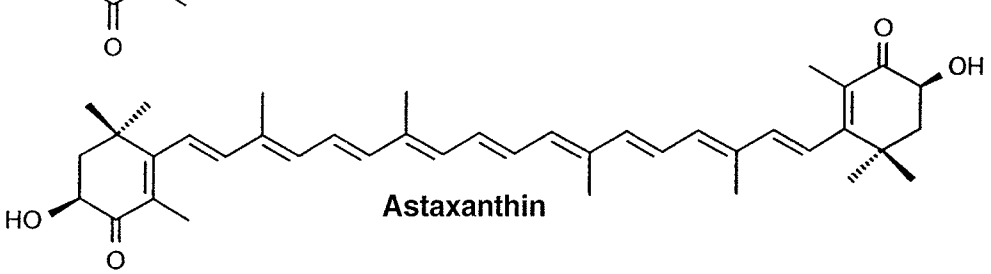
Citranaxanthin



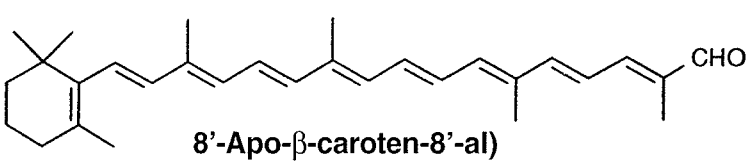
β-Carotene



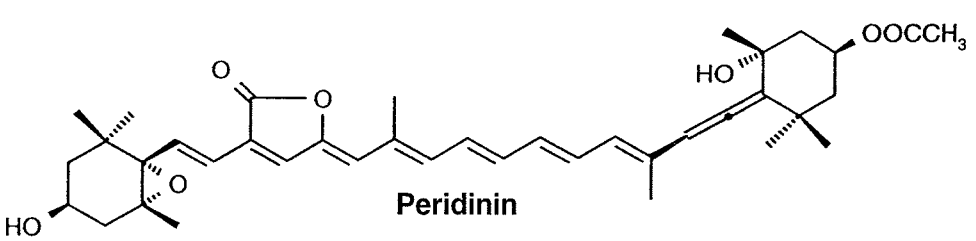
Canthaxanthin



Astaxanthin



8'-Apo-β-caroten-8'-al)



Peridinin

Table 1. Major food sources of carotenoids in five European (*n* = 80) and one US study (n = 1102)

Carotenoid	France		Ireland		Netherlands		Spain		United Kingdom		USA (women)	
	Food	%	Food	%	Food	%	Food	%	Food	%	Food	%
β-Carotene	Carrots	38	Carrots	60	Carrots	42	Carrots	24	Carrots	53	Carrots	25
	Spinach	14	Tomatoes and products	13	Spinach	14	Spinach	26	Soups	10	Cantaloupe melon	6
α-Carotene	Carrots	82	Carrots	90	Carrots	87	Carrots	60	Carrots	88	Carrots	51
	Oranges	6	Coleslaw	5	Oranges	5	Tangerines	17	Coleslaw	6	Soups	17
β-Cryptoxanthin	Orange juice	50	Oranges	42	Tangerines	41	Tangerines	53	Orange juice	45	Orange juice	38
	Oranges	30	Tangerines	28	Orange juice	33	Oranges	38	Oranges	26	Oranges	34
Lycopene	Fresh tomatoes	25	Tomatoes		Tomato soup	29	Tomatoes		Tomatoes		Tomatoes	
	Tinned tomatoes	16	Tinned	23	Fresh tomatoes	16	Puree	42	Fresh	21	Fresh puree/ sauce	18
	Pizza	16	Soup	17	Pizza	16	Sauce	42	Tinned	20	Pasta	12
Lutein	Spinach	31	Peas	19	Spinach	30	Spinach	34	Peas	36	Spinach	14
	Mixed vegetables	6	Broccoli	16	Broccoli	10	Lettuce	16	Broccoli	8	Collard/ mustard/turnip greens	14
	Cucumber	6							Eggs	8		

From Chug-Ahuja *et al.* (1993)

Table 2. Concentrations of β-carotene and lutein in green leafy vegtables in Europe, Malaysia and North America

Country	Vegetable	β-Carotene (% of total)	Lutein (mg/100 g)	β-Carotene (mg/100 g)	Reference
Germany	Green pepper (capsicum)	24	7.99	5.49	Gross (1991)
Spain	Lettuce (leaf type)	33	0.34	0.17	Granado *et al.* (1992)
	Green pepper (raw)	38	0.34	0.21	
	Spinach (raw)	41	4.23	3.25	
	Green beans (raw)	29	0.36	0.17	
United Kingdom	Spinach (raw)	41	5.87	4.02	Hart & Scott (1995)
	Watercress (raw)	36	10.71	5.92	
	Broccoli (raw)	36	1.61	0.92	
	Savoy cabbage (outside leaves, raw)	45	14.46	11.84	
	Peas (frozen, raw)	21	1.63	0.43	
	Lettuce (leaf type)	55	1.61	1.98	
Malaysia	Chinese cabbage	75	0.96	3.02	Tee & Lim (1991)
	Coriander	70	1.34	3.17	
	Drumstick leaves	51	7.13	7.54	
	Lettuce	57	< 0.01	< 0.01	
	S. androgumus	29	29.91	13.35	
North America	Broccoli (raw)	32	2.83	2.33	Khachik *et al.* (1992a)
	Spinach (raw)	31	9.50	8.90	
	Green beans	31	0.59	0.47	

intake of β-carotene; however, the importance of this source is difficult to quantify, and the availability of β-carotene in these products is probably much higher than that in vegetables. In tropical countries, mangoes, papaya and red palm oil are also important sources of β-carotene.

In green leafy vegetables, β-carotene and lutein can account for more than 80% of all carotenoids (Khachik *et al.*, 1992a; Ong & Tee, 1992; Hart & Scott, 1995). It has been suggested that green leafy vegetables contain approximately equal amounts of β-carotene and lutein (Heinonen *et al.*, 1989), but the proportions of the two carotenoids vary widely (Table 2).

2.1.1.2 Commercial sources

Pure β-carotene is synthesized industrially and is the major carotenoid available commercially. In addition, extracts rich in β-carotene but also containing other carotenoids are produced industrially from palm oil, algae (*Dunaliella*) and fungi (*Blakeslea trispora*).

2.1.1.3 Use and application

The use of β-carotene (EC160a) as a colour in foodstuffs is authorized under European Parliament Directive 94/36/EC (Klepsch & Baltas, 1994). For the purposes of the Directive, 'colours' are substances that add or restore colour to food. The addition of β-carotene to unprocessed foods or to various unflavoured milk and cream products is not allowed; however, β-carotene is one of very few colourants that may be added to margarine, butter and some cheeses. β-Carotene is also used as a colourant in e.g. fruit juices, soft drinks and

corn flakes, but its contribution in these products to total intake is probably minor. β-Carotene, lycopene and lutein are also used more generally as food colourants (Klepsch & Baltas, 1994).

In the United States, β-carotene is classified as 'generally recognized as safe' by the Food and Drug Administration (Select Committee of GRAS Substances, 1979). β-Carotene has been used since 1975 in the United States to treat photosensitivity in adults with erythropoietic protoporphyria (see section 5.1), at doses of < 180 mg/d (Mathews-Roth *et al.*, 1977).

2.1.1.4 Analysis

The carotenoids have a characteristic absorption spectrum, and their concentrations can be calculated from specific extinction coefficients (see Section 1). It was reported that the collection of plasma in containers coated with ethylenediamine tetraacetic acid depressed the concentrations by 50% (Stacewicz-Sapuntzakis *et al.*, 1987); however, it has also been reported that the concentrations in serum and heparinized plasma are indistinguishable (Thurnham *et al.*, 1988a).

Carotenoids are separated by liquid chromatography, and numerous methods have been developed to separate carotenoids in the organic solvent after extraction from foods and blood samples. Although some 20 or more carotenoids are found regularly in blood (Thompson *et al.*, 1985; Khachik *et al.*, 1997), usually only the five best-known ones, i.e. β-carotene, α-carotene, lutein, lycopene and β-cryptoxanthin, are analysed (Nells & De Leenheer, 1983; Ito *et al.*, 1987; Thurnham *et al.*, 1988a; Cantilena & Nierenberg, 1989; Kaplan *et al.*, 1990; Krinsky *et al.*, 1990a; Nierenberg & Nann, 1992; Stahl *et al.*, 1993). A variety of internal standards can be used, including tocopherol acetate, tocopherol nicotinate, tocol, retinyl acetate, echinenone and β-apo-8'-carotenal. The choice of the internal standard depends on the chromatographic technique and whether a single or two-channel detector is used. Quantification of β-carotene by liquid chromatography is associated with a coefficient of variation of 5–10%.

The methods used for extraction from food (Tee & Lim, 1991; Khachik *et al.*, 1992a; Hart & Scott, 1995) differ from those used to extract blood samples, since the preparation of the initial extracts of foods is more complicated: the sample must be representative of the edible fraction, and it must be e.g. homogenized, possibly cooked or saponified. The last step obviates the use of esters as internal standards, and echinenone and β-apo-8'-carotenal are the most commonly used. The method of liquid chromatography used depends on the degree of resolution of the carotenoids in the extract required. A useful summary of the factors that affect the liquid chromatography of carotenoids is available (Craft, 1992).

2.1.1.5 Human use or exposure

The best known human use for β-carotene is to provide vitamin A (Bendich & Olson, 1989), but it can also be used therapeutically in some cases of erythropoietic protoporphyria (Mathews-Roth *et al.*, 1977). Other possible uses are still poorly defined.

Exposure to carotenoids in the diet is universal. They appear to be absorbed by duodenal mucosal cells through a mechanism involving passive diffusion, similar to that for cholesterol and the products of triglyceride lipolysis (Parker, 1996; see also section 3.1.6). In most industrialized countries, fruit and vegetables provide an average of 2–3 mg/d of provitamin A carotenoids, of which β-carotene is the principal component (Granado *et al.*, 1996), although the intake may be much higher in persons who regularly eat e.g. carrots (Gregory *et al.*, 1990; Scott *et al.*, 1996). The availability of carotenoids from food sources is limited, however, by the physical matrix in which they are ingested and their dissolution in dietary lipids in the gut (de Pee *et al.*, 1995; Parker, 1996). β-Carotene is found in most blood samples. The amount present in samples in developing countries is often low, probably reflecting diets low in carotene and/or fat and poor bioavailability of carotenes from vegetable sources (de Pee *et al.*, 1995). An unknown amount is converted to retinol as it crosses the gut and little is left to pass into the bloodstream as β-carotene *per se*. Thus, only about 10% of

carotene was present as β-carotene in the blood of British adults (Thurnham & Flora, 1988); however, the concentration of plasma β-carotene in this survey was relatively low (median, 0.24 mmol/L in men, 0.32 μmol/L in women) in comparison with those in other parts of Europe, such as France (0.38 and 0.62 μmol/L; Howard *et al.*, 1996), Germany (0.43 and 0.61 μmol/L; Heseker *et al.*, 1991) and Northern Ireland (0.37 and 0.57 μmol/L; Howard *et al.*, 1996). This discrepancy probably reflects the relatively low consumption of vegetables (120 g/d) and fruit (60 g/d) in Great Britain (Gregory *et al.*, 1990) in comparison with the reported figure of 700 g/d in France (Drewnowski *et al.*, 1997; see also Table 3).

Since the availability of fruit and vegetables is often seasonal, the dietary concentrations of carotenoids may fluctuate during the year. Furthermore, seasonal factors such as light and heat (Takagi *et al.*, 1990) also affect the amounts of carotenoids in fruit and vegetables. The plasma concentrations of β-carotene frequently correlate with the dietary intake of carotenoids (Roidt *et al.*, 1988; Stryker *et al.*, 1988; Nierenberg *et al.*, 1989; Ascherio *et al.*, 1992; Cooney *et al.*, 1995; Saintot *et al.*, 1995) and vegetables (Drewnowski *et al.*, 1997). Seasonal variations in the intake of β-carotene have been reported by some authors (Nathanail & Powers, 1992; Rautalahti *et al.*, 1993; Olmedilla *et al.*, 1994; Cooney *et al.*, 1995) but not others (Thurnham & Flora, 1988; Cantilena *et al.*, 1992), perhaps due to differences in the amounts consumed. Thus, when seasonal differences are not found, vegetable intake may be low (Gregory *et al.*, 1990) or vary little during the year (Cantilena *et al.*, 1992). The amount of β-carotene converted to retinol may also affect seasonal fluctuations in plasma β-carotene. Scott *et al.* (1996) found no difference in the dietary intake of β-carotene among women in four-day, weighed samples, yet their plasma β-carotene was significantly lower in winter and spring than at other times of the year. Low intakes of β-carotene may be inadequate to support the synthesis of vitamin A and increase plasma β-carotene, as is the case in many developing countries (Ascherio *et al.*, 1992).

Another factor that affects the plasma concentrations of carotenoids is turnover. The half-lives of plasma β-carotene, α-carotene and β-cryptoxanthin are short (7–14 days) in comparison with those of lycopene (12–33 days) and lutein (33–61 days) (Micozzi *et al.*, 1992; Rock *et al.*, 1992). Plasma concentrations are probably maintained from deposits in adipose tissues, which in the case of β-carotene represent > 65% of the total body pool (Bendich & Olson, 1989; Kaplan *et al.*, 1990).

The plasma concentrations of β-carotene in humans vary considerably, due not only to differences in dietary habits. After supplementation with 20 mg/d β-carotene, the concentrations in plasma were increased by an average of 10-fold, but the concentrations after two months were strongly correlated with the baseline values ($r = 0.64$, $p < 0.001$) (Albanes *et al.*, 1992). As all subjects in this study had the same intake, individuals appear to differ substantially with regard to the absorption of β-carotene (Dimitrov *et al.*, 1988). Similar findings were reported by O'Neill and Thurnham (1988) in persons receiving single doses of 32–40 mg of supplemental carotenoids.

Smoking also influences β-carotene concentrations, which are generally lower in smokers than nonsmokers (Thompson *et al.*, 1985; Stryker *et al.*, 1988; Thurnham, 1990; Albanes *et al.*, 1991; Ascherio *et al.*, 1992; Brady *et al.*, 1996; Fukao *et al.*, 1996). Smokers tend to consume fewer vegetables than nonsmokers (Ascherio *et al.*, 1992; Margetts & Jackson, 1993; Brady et al., 1996), and it was suggested that diet alone accounts for the lower plasma carotenoid concentrations (Brady *et al.*, 1996); even after correction for differences in intake, however, plasma β-carotene concentrations are 20–50% lower in smokers (Gregory *et al.*, 1990; Thurnham, 1994). The effects of smoking is discussed in more detail in section 3.1.3.

2.1.2 α-Carotene

2.1.2.1 Occurrence

α-Carotene occurs in most vegetables and fruits but usually at concentrations much lower than those of β-carotene. Important natural sources of α-carotene are carrots (Bushway *et al.*, 1986; Micozzi *et al.*, 1992), red palm oil (Ong & Tee,

Table 3. Lutein and β-carotene concentrations (μmol/L) in plasma from men and women in studies in Europe, Japan and North America

Study	Lutein				β-carotene				Reference
	Men		Women		Men		Women		
	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	
UK National Survey	0.34[a] (0.177)	429	0.34[a] (0.19)	443	0.29 (0.29)	871	0.37 (0.262)	892	Gregory *et al.* (1990)
Belfast, Northern Ireland	0.27 (0.13)	88	0.29 (0.14)	79	0.37 (0.25)	88	0.57 (0.37)	79	Howard *et al.* (1996)
Cambridge, UK (middle aged women only)			0.41 (0.14)	168			0.54 (0.27)	168	Scott *et al.* (1996)
Toulouse, France	0.56 (0.23)	100	0.61 (0.28)	106	0.38 (0.26)	100	0.63 (0.53)	106	Howard *et al.* (1996)
Montpellier, France	NR		NR		1.0 (0.40)	30	1.6 (0.60)	68	Saintot *et al.* (1994)
Madrid, Spain	0.20 (0.09)		0.24 (0.21)	54	0.22 (0.14)	57	0.37 (0.23)	54	Olmedilla *et al.* (1994)
German National Survey	NR	57	NR		0.43 (0.10–1.6)[b]	278	0.61 (0.16–2.2)[b]	367	Heseker *et al.* (1991)
Washington, USA (middle-aged persons)	0.31 (0.14)		0.34 (0.16)	55	0.29 (0.18)	55	0.41 (0.26)	55	Stacewicz-Sapuntzakis *et al.* (1987)
Nurses Study, USA	0.35 (0.10)	55	0.33 (0.11)	186	0.46 (0.29)	121	0.58 (0.37)		Ascherio *et al.* (1992)
Rural Japan (middle-aged persons)	NR	121	NR		0.55 (0.49)	356	1.15 (0.74)	567	Thurnham *et al.* (1997)
Rural Pakistan (infants)	0.17 (0.10)	101	0.17 (0.12)	90	ND		ND		

NR, not reported; ND, not detected

[a] Median (Thurnham & Flora, 1988)

[b] Median and range

1992) and mangoes (Cooney *et al.*, 1995). In these foods, not only is the proportion of α-carotene almost equal to that of β-carotene, but the concentrations of carotenoids are much higher than in other foods. As reports from The Gambia (Villard & Bates, 1987), Malaysia and Thailand (Tee & Lim, 1991), however, indicate that mangoes contain no α-carotene, there may be some interspecies variation. The concentration of α-carotene in most fruits is usually < 0.1 mg/100 g of edible fraction (Tee & Lim, 1991; Ong & Tee, 1992).

2.1.2.2 Commercial sources

α-Carotene is not synthesized commercially, but it is present in variable amounts in carotene extracts from various plants.

2.1.2.3 Analysis

Because of the structural similarities between α- and β-carotene, the two analytes tend to run close together when analysed by liquid chromatography. Nevertheless, they are usually easily separated. Most of the chromatographic methods used to separate β-carotene from other carotenoids can be used to separate α-carotene as well (see section 2.1.1.4).

2.1.2.4 Human use or exposure

Human use of and exposure to α-carotene are similar to those for β-carotene. It is found in most blood samples, although the concentrations are usually much lower than those of β-carotene (Thurnham & Flora, 1988), except when the diet contains a large proportion of carrots (Micozzi *et al.*, 1992), red palm oil (Adelekan *et al.*, 1989) or mangoes (Cooney *et al.*, 1995). α-Carotene is a provitamin A carotene since it retains one of the β rings intact; however, it is considered to have one-half of the biological potency of β-carotene as a source of retinol (Bauernfeind, 1972).

2.1.3 Lycopene

2.1.3.1 Occurrence

Lycopene is the predominant carotenoid in tomatoes; it also occurs at high concentrations (2–4.5 mg/100 g) in several deep-orange or reddish fruits such as papaya, watermelon and pink grapefruit (Ong & Tee, 1992). Preparations like tomato paste contain more than 10 times more lycopene (33–68 mg/100 g) than fresh tomatoes (about 4 mg/100 g) (Khachik *et al.*, 1992a; Tonucci *et al.*, 1995). A wide range of concentrations has been reported in different types of tomatoes, the pear-shaped variety being a particularly rich source (62 mg/100 g; Granado *et al.*, 1992).

2.1.3.2 Commercial sources

Lycopene is not synthesized commercially, but an extract of tomatoes which contains mainly lycopene is available.

2.1.3.3 Use and application

The use of lycopene (EC160d) as a colour is authorized by the European Parliament in many fruit preparations, meat and fish products, confectionery and drinks in which a red colour is desired and tomatoes are not a constituent; however, the addition of lycopene to tinned and bottled tomatoes and tomato-based sauces is not permitted (Klepsch & Baltas, 1994). The integrity of carotenoids is often preserved under quite demanding conditions, for example in tomato paste, which is typically prepared by dehydration at high temperature over an extended period under slight vacuum (Khachik *et al.*, 1992a). The deep red colour of the lycopene pigment therefore lends itself to widespread use as a colourant in many processed foods such as beans, fish, soups and sauces.

2.1.3.4 Analysis

In developed countries, lycopene is one of the main carotenoids in blood samples, and several methods for its analysis have been described (Thurnham *et al.*, 1988a; Cantilena & Nierenberg, 1989; Olmedilla *et al.*, 1990; Nierenberg & Nann, 1992; Stahl & Sies, 1992; Stahl *et al.*, 1993; Ritenbaugh *et al.*, 1996; Scott *et al.*, 1996). Isomers of lycopene have been detected in both biological samples (Stahl & Sies, 1992; Stahl *et al.*, 1993; Clinton *et al.*, 1996) and foods (Stahl & Sies, 1992; Clinton *et al.*, 1996), but foods usually contain mainly all-*trans*-lycopene while biological samples have a higher proportion of *cis* isomers (Clinton *et al.*, 1996). Most liquid chromatographic methods

do not provide baseline separation of the isomers, although separation of a set of 15 geometric isomers of lycopene identified by NMR has been reported (Hengartner *et al.*, 1992).

2.1.3.5 Human use or exposure

A threefold difference in dietary intakes of lycopene has been reported, from 1 mg/d in Spain (Granado *et al.*, 1996) to 2–3 mg/d in the United States (Chug-Ahuja *et al.*, 1993; Yong *et al.*, 1994). The concentrations of lycopene in blood samples also vary considerably between countries. The predominant source of lycopene in developed countries is tomatoes, which are consumed both as the fruit and as a constituent of many processed foods. Lycopene appears to be better absorbed from processed tomato products than from the fresh fruit (Stahl & Sies, 1992; Gärtner *et al.*, 1997), perhaps because the *cis* isomers are absorbed better than all-*trans*-lycopene (Stahl & Sies, 1992). The latter accounts for 79–91% of all lycopene in tomatoes, tomato paste and tomato soup (Clinton *et al.*, 1996). Heating tomato juice with a small amount of oil has been reported to increase the formation of 9-*cis*-lycopene (Stahl & Sies, 1992). Khachik *et al.* (1992a), however, reported no difference in the chromatographic profile of raw and stewed tomatoes and tomato paste, but the *cis* isomers of lycopene formed during the processing of lycopene-containing foods may concentrate in the lipid fraction and be absorbed preferentially to all-*trans*-lycopene. This possibility is supported by reports of analyses of lycopene in biological samples, in which the *cis* isomers predominate. The proportion of *cis* isomers in serum ranged from 58 to 72% and that in prostate tissue from 79 to 88% (Clinton *et al.*, 1996).

The higher absorption of lycopene from processed foods may explain the higher serum concentrations of lycopene in blood from persons in developed countries such as France (Howard *et al.*, 1996), Great Britain (Gregory *et al.*, 1990), Northern Ireland (Howard *et al.*, 1996) and the United States (Ritenbaugh *et al.*, 1996) and the low concentrations or absence in blood from persons in developing countries such as China (Yang *et al.*, 1984), India (Das *et al.*, 1996), Pakistan (Thurnham *et al.*, 1997) and Thailand (Thurnham *et al.*, 1990). It has been reported that the plasma concentration of lycopene is better correlated with its dietary concentration than is that of β-carotene (Scott *et al.*, 1996). Unfortunately, the somewhat restricted distribution of sources of lycopene in the diet limits the usefulness of this carotenoid as a general marker of vegetable intake.

Smoking appeared to have no effect on serum lycopene concentrations in several studies (Thompson *et al.*, 1985; Stryker *et al.*, 1988; Thurnham, 1994; Brady *et al.*, 1996), in contrast to β-carotene. Lycopene also differs from β-carotene in showing no consistent difference in serum concentrations between the sexes (Stacewicz-Sapuntzakis *et al.*, 1987; Thurnham & Flora, 1988; Brady *et al.*, 1996). Furthermore, the concentrations of lycopene in serum appear to fall with age (Gregory *et al.*, 1990; Brady *et al.*, 1996), whereas this phenomenon has not been observed for other carotenoids. The plasma half-life is reported to be 12–33 days, which is longer than that of β-carotene (Rock *et al.*, 1992).

2.2 Xanthophylls

2.2.1 Lutein and zeaxanthin

2.2.1.1 Occurrence

As indicated earlier, lutein is one of the major carotenoids in green plants throughout the world (Khachik *et al.*, 1992a; Ong & Tee, 1992) and is the most prevalent carotenoid in blood samples (Thurnham *et al.*, 1997), although it is often not the most concentrated in plasma in industrialized countries (Thurnham & Flora, 1988; Micozzi *et al.*, 1992; Rock *et al.*, 1992; Brady *et al.*, 1996). Lutein is present in the serum of children with malnutrition or fat-absorption problems, even if no other carotenoid is detectable. The widespread occurrence of lutein in both vegetables and fruit, coupled with the high concentrations in some green vegetables (Table 2), undoubtedly accounts for its almost universal appearance in blood (Table 3). Zeaxanthin is usually only a minor component of plasma, except when maize is a staple dietary cereal.

Particularly rich sources of lutein in western diets are broccoli and spinach (containing 3 and 10 mg/100 g, respectively; Khachik *et al.*, 1992a) and watercress (10.7 mg/100 g; Hart &

Scott, 1995). Tee and Lim (1991) reported concentrations of 20–30 mg/100 g in certain leafy vegetables in Malaysia. Zeaxanthin is far less abundant in foodstuffs than lutein, although it is found especially in yellow corn (Gross, 1991), spinach (Granado *et al.*, 1992) and sweet red peppers (Granado *et al.*, 1992).

2.2.1.2 Commercial sources

The main supply of lutein is derived by extraction from marigold flowers (*Tagetes* sp.; lutein esters) or from dried, powdered alfalfa (Isler *et al.*, 1967; Nonomura, 1990).

2.2.1.3 Use and application

Lutein (E161b) is permitted for use as a food colour in the European Community and may be used in a variety of products, including alcoholic and nonalcoholic drinks, preserved fruits and vegetables, confectionery, baked wares and decorations, milk products and cheeses, sauces and seasonings, fish, meat and fish products and nutritional supplements in amounts of 50–500 mg/kg (Klepsch & Baltas, 1994). Another major use for lutein is as an additive to chicken feed to colour egg yolks.

2.2.1.4 Analysis

Concentrations of lutein and zeaxanthin are determined by spectrophotometry (see Section 1). Several methods have been reported for their analysis in plasma (Thurnham *et al.*, 1988a; Olmedilla *et al.*, 1990; Craft, 1992; Nierenberg & Nann, 1992; Ritenbaugh *et al.*, 1996; Scott *et al.*, 1996). Although zeaxanthin and lutein can be fully resolved chromatographically, the procedure requires considerable time. As a consequence, simpler methods in which the two compounds are eluted together, because of their similar polarity, have generally been used in surveys. The dissection of human and monkey retinas for extraction of lutein and zeaxanthin has been describedin detail (Bone *et al.*, 1988; Handelman *et al.*, 1992).

2.2.1.5 Human use or exposure

About 1–2 mg/d of lutein are reportedly consumed in European and American countries (Chug-Ahuja *et al.*, 1993; Yong *et al.*, 1994; Granado *et al.*, 1996; Scott *et al.*, 1996). Lutein must compete with the other carotenoids for entry into the mucosal cells lining the gut (Kostic *et al.*, 1995). Furthermore, the uptake of lutein into the triglyceride-rich lipoproteins is reported to be only 85% as efficient as that of β-carotene or lycopene when fed as an encapsulated supplement with a standard, low-carotene meal (O'Neill & Thurnham, 1998); however, long-term supplementation with a high dose of β-carotene did not affect the serum lutein concentration (Albanes *et al.*, 1997), and the concentrations of lutein in the serum in various population groups cover a wide range (Table 3).

Serum lutein can be used as a biomarker of vegetable intake (Thurnham *et al.*, 1997). Lutein is widely distributed in foods but is found predominantly in the leaves of green plants, which are eaten in almost all cultures. Although β-carotene is also present in green leaves in similar amounts to lutein and has also been proposed as a marker of intake (Drewnoski *et al.*, 1997), the concentrations in serum represent the amount left after the formation of retinol and retinoic acid in the gut. Plasma β-carotene also tends to be more labile than lutein, since β-carotene turns over more rapidly in blood, the half-lives being < 12 and 33–61 days, respectively (Rock *et al.*, 1992). The plasma concentrations of luteinare better correlated with estimate carotenoid intakes than for β-carotene (Scott *et al.*, 1996). The metabolism and distribution of lutein in tissues are discussed in Section 3.

2.2.2 Cryptoxanthin

2.2.2.1 Occurrence

Cryptoxanthin includes α-cryptoxanthin, β-cryptoxanthin and zeinoxanthin. Only β-cryptoxanthin is usually measured in blood plasma, because reference compounds are not readily available for the other cryptoxanthins. β-Cryptoxanthin is found mainly in fruits, in amounts ranging from 17 mg/100 g in jackfruit to 1.48 mg/100 g in papaya (Tee & Lim, 1991). Three fruits, papaya, starfruit and tree tomatoes, are reported to contain > 1 mg/100 g (Tee & Lim, 1991). Citrus fruit are a major source of β-cryptoxanthin in western countries, at concentrations of 80–1800 mg/100 g (Mangels *et al.*, 1993; Hart & Scott, 1995).

2.2.2.2 Commercial sources

β-Cryptoxanthin is not synthesized commercially. Extracts rich in β-cryptoxanthin are available in analytical quantities only.

2.2.2.3 Use and application

Cryptoxanthin is not listed in the EC Directorate for food colours.

2.2.2.4 Analysis

β-Cryptoxanthin is separated by HPLC and quantified by spectrophotometry (see Section 1). In some of the methods (Stacewicz-Sapuntzakis *et al.*, 1987; Thurnham *et al.*, 1988a), the peak of β-cryptoxanthin overlaps with that of α-tocopherol, but they are easily distinguished since they absorb at different wavelengths.

2.2.2.5 Human use or exposure

β-Cryptoxanthin is commonly found in plasma samples collected in western countries, the population means varying from 0.13 mmol/L in men in the United Kingdom (Thurnham & Flora, 1988) to 0.6 mmol/L in Spanish women (Olmedilla *et al.*, 1994). The plasma concentration of β-cryptoxanthin is usually lower than that of both β-carotene and lutein (Stacewicz-Sapuntzakis *et al.*, 1987; Thurnham & Flora, 1988; Cantilena *et al.*, 1992; Cooney *et al.*, 1995), unless oranges are particularly common in the diet, as reported from Spain (Olmedilla *et al.*, 1994). β-Cryptoxanthin has provitamin A activity. The plasma concentrations are usually higher in women than in men (Thurnham & Flora, 1988; Olmedilla *et al.*, 1994; Howard *et al.*, 1996). The half-life of β-cryptoxanthin in blood is < 12 days, similar to those of the other main provitamin A carotenoids (Rock *et al.*, 1992).

2.2.3 Canthaxanthin

2.2.3.1 Occurrence

Canthaxanthin is a red–orange pigment found in crustaceans, sea trout, algae, bacteria and some edible mushrooms. It is also present in the axion tissues of flamingos and other red-feathered birds (Hathcock *et al.*, 1990).

2.2.3.2 Commercial sources

β-Carotene is used as the starting material for the commercial synthesis of canthaxanthin.

2.2.3.3 Use and application

Canthaxanthin (EC161g) is approved for use as a food additive in the European Community but only for the colouring of Strasbourg sausages to a level of 15 mg/kg (Klepsch & Baltas, 1994). The predominant uses of canthaxanthin are in feed for laying hens, in order to colour egg yolks, in broiler production and in salmon and trout farming. Canthaxanthin has been marketed under a number of trade names as a tablet for 'tanning' the skin, in which deposition in subcutaneous tissues simulates the appearance of a tan, but this use has been discontinued in some countries. In Europe, capsules containing 10 mg β-carotene and 15 mg canthaxanthin are used at doses < 150 mg/d as a treatment for erythropoietic protoporphyria.

2.2.3.4 Analysis

Canthaxanthin can be measured by spectrophotometric methods (see Section 1). When the standard methods of reversed-phase liquid chromatography now adopted by many workers for the analysis of carotenoids in blood, tissue and food extracts are used, canthaxanthin elutes in the early part of the chromatogram, between lutein and β-crytoxanthin (Thurnham *et al.*, 1988a; van Vliet *et al.*, 1991). Canthaxanthin has also been reported to co-elute with β-apo-10′-carotenoid (van Vliet *et al.*, 1991). Canthaxanthin is unlikely to be a major component of plasma in North America or the United Kingdom, but no systematic study has been reported.