DOI 10.1002/mnfr.200800053 Mol. Nutr. Food Res. 2009, *53*, S194 – S218

Review

Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans

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Carotenoids are one of the major food micronutrients in human diets and the overall objective of this review is to re-examine the role of carotenoids in human nutrition. We have emphasized the attention on the following carotenoids present in food and human tissues: β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin; we have reported the major food sources and dietary intake of these compounds. We have tried to summarize positive and negative effects of food processing, storage, cooking on carotenoid content and carotenoid bioavailability. In particular, we have evidenced the possibility to improve carotenoids bioavailability in accordance with changes and variations of technology procedures..

Keywords: Bioavailability / Carotenoids / Epidemiological studies / Food source / Technology process

Received: February 5, 2008; revised: May 27, 2008; accepted: May 29, 2008

1 Introduction

Carotenoids are a widespread group of naturally occurring fat-soluble pigments. They are especially abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables. In plant cells, carotenoids are mainly present in lipid membranes or stored in plasma vacuols [1, 2].

Literature reports on the various aspects of the biosynthesis of carotenoids and the changes in their accumulation in plants through genetic and environmental factors. Food carotenoids have been compiled in several tables and databases, generally including provitamin A carotenoids such as β-carotene and β-cryptoxanthin, as well as others without that provitamin activity, such as lycopene and lutein, and others less studied in relation to human health such as phytoene or phytofluene [1–4].

In human beings, carotenoids can serve several important biological activities. The most widely studied and well-understood nutritional role for carotenoids is their provitamin A activity. Deficiency of vitamin A is a major cause of premature death in developing nations, particularly among children. Vitamin A, which has many vital systemic functions in humans, can be produced within the body from certain carotenoids, notably β-carotene [5].

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Abbreviations: FBS, food balance sheets; **FCTs,** food composition tables; **FFQ,** Food Frequency Questionnaire; **MP,** minimally processed

Carotenoids also potentially play an important role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen. Lycopene, the hydrocarbon carotenoid that gives tomatoes their red colour, is particularly effective at quenching the destructive potential of singlet oxygen [6]. Lutein and zeaxanthin and xanthophylls found in corn and in leafy greens such as kale and spinach, are believed to function as protective antioxidants in the macular region of the human retina, protection against cataract formation, coronary heart diseases and stroke [7–9]. Astaxanthin, a xanthophyll found in salmon, shrimp and other seafoods, is another naturally occurring xanthophyll with potent antioxidant properties [10]. Other health benefits of carotenoids that may be related to their antioxidative potential, include enhancement of immune system function [11], protection from sunburn [12] and inhibition of the development of certain types of cancers [13].

In this overview, food sources and intake, effects of food processing and bioavailability have been considered.

2 Food sources and intake

2.1 Carotenoid content of foods

In developed countries, 80–90% of the carotenoid intake comes from fruit and vegetable consumption. Of the more than 700 naturally occurring carotenoids identified thus far, as many as 50 are present in the human diet and can be absorbed and metabolized by the human body [14]; however only six (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin), representing more than 95% of total blood carotenoids, are present in the blood of people from different countries and have been studied and associated with some health benefits.

The most studied carotenoids are the following six: β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin, which are all important in human nutrition due to their biological activities. 'The Carotenoid Content of US Foods' is a comprehensive database, representative of US food consumption and including raw, processed and cooked forms, as described by Holden *et al*. in 1999 [15]. Similarly, O'Neill *et al*. [3] reported a European database covering the most commonly consumed carotenoid-rich foods in five European countries: UK, Ireland, Spain, France and The Netherlands. This database is a compilation of investigations from the 1990s. In 1995, Hart and Scott [16] investigated the carotenoid content of vegetables and fruits commonly consumed in the UK. Leth *et al*. [17] presented the carotenoid contents of Danish food, and Murkovic *et al*. [18] presented an Austrian Carotenoid Database comprising raw vegetables grown in Austria.

In this paper, only data from recent studies on the abovementioned six important carotenoids and their content in foods are reported, covering most of the period from about 2000 to March 2007. Foods included are vegetables, fruits and dairy products, representing the main part of carotenoid intake in Europe. Data about exotic fruits imported into Europe are also included. In Table 1, data on the content of carotenoids in raw and in a few processed foods are presented. Contents refer to the edible part of the food and are stated as μg *per* 100 g fresh weight (or volume). In some papers, contents were related to dry weight and those values were converted to fresh weight and included in Table 1 only when the moisture content of the food was documented. Furthermore, zeaxanthin was sometimes included in the reported lutein content, as the two carotenoids are not separated by all employed analytical methods.

The analytical methods are continuously being improved, leading to more specific data on carotenoids. This also results in data on contents of other carotenoids, *e.g*. phytoene and phytofluene, present in tomatoes and tomato products, and violaxanthin present in other vegetables and fruits, *e.g*. melons. Data for these carotenoids are not included in Table 1.

Several factors affect the composition and content of carotenoids in foods, *e.g*. variety, genotype, season, geographic location/climate, stage of maturity and growing conditions.

2.1.1 Genotype effects

The genotype affects the composition and content of carotenoids in different varieties and cultivars of fruit and vegetables. Lenucci *et al*. [40] showed that the content of lycopene and β-carotene varied significantly among 14 cultivars of cherry tomatoes. Likewise, the total carotenoid content ranged from 3700 to 12 200 μg/100 g among 50 cultivars of red-fleshed watermelons from US [45]. Wall [24] studied composition of different cultivars of banana and papaya. The major carotenoids found in bananas were lutein, α-carotene and β-carotene, and the average content of these carotenoids differed up to two-fold among the two cultivars investigated. Among papaya cultivars, lycopene was found in the red-fleshed samples but not in the yellow-fleshed ones, while β-carotene, β-cryptoxanthin and lutein were present in all samples. In conclusion, there is a high variability in the content of carotenoids in foods reported by different authors.

2.1.2 Seasonal, geographical and cultivation variation

The effects of season, geographic location and cultivation practise on carotenoid composition have been investigated in tomato cultivars. Raffo *et al.* [41] harvested greenhouse cherry tomatoes at full ripeness at six different times of the year. No definite seasonal trend nor correlation with solar radiation or temperature was found for total carotenoids (sum of eight carotenoids), nevertheless tomatoes harvested in mid-summer (July) had the lowest average level of lycopene (7061 μg/100 g), whereas tomatoes from March contained 11 969 μg/100 g. Toor *et al*. [46] also studied sea-

Table 1. Data for the content of major carotenoids in selected foods (μg/100 g or 100 mL fresh weight/volume)

Foods	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Lycopene	Reference
Plant origin							
Apricot	123–188	n.d.–39	–[a)]	n.d.[b)]–44	585–3800	54	[19–21]
Avocado	213–361	8–18	21–32	19–30	48–81	–	[22]
Banana	86–192	–	n.d.–5	60–156	43–131	n.d.–247	[23, 24]
Basil	7050	il[c)]	89	n.d.	4820	n.d.	[18]
Bean, green	883	–	–	–	503	–	[25]
Broccoli	707–3300	il	n.d.	n.d.	291–1750	n.d.	[17, 18, 25]
Cabbage, white	450	il	n.d.	n.d.	410	n.d.	[18]
Carrot	254–510	il	n.d.	2840–4960	4350–8840	n.d.	[17, 18, 26]
Chilli, red	n.d.	–	–	–	6530–15 400	–	[27]
Cornflakes	n.d.–52	102–297	n.d.	n.d.	n.d.	n.d.	[17]
Cress	5610–7540	–	–	–	2720–3690	–	[26, 28]
Cucumber	459–840	il	n.d.	n.d.	112–270	n.d.	[17, 18]
Dill	13 820	il	410	94	5450	n.d.	[18]
Egg plant	170	il	n.d.	n.d.	1110	n.d.	[18]
Endive	2060–6150	–	–	–	1340–4350	–	[26, 28, 29]
Fig	80	–	10	20	40	320	[30]
Grapefruit, red	–	–	–	–	–	750	[20]
Guava	–	–	19–118	n.d.	102–2669	769–1816	[23]
Kale	4800–11 470	–	–	–	1020–7380	–	[31]
Kiwi	–	–	–	–	<20	<10	[32]
Leek	3680	il	n.d.	n.d.	3190	n.d.	[18]
Lettuce	1000–4780	–	–	–	870–2960	–	[25, 26, 28, 29]
Mango	–	–	17–317	n.d.	109–1201	<10–724	[23, 32]
Mandarin juice	–	–	752	n.d.	55	–	[33]
Nectarine, peel	–	–	n.d.–31	–	5–307	–	[34]
Nectarine, flesh	–	–	n.d.–21	–	2–131	–	[34]
Olive oil, extra virgin	350	–	n.d.	n.d.	230	n.d.	[30]
Orange	–	–	74–141	n.d.	171–476	n.d.	[23]
Orange juice	–	–	16–151	n.d.–31	n.d.–98	–	[33]
Papaya	93–318	–	n.d.–1034	n.d.	81–664	n.d.–7564	[23, 24]
Parsley	6400–10 650	il	n.d.	n.d.	4440–4680	n.d.	[17, 18]
Pea	1910	il	n.d.	n.d.	520	n.d.	[18]
Peach	–	–	–	–	–	11	[20]
Peach, peel	–	–	n.d.–36	–	11–379	–	[34]
Peach, flesh	–	–	n.d.–16	–	4–168	–	[34]
Pepper, green	92–911	n.d.–42	n.d.–110	n.d.–139	2–335	n.d.	[18, 25, 26, 35]
Pepper, orange	245	n.d.	3	72	400	–	[35]
Pepper, red	248–8506	593–1350	248–447	n.d.–287	1441–2390	–	[35]
Pepper, yellow	419–638	n.d.	15–41	10–28	42–62	–	[35]
Pineapple		–	70–124	n.d.	139–347	265–605	[23]
Pistachio	770–4900	–	–	–	n.d.–510	–	[36, 37]
Plum, peel	–	–	3–39	–	217–410	–	[34]
Plum, flesh	–	–	3–13	–	40–188	–	[34]
Potato, sweet	50	–	–	–	7830	–	[27]
Pumpkin	630	–	60	–	490	500	[3, 20]
Rhubarb	–	–	–	–	–	120	[20]
Sage	6350	il	87	n.d.	2780	n.d.	[18]
Spinach	5930–7900	il	n.d.	n.d.	3100–4810	n.d.	[18, 38]
Tomato	46–213	il	n.d.	n.d.	320–1500	850–12 700	[17–20, 26, 32, 39]
Tomato, canned	n.d.	n.d.	n.d.	n.d.	217–283	8480–11820	[17]
Tomato, cherry	n.d.–25	–	–	–	300–1100	800–12 000	[32, 40, 41]
Tomato, concentrate	–	–	–	–	–	49 300–94 000	[20]
Tomato juice	29	–	–	–	369	1024–11 000	[20, 42]
Tomato ketchup	n.d.	n.d.	n.d.	n.d.	135–500	4710–23 400	[17, 20, 32, 43]
Tomato puree	n.d.	n.d.	n.d.	n.d.	383–548	13 160–26 110	[17]
Tomato sauce, instant	–	–	–	–	–	5600–39 400	[20]
Tomato soup, instant	–	–	–	–	–	12 400–19 900	[20]
Watermelon, red	–	–	n.d.	n.d.	314–777	4770–13 523	[20, 23]
Watermelon, yellow	–	–	59–110	n.d.	56–287	n.d.–109	[23]

Table 1. Continued

Foods	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Lycopene	Reference
Wheat flour	76–116	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Wheat flour, durum	164	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Animal origin							
Butter	15–26	n.d.–2	5–8	n.d.–2	296–431	–	[44]
Cheese, ripened	3	0.2	0.2	n.d.	48	–	[44]
Cheese, young	4	0.2	0.1	n.d.	62	–	[44]
Egg, yolk	384–1320	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Egg	182	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Milk, full fat	0.8–1.4	n.d.–0.1	0.3–0.4	n.d.–0.1	15–19	–	[44]
Milk, semiskimmed	0.5–0.8	n.d.–0.1	n.d.–0.1	n.d.	7–9	–	[44]

a) –: not included in the reference(s).
b) n.d.: not detected or quantified.
c) il: included in lutein (stated in the reference).

sonal variations in lycopene content of greenhouse tomatoes, and found lowest contents in the summer months (December–February), as temperatures above 30°C were found to inhibit lycopene synthesis more than the slightly positive effect of solar radiation. Sass-Kiss *et al*. [19] found significantly different contents of lycopene in tomatoes from two successive harvest years due to different weather conditions. In addition, processing varieties of tomatoes grown in open fields contained higher amounts of lycopene than table varieties from greenhouses.

Bergquist *et al*. [38] investigated carotenoids in baby spinach cultivated at three different times within two years. Contents of total carotenoids varied about 15% among the three cultivations at commercial harvest time and about 30% after 5 or 9 days storage at 10°C, while the content tended to increase or remained stable during storage because the metabolic patway of some carotenoids continues during the ripness.

In investigation of Setiawan *et al*. [23], three ripe samples from different regions were analysed, and minimum and maximum values found in *e.g*. mango and papaya were respectively: β-cryptoxanthin 17–317 and n.d.–425 μg/100 g, lycopene 49–724 and 4305–7564 μg/100 g, β-carotene 109–1201 and 322–664 μg/100 g.

Kimura and Rodriguez-Amaya [28] compared hydroponic and conventionally grown lettuce, and found a lower (10–30%) carotenoid content (including lutein and β-carotene) in hydroponic cultivated lettuce.

Caldwell and Britz [47] investigated the effect of supplemental UV radiation on the carotenoid composition of greenhouse leaf lettuce. In general, supplemental UV-B increased the carotenoid content of green leaf lettuce and reduced levels in the red-leaf varieties which may be attributed to light-dependent changes in xanthophilly carotenoids content. Furthermore, up to ten-fold cultivar differences were found in levels of carotenoids in plants grown under identical conditions.

Effects of nitrogen rate and form on the accumulation of carotenoid pigments in the leaf tissue of greenhouse-grown kale were investigated by Kopsell *et al.* [31]. Treatment with different amounts of nitrogen at a constant 1:3 ratio of NH_4–N and NO_3–N showed that concentrations of β-carotene and lutein were not affected by nitrogen rate on a fresh weight basis, however on a dry weight basis the carotenoids increased linearly to increasing nitrogen rate. Increasing NO_3–N from 0 to 100%, at a constant nitrogen rate, resulted in increases in both lutein and β-carotene.

Commercially available Spanish orange juices, including one mandarin juice, were investigated by Melendez-Martinez *et al*. [33]. Hulshof *et al*. [44] found an effect of season on the content of β-carotene in milk samples from the Netherlands. β-Carotene was the predominant carotenoid in all the analysed dairy products even if carotenoid levels in dairy products are extremely low and of very little significance to overall intakes. Milk sampled from January to April contained approximately 20% less β-carotene than milk sampled from July to October, probably due to seasonal differences in animal feeding practices. However, no regional differences as a consequence of homogeneous climate were found.

2.1.3 Stage of maturity and storage

de Azevedo-Meleiro and Rodriguez-Amaya [29] found large differences in the carotenoid contents between young and mature leaves from the same head of endive, lettuce and New Zealand spinach. In endive and lettuce, the carotenoid concentration of the mature leaves were about two to four times those of the young leaves. In contrast, the mature leaves of New Zealand spinach only contained about 75% that of the young leaves, and the principal carotenoids were β-carotene, lutein, violaxanthin, neoxanthin and lactucaxanthin. The coloured compounds in pistachio nuts from different geographical regions (Greece, Iran, Italy, Turkey), each presenting specific varieties, were studied by Bellomo

and Fallico [37]. The level of the main carotenoid, lutein, depended on type of cultivar, cultivation practise and *ripeness* as well as origin of the nuts, the lutein content diminishing with ripening. Among ripe nuts the Italian samples had the highest lutein content.

When storing greenhouse tomatoes at different temperatures for 10 days, Toor and Savage [39], like in earlier observations, found about two-fold more lycopene in tomatoes stored at 15° and 25°C than in refrigerated tomatoes at 7°C (7.5 and 3.2 mg/100 g, respectively).

2.1.4 Potential rich sources

In many developing countries, vitamin A deficiency is widespread, leading to a general need to increase the vitamin A intake of the population, even if the major food source of dietary vitamin A in these area are provitamin A carotenoids. This enhancement of carotenoids might be achieved, *e.g.* by cultivating crops containing higher amounts of provitamin A carotenoids, traditional plant breeding or by genetic engineering [48 – 50]. Likewise, Western countries focus on fruit and vegetable consumption and the associated health benefits. Carotenoids are among the active components of fruits and vegetables with potential health effects, and enhancement of carotenoid levels might thus be desirable. Examples of investigations into richer sources of carotenoids are outlined below.

Kidmose *et al*. [51] studied carotenoids in different genotypes of spinach. The total carotenoid content varied from 17.76 mg/100 g (in the lightest green genotype) to 22.63 mg/100 g (in the darkest one) with highest β-carotene, lutein and neoxanthin levels. Xu *et al*. [52] analysed the carotenoid composition of peel and juice of ordinary and lycopene-accumulating mutants of orange, pummelo, and grapefruit. Carotenoid profiles of 36 major carotenoids varied with tissue types, citrus species, and mutations. Profiles of peel and juice differed, and content of total carotenoids was much higher in peels.

We summarized the most relevant investigations about the principal food sources of carotenoids. New Zealand spinach are rich in carotenoids, and are one of the most popular leafy vegetables in Brazil and de Azevedo-Meleiro and Rodriguez-Amaya [29] reported levels of about 3800 μg β-carotene, 4800 μg lutein, 2200 μg violaxanthin and 1500 μg neoxanthin *per* 100 g mature leaves. Likewise, Rajyalakshmi *et al*. [53] studied contents of total carotenoid and β-carotene in South Indian forest green leafy vegetables, and found high contents in some varieties. Furtado *et al*. [54] analysed carotenoid content in common Costa Rican vegetables and fruits, and pointed out rich sources. Content of carotenoids in commonly consumed Asian vegetables was studied by Kidmose *et al*. [27]. Many varieties had high contents of β-carotene, lutein and other xanthophylls, *e.g.* drumstick leaves and edible rape turnip leaves. Lako *et al*. [55] reported carotenoid profiles of a wide selection of Fijian fruit and vegetables, and found many rich sources among green leafy vegetables, *e.g.* drumstick leaves as above.

It is also worth noting that the ongoing trend towards globalization is modulating both the availability of foods (*i.e.* exotic fruits, carotenoid-fortified foods), and the social habits in relation to food consumption in some European countries.

2.2 Sampling of foods for carotenoid analysis

In the field of nutrition, sampling is generally aimed at taking samples representative of the eating habits of certain consumers, *e.g.* of the population of a nation. Proper sampling is of utmost importance to avoid unintended variability. When designing the sampling plan for a study of carotenoids in vegetables and fruits, it is important to consider many aspects. Thus a sample plan should include conditions that might influence carotenoid composition and content, *i.e.* cultivation conditions like: choice of variety and cultivar, geographical location, season and year, agricultural practices – like nutrients and fertilizers at disposal, and cultivation in open field or in greenhouse – and stage of maturity. Furthermore, harvesting and postharvest handling, storing, possible processing or cooking, should also be taken into account for a sufficient sample description.

2.3 Analytical methods

Like the above-mentioned agricultural and sampling aspects, the analytical methods by which the carotenoids are determined influence the levels of the different carotenoids.

The general steps in the analyses of carotenoids include: sample preparation, extraction and saponification, separation, detection and quantification. Errors can be introduced in each of these steps.

Several considerations must be taken into account throughout the analysis to get reliable results, as carotenoids are highly susceptible to isomerization or degradation from light, heat, oxygen, acids, prooxidant metals and active surfaces [56 – 58]. Otherwise, the carotenoids might to some extent undergo isomerization or degradation.

2.3.1 Sample preparation

Before homogenization, an appropriate portion of the food, *e.g.* vegetables should be trimmed and cleaned and only those parts that are normally eaten should be included in the analyses [18]. The foods might be lyophilized or frozen to avoid changes in the carotenoid concentrations before preparation. These procedures should ensure that representative samples are ready for extraction.

2.3.2 Extraction and saponification

In food analyses, the procedure normally includes extraction of the carotenoids followed by alkaline saponification

of the ester forms present in certain foods. In addition, the saponification step removes interfering substances like chlorophylls and unwanted lipids before the final extraction of the carotenoids. Saponification is not necessary for samples without these compounds.

Several extraction procedures have been applied, and have been described in other reviews [57–60]. Numerous organic solvents have been used either alone or in mixtures for liquid-liquid extraction, which is the general procedure. As an alternative to the traditional method, supercritical fluid extraction (SFE) has been applied in some recent investigations [61]. To prevent carotenoid losses during extraction, antioxidants such as butylated hydroxytoluene (BHT) are usually added to the extraction solvent. Moreover, internal standards might be used to assess losses during the extraction [15, 62]. In some studies, an SPE is added as a further purification of carotenoids prior to the determination [17].

2.3.3 Separation, detection and quantification

Traditionally, determination of carotenoids in foods was performed by measuring the total absorption of the extract at a specific wavelength and calculating the amount using β-carotene as standard. This was later improved by separation of carotenes and xanthophylls by open-column chromatography (OCC). The introduction of HPLC equipped with UV and/or PDA detectors made the isolation, detection and quantification of the individual carotenoids possible, thus greatly enhancing the quality of the analytical results. More recently, the application of HPLC coupled with MS (LC-MS) has proven a powerful tool for identification of carotenoids. This technique is very sensitive and might also provide information about structure. By coupling HPLC with NMR the structure of the carotenoids might be completely elucidated.

There are no general HPLC conditions of choice neither for mobile phase nor column [30, 57, 60]. Both normal-phase and RP HPLC can be applied to separate the carotenoids [19, 63, 64]. However, the most frequently used systems are RP [59]. Many different solvents have been applied as gradient or isocratic mobile phases. To prevent oxidation of carotenoids, an antioxidant is often added to the mobile phase. The column selection depends on the requirements for the separation of the individual carotenoids and their isomers. Monomeric C18 columns separate most of the xanthophylls, but not lutein and zeaxanthin, whereas these components can be resolved with polymeric C18 columns [65]. Similarly, the nonpolar carotenoids, *e.g.* α- and β-carotene, are poorly resolved with the monomeric C18 columns and partly separated with the polymeric C18 columns. Since Sander and Wise [65] showed an improved separation of both polar and nonpolar carotenoids including geometric isomers with a polymeric C30 column, this type of column has been used for a variety of food analyses [19, 66, 67].

2.3.4 Quality assurance and standard methods

To get reliable results in analysis of carotenoids it is always advisable to include measures of quality assurance. Preferably, the method should be validated and, *e.g.* sensitivity, selectivity, recovery, repeatability and reproducibility estimated. Scott *et al*. [56] developed a vegetable mix reference material (RM), and the use of standard or in-house RMs is highly recommendable [18] for assuring the analytical quality. Furthermore, purity of the carotenoids should be considered and care taken in the standardization of carotenoid solutions [16].

As reported above, no generally applicable standard method for determination of individual carotenoids in food has been introduced. However, standard methods are available from the Association of Analytical Communities (AOAC) [68] using OCC with spectrophotometric determination of carotenes and xanthophylls, respectively and European Committee for Standardization (CEN) [69] has published a standard method for determination of total β-carotene by HPLC with UV–Vis detection.

2.4 Carotenoid intake

It is widely assumed that serum concentrations of carotenoids reflect, at least to some extent, the consumption of carotenoid-containing foods [70]. The influence of diet as a factor of serum carotenoid concentrations has long been known, although both dietary intake and serum concentrations of carotenoids have shown a high variability both within and between subjects in different populations [71–75]. Seasonal variations in individual carotenoid intake, and serum concentrations, have been reported in some European countries (*i.e*. Spain) while not in others (*i.e*. UK, Republic of Ireland, Finland) [3, 73, 74, 76], even when total carotenoid intake may not vary significantly (*i.e*. Spain) [3]. Although fluctuations between seasons may be observed for several carotenoids both in the diet and serum levels [74–77], in Spanish diet, these reach statistical significance only for β-cryptoxanthin (higher in winter) and lycopene (higher in summer); these changes are found to be in accordance with the availability and consumption of the major dietary contributors (*i.e*. citrus fruits and tomato and watermelon, respectively) [76, 77].

A European north–south gradient for the intake of some carotenoids and serum concentrations, both within and between European countries, have been reported [3, 75, 78, 79]. This pattern is consistent with food availability data (*i.e*. fruits and vegetables) among European countries since southern (Mediterranean) countries (*i.e*. Greece, Italy, Portugal, Spain) consume greater amounts of fruits and vegetables than northern countries (*i.e*. UK, Ireland, Scandinavian countries) [80, 81]. In some countries, this geographical trend has been reported for both total and individual carotenoid intake and, overall, it is associated with variations in fruit and vegetables consumption (*i.e*. in UK, low in the

North) and with socioeconomic status and cultural factors. In fact, the specific traditional and cultural factors between the two groups of populations, and in addition the changes in marketing could contribute to the change of life style [78]. Consistently, serum levels also show this distribution trend across north – south axis.

Time trends in carotenoid intake have been scarcely assessed in European countries. Nonetheless, changes in major dietary sources of carotenoids (fruits, vegetables, cereals and recently fortified foods) is known to have occurred in European countries during the last decades [81 – 83] which is partly explained by changes in socio-economical, demographic and cultural factors. Time variation, on a short-term basis, in carotenoid intake has been assessed in Denmark, where, apparently, intake pattern of carotenoids has not changed from 1995 to 1997 [17]. Similarly, in Spain, using almost the same methodology, a fairly consistent qualitative and quantitative pattern of carotenoid intake from fresh fruits and vegetables was observed on a short-term basis, *i.e.* between 1996 and 2004, although this pattern was different when data were calculated on a longer time scale, *i.e.* 1960 – 1980 (it could be due to changes in fruit and vegetables consumption of populations) [77].

2.5 Methodology

Estimated intakes of carotenoids vary widely both on an individual, regional and national level, and significant seasonal variations in intake of individual carotenoids have been also reported in some countries (*i.e.* Spain) [76, 77]. Carotenoid intake assessment, at both the individual and group level, has been shown to be complicated mainly for the high variability within-subject and between-subject intake, inaccuracies associated with methods of dietary assessment, and inconsistencies in food composition tables (FCTs) and databases [84 – 86].

The food balance sheets (FBS) [83] present a comprehensive picture of the pattern of a country's food supply during a specified reference period. The FBS shows for each food item – *i.e.* each primary commodity and a number of processed commodities potentially available for human consumption – the sources of supply and its utilization. The total quantity of foodstuffs produced in a country added to the total quantity imported and adjusted to any change in stocks that may have occurred since the beginning of the reference period gives the supply available during that period. On the utilization side a distinction is made between the quantities exported, fed to livestock, used for seed, put to manufacture for food use and nonfood uses, losses during storage and transportation, and food supplies available for human consumption. The *per capita* supply of each such food item available for human consumption is then obtained dividing the respective quantity by the related data on the population actually partaking of it. Data on *per capita* food supplies are expressed in terms of quantity and – by applying appropriate food composition factors for all primary and processed products – also in terms of caloric value and protein and fat content.

Table 2. Sources of nutritional data

Level	Source	Type of data
Population	Food balance sheets	Ecological; large units
Household	House budget survey	Ecological; small units
Individual	Nutrition survey	Analytical; individuals

Source: EURONUT, Report 9, 1987 [87].

Table 3. Availability of data for lutein and/plus zeaxanthin content in foods nutritional and epidemiological studies

Ref.	Type of report	Country (food origin)	Lutein	Lutein + Zeaxanthin
[14]	HPLC report	USA	Yes	
[89]	HPLC report	Finland		Yes
[90]	HPLC report	Malaysia	Yes	
[91]	HPLC report	Spain	Yes	
[92]	Database	World wide		Yes
[2]	Database	USA (several)		Yes
[16]	HPLC report	UK	Yes	
[93]	Database	Spain	Yes	
[94]	HPLC report	USA	Yes	
[15][a)]	Database	USA (USA)		Yes
[18]	Database	Austria		Yes
[3]	Database	Europe (several)		Yes

a) Zeaxanthin values reported independently for selected foods.

Source: Permission *Brit. J. Nutr.*: Granado *et al.* 2003 [88].

Carotenoids content has been calculated applying USDA FCTs.

Sources of nutritional data have been classified at different levels and data obtained are of different type (Table 2) [87].

Regardless of the confidence in the method used for dietary assessment, evaluation of nutrient exposure by dietary means is based on the availability of reliable food composition data. Since the nutritional interest in carotenoids was largely due to their provitamin A activity, traditionally, FCTs and databases have, traditionally, not included values for individual carotenoids in foods, although they have considered vitamin A (retinol equivalents) content. However, the increasing evidence of the potential role of several constituents present in fruits and vegetables (carotenoids) in human health led to a revision of former data and the inclusion of nonprovitamin A carotenoids (*i.e.* lutein) in the new FCTs and databases during the 1990s (Table 3) [88].

2.6 Available data of dietary intake

Few studies have been carried out to ascertain the total intakes of carotenoids in the European diet. A European

Table 4. Comparison of carotenoid intake (mg/day) in adults in five European countries (data are medians and interquartile ranges)

	β-Carotene		Lutein (+ Zeaxanthin)		Lycopene		α-Carotene		β-Cryptoxanthin		Total carotenoids	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Spain (n70)	2.96	1.58 – 4.41	3.25	1.75 – 4.34	1.64	0.50 – 2.64	0.29	0.15 – 0.51	1.36	0.74 – 2.16	9.54	7.16 – 14.46
France (n76)	5.84	3.83 – 8.00	2.50	1.71 – 3.91	4.75	2.14 – 8.31	0.74	0.37 – 1.36	0.45	0.17 – 0.88	16.06	10.3 – 22.1
UK (n71)	5.55	3.66 – 6.56	1.59	1.19 – 2.37	5.01	3.2 – 7.28	1.04	0.71 – 1.66	0.99	0.32 – 1.64	14.38	11.77 – 19.1
Rep of Ireland (n76)	5.16	3.47 – 7.42	1.56	1.14 – 2.1	4.43	2.73 – 7.13	1.23	0.69 – 1.78	0.78	0.4 – 1.44	14.53	10.37 – 18.9
The Netherlands (n75)	4.35	2.93 – 5.7	2.01	1.42 – 3.04	4.86	2.79 – 7.53	0.68	0.30 – 0.90	0.97	0.50 – 1.75	13.71	9.98 – 17.7

Source: Permission *Brit. J. Nutr.*: O'Neill *et al.* 2001 [3].

Table 5. Intake (mg/person/day) reported in several European countries

Ref.	Lutein (+ zeaxanthin)	β-Cryptoxanthin	Lycopene	α-Carotenoid	β-Carotenoid	Dietary method/database	Foods/population assessed (subjects)
[73]	0.92	–	1.03	–	2.21	4 days collection HPLC data	Vegetables UK; *N* = 79
[95]	0.67	0.14	0.74	–	1.51	7 days diary carotenoid database	Total diet UK (EPIC Norfolk cohort) N = 176 controls
[78]	–	0.022 – 0.033	–	0.31 – 0.34	1.47 – 1.70	4 days weighed records (+ eating out)	Total diet UK (National Diet and Nutrition Survey, *N* = 1.478 (<65 years)
[96]	2.45/2.55 (w/m)	0.21/0.16 (w/m)	1.30/1.05 (w/m)	0.69/0.69 (w/m)	2.90/2.96 (w/m)	Dietary questionnaire; energy-adjusted intake Harvard School of Public Health database	Total diet The Netherlands *N* = 120.693[a]
[96]	1.15	0.03	0.65	0.53	1.76	Dietary questionnaire; energy-adjusted intake Harvard School of Public Health database	Total diet Finland (ATBC study, placebo branch); *N* = 6.771 men
[76]	0.58	0.41	1.25	0.22	1.00	Family Budget Survey HPLC data	Fresh fruits and vegetables Spain; *N* = 72.279
[97]	–	–	–	–	3.1 – 5.0	Two 24 h recalls; CIQUAL database	Total diet Spain; *N* = 2.346
[98]	1.47	–	0.95	0.24	2.11	Dietary history questionnaire EPIC database (2nd Edn.)	Total diet Spain; *N* = 354[b]
[99]	0.90	0.64	2.09	0.26	1.99	Dietary history questionnaire EPIC database (2nd Edn.)	Total diet Spain (EPIC cohort), *N* = 41.446
[77]	0.45	0.31	1.16	0.26	1.07	Family Budget Survey HPLC data	Fresh fruits and vegetables Spain; *N* = 6.000 households
[100]	4.01	0.17	7.38	0.15	2.6	Seven-day dietary diary HPLC data	Total diet Italy (INN-CA Study); *N* = 1.968

a) Netherlands Cohort study; 62.412 men, 58.279 women (assessed at baseline).
b) Subjects considered as controls were patients admitted to the hospitals with a variety of diagnosis unrelated to the principal study factors (gastric cancer).

carotenoid food database was published along with the assessment, by a Food Frequency Questionnaire (FFQ) at individual level, of the carotenoid intakes of people groups in a five-country comparative study [3]. Main results are presented in Table 4. However, it should be noticed that the population used in this study was a group in a determined area of each of the five participant countries (*ca*. 80 subjects *per* country). When interpreting the data provided by that study, it should be considered that the levels of intake reported in this study are somewhat consistent with the findings in serum of the same individuals. That is, the relative crude intake and the relative contribution of xanthophylls and carotenes indicate 'true' differences in carotenoid intake (and food sources) among European countries. Participants may not necessarily be representative of the overall population although it was assumed that they followed a typical food intake pattern characteristic of their country. In addition, all subjects filled out a common FFQ.

Table 5 summarizes carotenoids intake in some European countries (UK, Finland, The Netherlands, Spain and Italy) from representative literature. To have an overall view of other countries, we have to take into account the analysis of FBSs. However, both crude data and comparisons should be considered with caution since, as shown, sample size and methodology differ between studies.

Table 6 shows the percentage contribution of individual food items evaluated by FFQ to the total intake of each of the five carotenoids in parentheses.

Table 6. Major foods contributing to carotenoid intake in adults in five European countries

	France (Grenoble)	Republic of Ireland (Cork)	UK (Coleraine)	The Netherlands (Zeist)	Spain (Madrid)
	Name (%)	Name (%)	Name (%)	Name (%)	Name (%)
β-Carotene	Carrots (38)	Carrots (60)	Carrots (53)	Carrots (42)	Spinach (26)
	Spinach (14)	Tomat. prod (13)	Soups (10)	Spinach (12)	Carrots (24)
Lutein	Spinach (31)	Peas (19)	Peas (36)	Spinach (30)	Spinach (34)
	Lettuce (8)	Broccoli (16)	Broccoli (8)	Broccoli (10)	Lettuce (16)
	Eggs (8)	Eggs (10)	Eggs (8)	Peas (9)	Oranges (7)
	Mix vegetables (6)	Carrots (9)	Sweetcorn (7)	Chicory (8)	Eggs (7)
Lycopene	Tomatoes (25)	Tomatoes canned (23)	Tomatoes (21)	Tomato soup (29)	Tomatoes (55)
	Tomatoes canned (16)	Tomato soup (17)	Tomatoes canned (20)	Tomatoes (16)	Tomato puree (42)
	Pizza (16)	Pizza (16)	Pizza (15)	Pizza (16)	
α-Carotene	Carrots (82)	Carrots (90)	Carrots (88)	Carrots (87)	Carrots (60)
	Oranges (6)	Coleslaw (5)	Coleslaw (6)	Oranges (5)	Tangerines (17)
β-Cryptoxanthin	Orange juice (50)	Oranges (42)	Orange juice (45)	Tangerines (41)	Tangerines (53)
	Oranges (30)	Tangerines (28)	Oranges (26)	Orange juice (33)	Oranges (38)

Source: Permission *Brit. J. Nutr.*: O'Neill *et al.* 2001 [3].

Table 7. Ten top contributors (%) to lutein (+ zeaxanthin) intake in five European countries[a)]

France ($N = 76$)	Republic of Ireland ($N = 76$)	UK ($N = 71$)	The Netherlands ($N = 75$)	Spain ($N = 70$)
Spinach (31)	Peas (19)	Peas (36)	Spinach (30)	Spinach (34)
Lettuce (8)	Broccoli (16)	Broccoli (8)	Broccoli (10)	Lettuce (16)
Eggs (8)	Eggs (10)	Eggs (8)	Peas (9)	Oranges (7)
Mix vegetables (6)	Carrots (9)	Sweetcorn (7)	Chicory (8)	Eggs (7)
Cucumber (6)	Tomato (8)	Lettuce (6)	Lettuce (4)	Broccoli (6)
Green beans (4)	Oranges (7)	Carrots (4)	Tomato (4)	Peas (6)
Courgette (4)	Peppers (6)	Tomato (4)	Oranges (4)	Potatoes (3)
Peas (3)	Sweetcorn (4)	Tangerines (4)	Eggs (4)	Tangerines (3)
Tomato (3)	Spinach (3)	Celery (4)	Green beans (4)	Peppers (3)
Sweetcorn (2)	Lettuce (3)	Spinach (3)	Potatoes (4)	Leeks (2)
Total (%) 75	*Total (%) 85*	*Total (%) 84*	*Total (%) 81*	*Total (%) 97*
Green veg. 56%	Green veg. 47%	Green veg. 57%	Green veg. 65%	Green veg. 67%

a) Assessed in winter.
Source: Permission *Brit. J. Nutr.*: Granado *et al.* 2003 [88].

Table 7 shows estimations using data obtained in a European multicentre study where dietary intake was estimated using a common FFQ and database of carotenoids in food [88].

As shown, although green vegetables are important contributors to lutein intake in five European groups, relative contribution differs substantially among them. It is also worth noting the relative contribution of nongreen vegetables and fruits and the fact that nongreen foods may account for almost half of the total lutein intake in some groups. More importantly, zeaxanthin, is mostly provided by nongreen vegetables and fruits [76, 88].

In Table 8, a comparison between countries on the relative contribution of each carotenoid to total carotenoids intake calculated from FBS is reported [4, 83].

While several methodological constraints (databases, groups assessed and method for dietary assessment) limit the comparability of crude intakes of carotenoids among groups, an alternative approach to compare groups/populations is to estimate the relative contribution of each carotenoid to the total intake. This approach does not overcome all the constraints regarding the reliability of the data used for comparison but may provide an interesting picture for comparative (ecological) purposes. This approach is based on several facts:

(i) The relative contribution of each carotenoid has some association with its crude intake (g/person/day), and therefore the intake of its major dietary sources, and provides information for each carotenoid (and food sources) within the context of the total diet. For example, intake of β-cryptoxanthin may be similar in two groups but the contribution to total carotenoid intake may be significantly different.

(ii) The above point relates to other nutritional and physiological facts. Carotenoids may interact with each other (synergistic and antagonistic) during absorption, transport, deposition and biological action. Thus, the relative amount of each class and type of carotenoid in the total diet become relevant [101].

Table 8. Relative contribution (%) of each carotenoid intake to total carotenoid intake according to FBSs data [83].

Country	Total intake μg/day[a)]	Lutein (+ zeaxanthin)	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
Germany	9.368	52	3	8	3	33
Denmark	10.092	52	4	7	3	34
Italy	15.753	45	4	15	3	33
Sweden	7.521	48	5	11	3	32
UK	8.654	50	4	9	3	33
Greece	20.968	40	3	21	4	32
France	13.984	50	4	9	3	34
The Netherlands	8.761	48	5	10	3	33
Spain	12.789	45	4	14	3	34
Europe	*11.786*	*48*	*4*	*12*	*3*	*33*

a) Sum of lutein (zeaxanthin), β-cryptoxanthin, lycopene, α-carotene and β-carotene.
Based on data from USDA Food Composition Tables [4].

Table 9. Relative contribution (%) of each carotenoid intake to total carotenoid intake[a)]

Country (ref.)	Carotenoid intake (mg/person/day)[a)]	Foods assessed	Lutein (+ zeaxanthin)	β-Crypto-xanthin	Lycopene	α-Carotene	β-Carotene
Spain							
[76]	3.5	Fresh fruits and vegetables	17	12	36	6	29
[3]	9.54	Total diet	37	14	17	3	31
[99]	5.88	Total diet	15	11	36	4	34
[102]	3.25	Fresh fruits and vegetables	14	10	36	8	33
The Netherlands							
[103]	6.1						21
[3]	13.71	Total diet	15	7	35	7	32
[96]	7.55	Total diet	32 (M)	3	17	9	38
	7.41		34 (W)	2	14	9	40
Finland							
[104]	4.0	Total diet	28	<1	20	3	50
[96]	4.12	Total diet	28	<1	16	13	43
France							
[3]	16.06	Total diet	16	3	30	5	36
UK							
[3]	14.38	Total diet	11	7	35	7	39
Rep. Ireland							
[3]	14.53	Total diet	11	5	30	8	36
Overall range (%)	–		*11–37*	*0–14*	*14–36*	*3–13*	*21–50*

a) Mean or median values; total carotenoid intake = sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene.

(iii) Finally, because of each carotenoid may display different biological functions, actions and associations, relevant both at individual and population level, the relative occurrence of each carotenoid within the total diet may become important when comparing groups within an epidemiological context.

For example, as shown in Table 9 based on the data reported by O'Neill *et al*. [3], using the same dietary method and database, α-carotene and β-carotene show a consistent contribution in the European countries (3–9 and 31–39%, respectively), regardless of the dietary habits and geographical origin of the groups assessed. On the contrary, for lutein and lycopene, a different contribution pattern is observed between Spain (37 and 17%, respectively) and the rest of the European countries (11–16 and 30–35%, respectively). Regarding β-cryptoxanthin, a clearly distinct relevance is observed with Spain showing two- to three-fold more contribution than in others, especially north European countries. All these values are consistently below the mean/median values reported, for example, in Spain (0.3–0.6 mg/day) [3, 76,

77]. Thus, regardless of the method of dietary assessment and used database, sample size and endpoint measured, it is interesting to note that, compared to other dietary carotenoids, β-cryptoxanthin contribute marginally (0 – 7%) in North European countries (Finland, Denmark, Germany, England, Ireland), whereas in the south (*i.e.* Spain) it accounts for 10 – 14% (annually) and up to 20% (*i.e.* winter) to total carotenoid intake [3, 76, 77]. While this approach seems to be useful to compare exposure (nutrient intake) in different groups, it also depends on the method of assessment. This fact is highlighted when this is approached using FBSs as shown in Table 8. The apparent lack of variation in the relative contribution among European countries (except for lycopene and lutein) contrasts with the figures obtained in the individual studies performed in the same countries. This may be apparently due to the method used to estimate dietary intake since FBS provide figures on food availability (not consumption) while the individual studies provide information about 'true' nutrient intakes of the individuals although by different methods.

3 Effects of food processing on carotenoid stability and/on bioavailability

Carotenoid content and pattern of food material are modified during postharvest storage of plant materials, as well as during processing – at home or industry – and storage of food products. Particularly, thermal processing (*i.e.* blanching, pasteurization, cooking, canning, frying and drying) may decrease carotenoid contents, but at the same time may be beneficial through the disruption of food matrices (*e.g.* cell walls and membranes) and so facilitating the liberation (bound) and solubilization of carotenoids (free and ester forms) resulting in an increased carotenoid bioavailability. Processing operations that reduce the particle size of food material (*e.g.* chopping, grinding, milling or homogenation) or the incorporation of an oil-phase in food formulations (*e.g.* addition of oil to salads, emulsioning), may also enhance carotenoid bioaccessibility [105 – 109]. Emerging technologies (*e.g.* high pressure-low temperature, pulse electric fields) and several new approaches in food packaging (*e.g.* modified atmospheres, addition of antioxidants and active packages) in addition may modify carotenoid contents of food [110, 111]. Therefore, food processing implies a relevant impact on the nutritional quality of food and the stability of micronutrients in foods during food supply. Thus, food processing has a relevant impact on the dietary patterns of the population.

3.1 Postharvest storage

Mayer-Miebach and Spieß [112] reported that the total carotenoid content of *Kintoki* carrots was reduced by about 30% of the initial amount during 8 wks of storage at 1°C with 97% humidity. Lycopene content was reduced to about 60%, while only 20% of the β-carotene content was lost.

Kopas-Lane and Warthesen [113] found that the lutein content in spinach was nearly stable during storage at 4°C for 8 days in the dark, whereas up to 22% was lost when exposed to light.

3.2 Thermal processing

The scientific literature shows a wide variability of effects depending on the time/temperature conditions used (Table 10). The effects of important unit operations often used in industry are described below.

3.2.1 Kinetics of thermal degradation/ isomerization

Studies towards the kinetics of thermal degradation and isomerization of carotenoids in food matrices are scarcely found in literature. Dewanto *et al.* [146] showed that the amount of all-trans-lycopene extracted from tomato homogenates, subjected to heat treatment at 88°C increased significantly 1.6-fold after 2 min and 2.7-fold after 15 or 30 min, as compared to non heated homogenates. The total (*Z*)-lycopene content increased by 6, 17 and 35% after 2, 15 and 30 min, respectively. After subjecting *Nutri Red* carrot purees with a 1% oil supplement to 2 h heat treatments at 100, 110, 120, 130 and 140°C, (all-*E*)-lycopene content decreased to 60, 63, 63, 38 and 25% of the initial value, respectively. Oil supplements had no effect on (all-*E*)-lycopene but slightly reduced isomerization. In samples without oil, (9*Z*)-lycopene increased by 10-, 30-, 41-, 43- and 38-fold at 110, 120, 130 and 140°C, respectively. Heat treatment at 70°C degraded only slight amounts of (all-*E*)-lycopene even after a 5 h heating time [121]. In homogenates of a zeaxanthin and lutein containing potato variety, the treatment temperature (25 – 150°C) had a much more marked effect on the carotenoid pattern than treatment time (0 – 5 h). The potato variety used for all experiments contained several carotenoids, mainly zeaxanthin (0.2 – 0.8 mg/100 g) and lutein (0.04 – 0.16 mg/100 g) [147]. At temperatures above 70°C lutein was totally degraded, while zeaxanthin was stable even for high-temperature and long-time treatments, regenerating 9-*cis*-zeaxanthin.

3.2.2 Blanching/pasteurization

Blanching (70 – 105°C) and pasteurization (60 – 85°C) are mild heat treatments for short time periods used to inactivate enzymes and vegetative microorganisms. Data obtained by Aman *et al.* [114], analysing spinach after steam-blanching for 2 min, have shown a decrease of total lutein (17%) and (9*Z*)-lutein contents (7%), while the (13*Z*)-isomer level was unaffected. According to Choe *et al.* [115], the lutein content of spinach was stable during blanching and steaming for 2 and 5 min, respectively. Control samples contained 30.99 mg lutein and 42.86 mg β-car-

Table 10. Effect of thermal processing on stability of some nonprovitamin A carotenoids

Technology	Product	Bioactive compound	Effect	Ref.
Blanching	Carrot (*Kintoki*)	(all-*E*)-lycopene	–	[112]
	Spinach	(all-*E*)-, (13*Z*)-lutein	–	[114, 115]
		(all-*E*)-, (9*Z*)-lutein	↓	[114, 115]
Pasteurization	Tomato (puree)	(all-*E*)-lycopene, -lutein	–	[116]
	Orange (juice)	(all-*E*)-lutein	↑	[117]
		(all-*E*)-zeaxanthin	–	[117]
	Orange – carrot (juice mix)	(all-*E*)-lutein	–	[118]
		(all-*E*)-zeaxanthin	↑	[118]
Cooking	Tomato (homogenates)	(all-*E*)-, *cis*-lycopene	↑	[112]
	Tomato (juice)	Total lycopene	–	[119]
		cis-Lycopene	↑	[119]
	Tomato (pulp)	(all-*E*)-lycopene	↓	[120]
	Carrot (*Nutri Red*) with/without oil	(all-*E*)-lycopene (9*Z*)-lycopene	↓↑	[121]
	Broccoli, spinach, green beans	(all-*E*)-lutein	↑	[122]
Canning	Tomato (pulp)	(all-*E*)-lycopene	↑	[123]
	Carrot (*Nutri Red*) with/without oil	(all-*E*)-lycopene (9*Z*) lycopene	↓↑	[121]
	Kale, corn, spinach, green peas	Total lutein, zeaxanthin	↑	[124]
	Corn	(all-*E*)-lutein, -zeaxanthin	–	[125]
	Sweet corn	(all-*E*)-lutein, -zeaxanthin	↓	[114]
Osmotic treatment	Carrot (*Nutri Red*)	(all-*E*)-lycopene	↑	[126]
Hot air drying	Tomato, carrot (*Nutri Red*)	(all-*E*)-lycopene	↑	[121, 127, 128]
	Tomato	(all-*E*)-lycopene	–	[129, 130]
	Tomato (paste)	(all-*E*)-lycopene	↓	[131]
	Tomato	(all-*E*)-lutein	↑	[129, 130, 132]
	Potatoes	(all-*E*)-zeaxanthin	↑	[133]
	Red pepper (whole/cut pods)	(all-*E*)-zeaxanthin	↑	[134, 135]
	Pepper (whole pods), Paprika	(all-*E*)-zeaxanthin	↓	[136, 137]
Frying	Potatoes	(all-*E*)-lutein	↑	[138]
	Carrot (chips)	Total carotenoids	↓	[139]
Microwave heating	Carrot (*Nutri Red*) (slices)	(all-*E*)-lycopene	–	[12]
	Broccoli	(all-*E*)-lutein	↑	[124, 140]
	Spinach, green beans, broccoli	(all-*E*)-lutein	–	[122]
	Sweet potatoes (leaves)	(all-*E*)-lutein	↓	[141]
	Papaya, broccoli (florets)	Total carotenoids	↓	[140, 142]
Multistep heat-treated products	Tomato (various commercial products)	(all-*E*)-, *cis*-lycopene	–	[119]
	Tomato (paste)	(all-*E*)-lycopene	↑	[143]
			↓	[144]
		cis-Lycopene	–	[143]
		(all-*E*)-lutein	–	[143]
	Orange (juice)	Total carotenoids	↓	[145]

–, No changes; ↑, increase; ↓, decrease.

otene *per* 100 g sample. In an orange – carrot juice mixture, no variations in lutein content were observed after pasteurizing at 98°C for 21s, while about 45% more zeaxanthin was detected due to an enhanced extractability [118]. The same effect was shown after blanching of lycopene containing *Kintoki* carrots at 90°C for 15 min, which raised lycopene content for about 15% [107]. In tomato puree, lycopene and lutein contents were not affected by pasteurization [116].

3.2.3 Cooking/canning

A prolonged heating time of 2 h at 100°C caused a partial decrease (18%) of lycopene content in tomato pulp; (*Z*)-isomers were not detected [120]. The amount of lutein extracted from green peas increased by about 10 – 15% after boiling for 1 h [122]. In sweet corn, canning at 121°C in a rotary retort decreased total lutein and zeaxanthin content by 26 and 29%, respectively, while the amounts of (*Z*)-lutein and (*Z*)-zeaxanthin increased from 12 to 30% and from 7 to 25%, respectively. (13*Z*)-isomers of both lutein and zeaxanthin prevailed as individual stereoisomers [143]. So, from examples above mentioned, the different way of cooking could lead to a decreasing, an increasing or no variations in the content of single carotenoids; in addition, the way of cooking could modify the profile of carotenoid content in relationship with food matrix and stability of specific carotenoids in the foods.

3.2.4 Multistep heat treatment

After a commercial hot-break extraction of tomato paste at 90°C for 5 – 10 min followed by concentration under vacuum at 60 – 70°C and final sterilization at 121°C for

30 min, the amount of (all-*E*)-lycopene extracted was enhanced about 1.4-fold, while the (*Z*)-lycopene and lutein contents remained unchanged [115]. Also, results obtained by Agarwal *et al*. [119] indicated the stability of (all-*E*)-lycopene under industrial processing conditions: raw tomatoes and various commercial tomato products, after a multi-step heat-treatment, were found to contain 5 – 10% (*Z*)- and 90 – 95% (all-*E*)-lycopene; no difference was observed.

3.2.5 Drying

For hot air drying, whole or chopped plant material is generally exposed to temperatures not exceeding 80°C. Therefore, no significant carotenoid losses or generation of (*Z*)-isomers are expected. However, oxidative losses may occur in some traditional slow drying methods that last over a period of few days. Much higher inlet temperatures are used for spray-drying, thus raising the probability of (*Z*)-isomer generation. Goula and Adamopoulos [131] observed oxidative lycopene losses (up to 32%) during spray-drying (air inlet temperature: 110 – 140°C) of tomato paste. On the other side, no significant carotenoid losses were observed in tomatoes dried at lower temperature (42°C) [130]. Enhanced carotenoid extractability after hot air drying has been reported by various authors: lycopene [121, 127, 128], lutein [132], zeaxanthin [133].

3.2.6 Frying

For frying, the material is cut, blanched, sometimes soaked in an antioxidant solution and, finally fried in fat or oil preheated to temperatures of 150 – 180°C. Food material is heated rapidly in the surface layers to the temperatures of the frying medium; however temperature does not exceed 100°C in inner layers. Lutein remained stable after frying of eight different potato varieties and a higher extractability of lutein was reported [138].

3.2.7 Microwave heating

The main industrial applications of microwave heating are tempering, baking and drying; other uses include blanching and cooking. In papaya, microwave blanching induced small losses of the total carotenoids [142]. Khachik *et al*. [122] studied the effect of microwave cooking on lutein retention and its (*Z*)/(*E*) ratio for different vegetables. Under mild cooking conditions (750 W; spinach: 1.5 min; green beans: 4 min; broccoli: 5 min), the lutein levels and (*Z*)/(*E*) ratios remained unchanged. During microwave cooking (700 W) of sweet potato leaves, (all-*E*)-lutein losses increased with increasing cooking times of up to 56% after 8 min; no (*Z*)-lutein isomers were formed. The (9*Z*)-lutein, contained in the fresh leaves, was completely degraded, and two lutein dehydration products were identified [141]. After microwave vacuum drying with a microwave power program of 400 W continuously, (all-*E*)-lycopene content remained stable in *Nutri Red* carrot slices. However, significant losses of carotenoids were observed, when a combined microwave power programme (600/240 W), by which high temperatures were generated, was used. No (*Z*)/(*E*) isomerization took place [121].

3.3 Product storage

The effects of food storage are summarized in Table 11.

3.3.1 Frozen storage

Long term frozen storage has been found to cause a reduction of the carotenoid content. For example, for watermelons, a decrease of up to 40% of the lycopene content was observed after 1-year storage at temperature ranges between –20 and –80°C [148]. However, lycopene was stable for three months in diced tomatoes stored at –20 and –30°C [149]. The exclusion of oxygen during frozen storage of tomato products reduces the rate of lycopene degradation [150, 151]. During frozen storage of pizza, the rate of degradation of the lycopene contained in the tomato ingredient is much faster than during frozen storage of the ingredient (tomato dices or purees) [150]. Depending on the packaging method (with/without oxygen exclusion; with/without paper box) up to 70% of lycopene may be destroyed.

3.3.2 Cold storage

The cold storage of minimally processed (MP) plant material – generally freshly cut and washed – has been studied by several authors. de Azevedo-Meleiro and Rodriguez-Amaya [29] reported a 19% reduction of the lutein content of MP endive after 5 days storage at 7 – 9°C. The lycopene content of MP watermelon (75% of the total carotenoids) slightly decreased during storage at 9°C, however stored at 5°C under light the lycopene losses were lower [34].

3.3.3 Storage at room temperature

During 1-year storage of commercially canned tomato juice no significant lycopene loss was observed at 25°C either at 37°C [119]. In commercially prepared tomato pulp, puree and paste lycopene remains stable even when stored under conditions of accelerated aging at 30, 40 and 50°C up to 90 days [154]. Light has an effect on the isomerization of lycopene in tomato juice: after 12 wks storage at 25°C in the dark the formation of (9*Z*)- and (13*Z*)-lycopene was favoured, while after the same time at the same temperature but using light storage (13*Z*) and (15*Z*) were the predominant lycopene isomers [159].

During storage of dried tomato products oxidation and isomerization are the main mechanisms of (all-*E*)-lycopene loss. In powders, with a great specific surface exposed to the storage conditions, an increased sensitivity for oxidative lycopene losses can be expected. Isomerization increases with increasing storage time and under illumination conditions; however oxidation increases mainly due to increased storage temperature. The residual moisture of the product

Table 11. Effect of storage on stability of some nonprovitamin A carotenoids

Technology	Product	Bioactive compound	Effect	Ref.
Storage <0°C	Watermelon	(all-*E*)-lycopene	↓	[148]
	Tomato (diced, pulp, puree)	(all-*E*)-lycopene	↓	[120, 149, 150]
	Pizza	(all-*E*)-lycopene	↓	[150, 151]
	Green beans	(all-*E*)-lutein	↓	[152]
	Red grapefruit (juice concentrate)	Total carotenoids	↓	[153]
Storage 0 – 10°C	Tomato	(all-*E*)-lycopene	–	[39]
	Carrot (*Kintoki*)	Total carotenoids	↓	[112]
		(all-*E*)-lycopene	↓	[112]
	Watermelons	(all-*E*)-lycopene	↓	[34]
	Spinach	(all-*E*)-lutein	– (*dark*)	[113]
		(all-*E*)-lutein	↓ (*light*)	[113]
	Endive	(all-*E*)-lutein	↓	[29]
Storage >10°C	Tomato	(all-*E*)-lycopene	↑	[39]
	Tomato (juice, canned juice, paste, soup, sauce, pulp, puree)	(all-*E*)-lycopene	–	[119, 154, 155]
	Tomato (powder)	(all-*E*)-lycopene	↓	[156 – 158]
	Tomato (juice)	(all-*E*)-lutein	↓	[159]
	Red pepper (whole/cut pods, powder)	(all-*E*)-lutein	↓	[135]
	Carrot (spray dried pulp)	(all-*E*)-lutein	↓	[160]
		(9*Z*)-lutein	–	[160]
		(13*Z*)-lutein	↑	[160]

–, No changes; ↑, increase; ↓, decrease.

plays an important role in lycopene stability. Under inert atmosphere (nitrogen) storage, much greater lycopene losses were observed in foam-mat dried tomato powder with a moisture content <1% than in powders with ≈3% moisture content, confirming that the enhancement of oxidative reactions are associated with very low moisture materials [156]. However, in products with higher moisture contents (9 – 23%), an increase of moisture enhances the oxidative lycopene losses [157]. At very low moisture contents, lipid auto-oxidation is enhanced leading to important lycopene losses. In the intermediate moisture range nonenzymatic browning reactions are favoured, which could provide some protection against carotenoid oxidation.

3.4 Summary

It is evident that different processes have different effects on specific carotenoids probably due to: (i) the chemical/stereochemical structure of the carotenoid (*e.g.* carotene, alcohol, epoxide, (*Z*)/(*E*)-isomer), (ii) its integration into a specific food matrices (*e.g.* free or esterified, as crystals or lipid droplets), (iii) the presence of pro-oxidants (Cu^{2+}, Fe^{2+}) and/or antioxidants (ascorbic acid, vitamin E) therein and (iv) its stability upon heating time and temperature, light as well as oxygen. Therefore, it is difficult to assess a general effect of food processing. In conclusion, the effects of thermal processing and storage on stability and bioavailability of carotenoids depend mainly on the severity of the thermal treatments applied. At lower temperatures (60 – 100°C), most carotenoids are stable and isomerization is negligible during blanching, pasteurization, cooking, low temperature drying and frying. Due to the disruption of the matrix of plant tissues and the destruction of the integrity of cell walls and membranes, carotenoid extractability is often increased. At temperatures above 100°C, practised for canning and sterilization, total carotenoid contents are decreased, major (*Z*)-isomerization occurs and bioavailability is improved due to enhanced matrix disruption and oil supplements. The fairly high bioavailability rise at processing temperatures above 100°C may be also due to isomerization rather than matrix disruption alone. In contrast, as an effect of oxygen, carotenoids are instable during drying processes as well as during storage of fresh, dry or frozen products. Further studies about processing and storage effects on carotenoids should focus on specific carotenoids in specific vegetables/fruits with the objective of optimizing industrial processes in order to improve the bioaccessibility and bioavailability of carotenoids (see Section 4).

4 Bioavailability

Bioavailability is defined as the fraction of a dietary component capable of being absorbed and available for use or storage. This is a crucial point in the assessment of the role of provitamins in human health, both to overcome deficiency and to potentially decrease the risk for several chronic diseases.

4.1 Preabsorptive processes and absorption

Studies on absorption of carotenoids started in the early 1960s [161], however the molecular mechanisms involved in their passage through the enterocytes still remain a mat-

ter of debate [162]. Bioaccessibility of carotenoids in vegetables is remarkably low and these compounds are characterized by a slow rate of absorption both in animals and humans because their chemical structure deeply interacts with macromolecules within the plant food matrix [162]. As an example, an *in vitro* digestion model system reported that only 1–3% of the β-carotene in raw carrots is accessible for absorption; and the accessibility of lycopene in canned and fresh tomatoes was <1% [163, 164]. Further studies indicated that more than 70% of the carotenoids remained in the final digesta [165].

4.1.1 Storage factor influencing the release of carotenoids from food matrix

A lot of factors can influence the initial release of carotenoids from the food matrix and their subsequent dissolution in lipidic drop in the stomach and duodenum [166]. Release from the food matrix is the initial and important step in the absorption process of carotenoids. Generally they are present in complexes with proteins as in green leaf vegetables or in semicrystalline structure as in carrots and tomatoes. Then they have to be transferred or dissolved in the lipid phase before they are absorbed. Physically altering food by cooking, blending or finely chopping improves release from the food matrix [132, 164]. Furthermore, the gastric hydrolysis of dietary lipids and proteins increases the release of carotenoids from the food matrix, and begins the process of solubilization of carotenoids into mixed lipid micelles in the gut lumen. The transfer of carotenoids from the predominantly aqueous environment to bulk lipid or micelles requires very close proximity of carotenoids to lipid micelles that starts to happen during the gastric digestion [167]. In this phase, the roles of bile salts and pancreatic secretion are critical for the emulsification, and during solubilization of carotenoids in the mixed micelles. Furthermore, Serrano *et al*. [168], showed a significant inverse correlation between small intestine availability of carotenoids (lutein + β-carotene) and content of klason lignin, non-starch polysaccharides and resistant protein in green leafy vegetables that should directly affect the intestinal availability of carotenoids acting as a barrier to the action of digestive enzymes and to the release of carotenoids from the food matrix.

Xanthophylls present in fruits, however, seem to be more efficiently released than β-carotene. *In vitro* studies indicated that, in green vegetables, epoxy-xanthophylls and their ester derivatives present in fruits are transferred more easily into the micellar phase [165, 169]. Furthermore, in the case of dietary ester of zeaxanthin, the partial hydrolysis promoted by carboxyl ester lipase during the small intestinal phase of digestion enhances the bioavailability of this carotenoid [170].

4.1.2 Postharvest factors influencing the carotenoid bioavailability

The effect of food processing on carotenoids bioavailability can be illustrated by comparing the blood response after heating a raw food compared with food that has been heat-treated and/or mechanically homogenized to disrupt the food matrix. Stahl and Sies [171] found that boiling tomato juice with 1% corn oil for 1 h before consumption led to a two-fold increase in lycopene plasma concentrations compared to the consumption of tomato juice not further heated. Porrini *et al*. [172] demonstrated that plasma total lycopene levels were higher after the intake of a commercial tomato puree that had undergone a process of heating and homogenization than after raw tomato consumption, thus demonstrating a significant effect of thermal treatment on food matrix and on absorption. On the same way, van het Hof *et al*. [173] observed that both, heating tomato for 1 h at 100°C and homogenization under high pressure, enhanced the lycopene response in both, triglyceride-rich lipoproteins and plasma, significantly. During sterilization of a *Nutri Red* carrot homogenate with a 1% oil supplement at 130°C for 30 min, the isomeric ratio of (all-*E*)- to total (*Z*)-isomers changed from 90:10 to 50:50. Isomeric ratio of the same homogenate, cooked at 100°C for 30 min without oil supplementation, was not altered. For consumption, oil content of all samples was 1%. Compared to the ingestion of an untreated control (blanched and stored at −50°C), a nine-fold increase with the lycopene content of the chylomicron fraction was found in the sterilized sample; bioavailability of the cooked samples increased by only 2.5-fold. Although no (5*Z*)-lycopene was generated in the homogenates during any of both thermal treatments, this isomer accounted for about 20% of the total lycopene in chylomicrons [174]. A remarkable enrichment of the relative contents of (5*Z*)-lycopene was also observed after ingestion of tomatoes, tomato juice and purée, respectively [175]. In contrast, lycopene uptake from whole cherry tomatoes, ingested either fresh or cooked at 100°C for 15 min without previous mechanical disruption, was not altered [176].

4.1.3 The composition of the meal on bioavailability

Experimental evidence has been accumulated on the role of dietary fat in the absorption and bioconversion of provitamin A carotenoids to vitamin A [14, 177]. The dietary fat intake plays an important role in the plasma responses to β-carotene supplements [178]. Recently, Brown *et al*. [179] showed that use of fat-free or reduced-fat salad dressings limited the absorption of carotenoids, which are abundant in fresh vegetable salads. In a view of these results, the authors suggested the threshold of 3–5 g fat *per* meal reported by Roodenburg *et al.* [180] and adopted as a guide-

line to promote optimal absorption of β-carotene [181]. In the study by Roodenburg *et al.*, α-carotene and β-carotene were provided in the form of pure supplements dissolved in fat, and not from plant foods. Other investigators used plant sources and found that minimal dietary fat (2.4 g/meal) is sufficient for optimal absorption of provitamin A carotenoids and their bioconversion into vitamin A [9]. The effects of lipid intake on the absorption of carotenoids was confirmed by the observation that the addition of avocado fruit or avocado oil as a lipid source enhances absorption of lycopene and β-carotene and α-carotene, β-carotene and lutein, respectively in humans [182]. Dietary fibre intake is another factor that could regulate carotenoid bioavailability. It is a known fact that fibre decreases the absorption of carotenoids by entrapping them and interacting with bile acids; this leads to an increase of faecal excretion of fats and fat-soluble substances such as carotenoids [183, 184].

The inter-relationship of the different carotenoids present in the food matrix also affects carotenoid absorption. A competitive inhibition, towards the absorption mechanism of a single carotenoid derivative, in fact, may occur at the level of micellar incorporation, intestinal uptake, or lymphatic transport or at one or more of the later steps. It has been proposed that a high-dose intake of carotenoids may antagonize the bioavailability and absorption of other carotenoids. For example, studies on simultaneous ingestion of carotenoids indicate that β-carotene may interfere with absorption of lutein and canthaxanthin, while high doses of simultaneous combination between lycopene and β-carotene decrease bioavailability of both [185, 186]. In contrast, Hoppe *et al*. [187], showed no interaction towards lycopene absorption by β-carotene, β-cryptoxanthin, α-carotene, lutein and zeaxanthin.

4.1.4 Physiological state of the consumer

Parasitism and disease resulting in intestinal dysfunction may have profound effects on carotenoid uptake and bioconversion, but these pathological states have not yet been adequately quantified. For example, in some studies, the lack of observed improvement in vitamin A status in individuals consuming dark green, leafy vegetables may be attributable, at least in part, to concomitant infection with intestinal helminths, *Helicobacter pylori*, or other organisms [188]. Persistent diarrhoea, lipid malabsorption, and deficiencies of vitamin A, protein and zinc also appear to be important factors that impair provitamin A-carotenoid utilization, in addition to their effects on vitamin A metabolism and turnover [166]. Carotenoid-rich fruits and vegetables may indeed provide sufficient vitamin A to meet physiological requirements and even replete body stores under conditions of relatively good health and hygiene. However, debilitating infections and parasitic infestations which are endemic in the tropics and subtropics both compromise carotenoid utilization and increase the individual's requirement for vitamin A. Thus, programs which seek to improve community vitamin A status through food-based interventions will be complemented and strengthened by public health measures which decrease the burden of infection and illness.

Also, age is an other factor that contributes to carotenoid bioavailability [189]. Carroll *et al*. [190] estimated from the analysis FFQ that β-carotene and lycopene are the major dietary carotenoids obtained from a younger and older Irish population. The profile of plasma carotenoid concentrations showed that β-carotene is the major carotenoid in both age groups. Younger groups have higher plasma concentrations of lycopene, β-cryptoxanthin, lutein + zeaxanthin. As described in other European populations these moderate positive associations exist between several plasma carotenoid concentrations and estimated record dietary carotenoids in younger but not in older groups [191].

4.2 Methodology to assess bioavailability

Several confounding factors are present in the literature regarding the assessment of carotenoid bioavailability in humans. Generally the pharmacokinetic studies only provided information on relative bioavailability (relative to reference dose or control) and not on the absolute bioavailability of the carotenoids. Moreover, acute studies need to use large doses of carotenoids to elicit a quantifiable change in blood or urine excretion.

Frequently the approaches used in human studies are short-term, single-dose, pharmacokinetic studies or long-term, multiple-dose supplementation assays. In the latter, the information obtained, relative to nutritional status, depletion and/or saturation processes, could be affected by the typology of the protocol used (*i.e*. on samples collections, 'acute', postprandial metabolism or 'chronic') [192]. Furthermore, these studies could be broadly divided into those using large pharmacological doses, which are only partly available due to limitations in the absorption process, and those using more physiological carotenoid doses, either using pure substances, and different matrices, including foods.

Another critical point is the individual response. Based mostly on plasma concentrations observed after carotenoid administration, there is evidence to suggest that there are 'poor' and 'good' absorbers. This fact is frequently observed in single-dose kinetic studies whereas in long-term studies most of the subjects show significant, though highly variable responses. Thus, this discrimination of subjects based on plasma responses has been criticized since a lack of acute plasma response does not necessarily mean absence of absorption.

Studies of bioavailability of carotenoids, however, are difficult for the endogenous presence in plasma and tissues of carotenoids. In most cases, larger doses than those provided by mixed diets need to be supplied in order to observe variations in plasma. To overcome this problem stable iso-

tope-labelled carotenoids are being increasingly used to assess nutrient bioavailability [193]. In this regard, stable isotope labelling can be performed both intrinsically (in growing foods) and extrinsically (single compounds), allowing the study of carotenoid bioavailability (*i.e.* absorption, transport, distribution, storage, excretion, turn-over, …) at dietary levels and regardless of endogenous presence.

These methods, however, have limitations (*i.e.* the perceived health risk and the costs associated with the necessary methodology). Because of these limitations, many studies have been performed using *in vitro* and animal models. Although animal models may provide relevant information with regard to bioavailability in man, no one animal model completely mimics human absorption and metabolism of carotenoids [194]. Extrapolation of these results and their relevance to humans should, therefore, be considered with caution.

In vitro models based on human physiology have been developed as simple, inexpensive and reproducible tools to study digestive stability, micellization, intestinal transport and metabolism and to predict the bioavailability of different food components. *In vitro* models have been used in studies on vitamin and carotenoid absorption mechanisms and, recently, models of *in vitro* digestion, micellarization and uptake by cell culture (Caco-2 cells) have been used as a model to assess carotenoid bioavailability from foods [195]. This approach is useful for studying preabsorptive processes and thus food related factors that affect bioavailability. Nonetheless, some type of standardization is needed and a wider use of these protocols will determine whether they are valid in predicting absorbability and/or bioavailability in humans.

Finally, an interesting alternative to estimate carotenoid bioavailability could be the evaluation of compartmental modelling that allows us to describe the absorption, redistribution and disposal of nutrients in the body [196, 197].

4.3 Tissue culture experiments for cellular uptake and metabolism

Although the intestinal uptake of carotenoids has been thought to occur by simple diffusion [198], recent studies have reported the existence of protein-mediated transport of carotenoids in enterocytes. Studies in Caco-2 cell monolayers indicate [199 – 201] that carotenoids and cholesterol could share common mechanistic pathways across the intestinal cell. In fact ezetimibe (EZ), an inhibitor of cholesterol transport as well as cholesterol itself inhibited (in a concentration-dependent manner) β-carotene transport, but did not affect retinol transport. This suggests that β-carotene and cholesterol interact during their transport through Caco-2 cells, and, therefore, nonpolar carotenoids and cholesterol share one (or more) common transporter(s). The scavenger receptor type B1 (SR-BI) was postulated to play a role in intestinal cholesterol [202, 203], and β-carotene absorption. In a similar manner, the putative proteins involved in the facilitated diffusion of carotenoids are identified in the Niemann-Pick C1Like 1(NPC1L1) and the adenosine triphosphate (ATP)-binding cassette (ABC) A1 transporter.

A similar *in vitro* system was proposed to study lutein absorption. Lutein was added to Caco-2 cell culture and the absorption of lutein was measured. The rate of transport of lutein micelles (lutein mixed with phospholipids, lysophospholipids, cholesterol, monoolein, oleic acid and taurocholate) was time- and concentration-dependent and was inhibited by coincubation with anti-SR-BI antibody and BLT1 (a leukotriene receptor). Coincubation with β-carotene, but not lycopene, decreased the lutein absorption rate (approx. 20%) significantly. These results suggest that lutein absorption is, at least partly, protein-mediated and that some lutein is taken up through SR-BI [204]. Although a binding protein specific to lycopene has not yet been verified, *in vivo* studies in rats suggested that one may exist. This could explain the preferential uptake of 14C-lycopene in some tissues [205].

Once the carotenoid is inside the enterocyte its fate depends on its structure. If the carotenoid contains an unsubstituted β-ionone ring with a polyene side-chain of at least 11 carbon atoms, it can be cleaved enzymatically to vitamin A. This central cleavage pathway, which requires molecular oxygen, is catalysed by the enzyme carotenoid 15,15′-monooxygenase, and yields two molecules of (all-*E*)-retinal from (all-*E*) β-carotene. This enzyme apparently cleaves (9*Z*) β-carotene also, yielding a 1:1 mixture of (all-*E*) and (9*Z*) retinal, which can be further oxidized to (9*Z*) retinoic acid. The (9*Z*) and (all-*E*) isomers of β-carotene can also be interconverted [206].

The second pathway of β-carotene metabolism is the eccentric cleavage, which occurs at double bonds other than the central 15,15′-double bond of the polyene chain of β-carotene to produce β-apo-carotenals with different chain lengths. However, given that only trace amounts of apocarotenals are detected in *in vivo* treatment [207] and that they can be formed nonenzymatically from β-carotene auto-oxidation [208], the existence of this pathway has been the subject of debate. The two major sites of β-carotene conversion in humans are the intestine and liver. By direct determination of β-carotene oxygenase activity in human small intestine and liver samples, it was estimated that in a human adult the maximum capacity for β-carotene cleavage by the two tissues would be 12 mg β-carotene *per* day [209]; this amount is much higher than the observed average daily intake of 1.5 mg *per* day in the United States or even the higher daily intake of 6 mg β-carotene/day suggested by some authors as being needed to meet the goal of 90% of vitamin A intake [210].

Very little is known about cellular events that regulate or facilitate the incorporation of carotenoids into lymphatic lipoproteins. Still unsolved is how the flow of hydrophobic

carotenoids within the enterocyte is controlled. The poor solubility of carotenoids in aqueous solutions suggests the need for a cytosolic binding protein, but to date no specific binding protein for carotenoids in the intestinal mucosa has been reported. Under normal dietary conditions both the retinyl esters formed from carotenoids in the enterocyte and the intact absorbed carotenoids are incorporated into lymphatic chylomicron [211].

4.4 Human studies

The wide presence of carotenoids in foods have attracted the researchers' attention towards human intervention studies. Up till now, many papers have been published in this area and, considering the wide variety of parameters and factors evaluated, it becomes quite difficult to be exhaustive in the description of the so many aspects of carotenoid bioavailability.

In the EPIC study [212], a typical population groups study, the mean of the sum of the six measured carotenoids (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin) varied two-fold between regions in men and women (1.35 μmol/L for men in Malmö *vs*. 2.79 μmol/L for men in Ragusa/Naples; 1.61 μmol/L for women in The Netherlands *vs*. 3.52 μmol/L for women in Ragusa/Naples). Women had higher plasma levels of carotenoids than men, except in the case of lycopene. This is in agreement with data reported earlier [74, 213]. Mean carotenoid levels in plasma, in population groups of several regions, showed broader distributions: Italian regions, Athens and UK vegetarians had the highest lycopene and lutein levels while β-carotene and α-carotene were highest among UK vegetarians and β-cryptoxanthin levels were higher in the Spanish regions [212].

Supplementation studies represent another way to test the carotenoids bioavailability in humans; within a multicentre study, serum responses to carotenoid supplementation (lutein, lycopene or α-carotene + β-carotene) were assessed in a randomized, placebo-controlled intervention study [214]. The trial involved 400 apparently healthy men and women (40 men, 40 women/region) from five European regions (France, Northern Ireland, Republic of Ireland, The Netherlands and Spain) and it was conducted using identical time protocols (16 months), capsule preparations and very similar doses (approx. 15 mg carotenoids), allowing relative comparisons between each carotenoid treatment. In addition, the centralization, randomization and quality control of analysis eliminate interlaboratory analytical bias and improve reliability of the results. Carotenoid supplementation was set at dietary achievable levels and then, the supplement of α- and β-carotene supplied an amount equivalent to that contained in 100 g cooked carrots; lutein amount was similar to that present in 200 g cooked spinach and lycopene was equivalent to that provided by 600 g raw tomato or 100 g tomato paste. Data from this study showed that supplementation with α + β-carotene (carotene-rich palm-oil) resulted in a 14- and 5-fold increase in serum levels respectively. Supplementation with lutein (from marigold extracts) elevated serum lutein (about five-fold), zeaxanthin (about double) and ketocarotenoids (not supplied), whereas lycopene supplementation (derived from tomato paste) resulted in a two-fold increase in serum lycopene. Isomer distribution of β-carotene and lycopene in serum remains constant regardless of the isomer composition in the capsules. In Spanish volunteers, additional data [215] showed that serum response to carotenoid supplementation reached a plateau after 4 wks of supplementation whereas no significant side-effects (except carotenodermia) nor changes in biochemical or haematological indices were observed. The presence of a chromatographic peak (tentatively identified as lutein monopalmitate) was only detected in subjects with relatively high serum lutein levels (>1.05 μmol/L). This peak may be indicative of a ceiling effect on saturation of the transport capacity of lutein, which may be re-esterified *in vivo* when it is supplied in excess of normal dietary intake [214, 215].

A lot of human epidemiological studies suggest a protective effect of diets rich in carotenoids, composed mainly of fruit and vegetables, against cancers at various sites. In contrast, intervention studies with higher concentrations of synthetic β-carotene more available than that in fruit and vegetables, have failed to provide the expected protection [216 – 221]. In addition, β-carotene is an important antioxidant in our daily diet which might be significant for health promoting even if its role for disease prevention is still not clear. Concerning lycopene, a correlation between lycopene derived from tomato products supplementation and risk of prostate cancer, was reported by Basu and Imrhan [222] in a recent review of 20 studies, even if future investigations are required to clarify the lycopene role and its action mechanism.

These results suggest that at present there is still insufficient evidence to advocate the consumption of isolated carotenoids for prevention of several chronic diseases [79, 223 – 227]. In fact, data collected with the same methodology, comparable and representing a large number of population are required to quantify the intake of carotenoids and to represent the consumption of the population.

5 Concluding remarks

Carotenoids are a wide variety of molecules present in the human diet so our review is extensive and covers different aspects. The main dietary sources of carotenoids were reviewed. We have tried to summarize positive and negative effects of food processing, storage, cooking on carotenoid bioavailability. In particular, we have evidenced the possibility to improve carotenoids bioavailability in accordance with changes and variations of technology procedures.

We focused our attention on several factors influencing carotenoid accumulation and bioavailability and on the potential health properties and possible biological role of these phytochemicals in human physiology.

The metabolism, absorption and excretion of carotenoids have been studied extensively *in vitro*, in animal models and in humans.

Although a lot of literature data are available for the design and interpretation of intervention studies [228, 229], further investigations are required to understand the absorption and metabolism pathways and the action mechanism of carotenoids in humans. From this point of view, this paper could be a useful updated knowledge for both expert and not expert readers. It also highlights the need for further research with appropriate approaches (*i.e.* dietary intake evaluation, development and update of a carotenoid database for different countries).

The authors are grateful to Editorial Office of British Journal of Nutrition to reuse the Tables 3, 4, 6 and 7.

The authors have declared no conflict of interest.

6 References

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