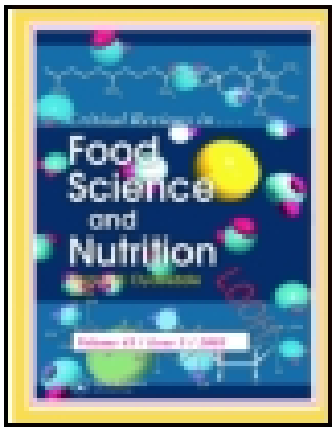

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Carotenoid Deposition in Plant And Animal Foods and Its Impact on Bioavailability

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Accepted author version posted online: 26 Jun 2015.

CAROTENOID DEPOSITION IN PLANT AND ANIMAL FOODS AND ITS IMPACT ON BIOAVAILABILITY

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ABSTRACT

Over the past decades, an enormous body of literature dealing with the natural deposition of carotenoids in plant- and animal-based foods has accumulated. Prominent examples are the large solid-crystalline aggregates in carrots and tomatoes or the lipid-dissolved forms in dairy products and egg yolk. Latest research has identified lipid-dissolved forms in a rare number of plant foods, such as tangerine tomatoes and peach palm fruit (*Bactris gasipaes* Kunth). In addition, liquid-crystalline forms were assumed in so-called tubular chromoplasts of numerous fruits, e.g., in papaya, mango, and bell pepper. The bioavailability of carotenoids from fresh and processed foods strongly depends on their genuine deposition form, since their effective absorption to the human organism requires their liberation from the food matrix and subsequent solubilization into mixed micelles in the small intestine. Consequently, a broad overview about the natural array of carotenoid deposition forms should be helpful to better understand and modulate their bioavailability from foods. Furthermore, naturally highly bioavailable forms may provide

biomimetic models for the improved formulation of carotenoids in food supplements. Therefore, this review article presents scientific evidence from human intervention studies associating carotenoid deposition forms with their bioavailability, thus suggesting novel technological and dietary strategies for their enhanced absorption.

Keywords: absorption, vitamin A, antioxidants, crystalline, liquid-crystalline, lipid-dissolved, protein-complex, health, nutrition.

1 Introduction

The consumption of carotenoid-rich foods of animal and plant origin has been associated with numerous health benefits. The most prominent bioactivity of carotenoids is the metabolic conversion of some representatives called provitamin A carotenoids to vitamin A (Von Lintig, 2010, Harrison, 2012). This vitamin is of essential importance for the human and mammalian visual system as well as for maintaining growth and soundness of cells. Although being recognized as a worldwide nutritional problem for many decades, vitamin A deficiency and its severe implications to human health still represent a major issue today. This nutrition-related and thus preventable deficiency has been called "Hidden Hunger" (Biesalski, 2013), since low vitamin A blood levels often remain undetected for a long time due to lacking obvious symptoms at early stages of deficiency. Later, specific alarming diseases like xerophthalmia, anemia, and night blindness are important indicators preceding most severe implications like dysplasia and death. An increased morbidity and mortality from infections is observed among vitamin A-deficient pregnant women, infants, and young children of the poorer populations of developing countries. Animal-based food rich in preformed and highly bioavailable vitamin A is often too expensive or simply unavailable in many affected countries. For instance, in Africa and Asia, up to 85% of dietary vitamin A comes from plant sources containing provitamin A carotenoids. However, provitamin A from many fruits and vegetables often cannot fully compensate this lack in dietary preformed vitamin A, particularly when their intake is low and dietary measures for enhancing their low bioavailability are not taken (WHO, 2009, FAO/WHO, 2002, Veda et al., 2007, Institute of Medicine, 2001, Biesalski, 2013). In this review, we highlight the striking difference in the deposition forms of carotenoids and vitamin A in animal and plant foods,

representing an inherent key factor of their bioavailability. Knowledge about the deposition in some plant foods is a prerequisite for understanding and enhancing the low bioavailability of provitamin A as well as for the identification of highly bioavailable sources.

Beyond vitamin A supply, the consumption of carotenoid-rich fruits and vegetables was related to numerous further health benefits. A protective effect of carotenoids against the development of sun-induced erythema (Lee et al., 2000, Stahl et al., 2000, Heinrich et al., 2003, Goralczyk and Wertz, 2009), premature skin aging (Goralczyk and Wertz, 2009), and beneficial effects on the functionality of the human immune system have been shown in several human trials (Chew and Park, 2009). In the early 1980's, first key publications dealing with the strong antioxidant properties of carotenoids like β-carotene have postulated a potential relationship of carotenoid intake and the prevention of chronic diseases like cardiovascular disease and cancer (Britton et al., 2009b). Since then, several epidemiological studies suggested inverse relationships between lung, breast, prostate, and colorectal cancer risks and daily intake or serum concentrations of carotenoids (Giovannucci, 1999, Schalch et al., 2009, Britton and Khachik, 2009). For instance, the consumption of lycopene-rich foods like tomatoes and tomato-based foods was associated with a 30 to 40% reduction in risk for prostate cancer (Giovannucci, 1999), although more recent studies questioned the assumed causal association to lycopene (Wei and Giovannucci, 2012). A most recent epidemiological trial with ca. 50.000 male participants related the dietary intake of lycopene with reduced risk of lethal prostate cancer, also discussing the controversial findings of past studies (Zu et al., 2014). A carotenoid-rich diet may also be helpful for the prevention of coronary heart disease, but, to date, results of corresponding studies are still inconsistent (Johnson and Krinsky, 2009). Another potentially important target organ of carotenoids is the

human eye, where lutein and zeaxanthin specifically accumulate in the *Macula lutea*. It is widely accepted that the intake of these two carotenoids is essential for the functioning of the visual system. However, a significant reduction of the risk in age-related macular degeneration (AMD) and cataract by a higher intake of lutein and zeaxanthin appears likely, but is subject to an ongoing scientific discussion (Schalch et al., 2009).

Prior to exerting any of the above-mentioned or yet unknown potential health effects, carotenoids need to be released from the food matrix, solubilized, micellized, and then absorbed to the blood stream during digestion. The effectiveness of this process depends on numerous factors (Canene-Adams and Erdman Jr., 2009, Castenmiller and West, 1998, Yeum and Russell, 2002). When comparing carotenoid bioavailability from different foods, a most important inherent factor is their highly variable deposition form within in the food matrix. Astonishing differences were observed in and among plant- and animal-based foods, such as, e.g., the large solid-crystalline aggregates in tomato and carrot or lipid-dissolved forms in most animal foods and some rare plant foods. Extensive knowledge about these deposition forms has accumulated over the past decades, and a broad overview might be helpful for developing strategies to modulate carotenoid bioavailability from foods. Furthermore, this knowledge may be useful for the formulation of most effective food supplements. Therefore, after compiling available information on the substantially variable deposition forms in plant and animal foods, scientific evidence was extracted from human intervention studies that related the deposition form of carotenoids with their bioavailability, including technological and dietary measures for it’s improvement.

2 Carotenoid deposition and accumulation in plants and animals

2.1 Brief overview on carotenoid biosynthesis

Understanding the accumulation and deposition of carotenoids in biological tissues requires a brief introduction into their biosynthesis. Humans and animals are unable to biosynthesize carotenoids *de novo,* but derive them from their food and feed, respectively. Biosynthetic organisms, among them higher plants, algae, fungi, and bacteria, annually produce approximately 10 million tons of carotenoids (Britton et al., 1998).

In higher plants, carotenoid biosynthesis takes place in plastids starting from isopentenyl diphosphate (IDP). The mevalonic (MVA) pathway and an MVA-independent pathway have been well-characterized for the formation of IDP, and the latter is considered the major route to carotenoids in higher plants (Britton, 1998). Four IDP (C_5) units are merged in various head-to-tail condensation reactions, yielding the immediate C_{20} precursor of carotenoids, i. e. geranylgeranyl pyrophosphate (GGPP). A head-to-head condensation of two molecules GGPP generates the colorless phytoene (7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene). Consecutive enzymatically-catalyzed dehydration reactions lead from phytoene to phytofluene, ζ-carotene, neurosporene, and finally to lycopene. Subsequent cyclization of one or two of lycopene's terminal ψ-groups results in a variety of mono- and bicyclic carotenoids. Bicyclic carotenoids with β- and ε-groups (Figure 1) are found in higher plants, such as α- and β-carotene (β,ε-carotene and β,β-carotene, respectively). Such pure hydrocarbon carotenoids are commonly called "carotenes", whereas carotenoids containing one or several oxygen functions are known as "xanthophylls". Hydroxyl groups are introduced by specific subsequent reactions catalyzed by ε-ring and β-ring hydroxylases. For instance, hydroxylation at position 3 of β,β-carotene leads to 3-hydroxy-β,β-carotene, also known as β-cryptoxanthin (Sandmann, 1994, Britton, 1998, Fraser

and Bramley, 2004). In plant tissues, hydroxylated carotenoids like β-cryptoxanthin, lutein, and zeaxanthin are frequently observed as fatty acid esters. Although scientific evidence is lacking, the formation of carotenol esters from the carotenoid and an activated fatty acid (acyl-coenzyme A) is most likely catalyzed by esterases (Britton, 1998). Keto-carotenoids are found less often in higher plants, but prominent examples are bell pepper and sapote (*Capsicum annuum* L. and *Sapota pouteria* (Jacq.) H. E. Moore & Stearn, resp. (Murillo et al., 2011, Schweiggert et al., 2005)). They are derived from oxygenation reactions catalyzed by carotene ketolases, such as the β-(C4)-oxygenase. The cDNA of this enzyme has been cloned from green algae (Britton, 1998), where keto-carotenoids like astaxanthin are more frequently encountered. For instance, the green microalgae *Haematococcus pluvialis* is used for commercial astaxanthin production (Li et al., 2011). Some examples of nutritionally-relevant and commonly encountered carotenoids in plant and animal foods are displayed in Figure 2.

2.2 Green plant foods

In order to understand the compositional similarity and the bioavailability of carotenoids from green plant tissues, knowledge about their localization and biological functions is a prerequisite. While chlorophylls in the chloroplasts (from ancient Greek *chloros* = green) impart the green color to plants, they mask high amounts of concomitant carotenoids. Not until the chlorophylls of autumn foliage are degraded, the bright orange and yellow colors of the previously “hidden” carotenoids become visible. Nevertheless, carotenoids are most important for the functioning of the light harvesting photosystems. After absorption of radiation in the high energy range of the visible electromagnetic spectrum (400-500 nm), they are able to pass their excitation energy on

to the chlorophylls. Thus, carotenoids extend the wavelength range of light that can be utilized for the light reaction of photosynthesis. In addition, carotenoids play an important role for the photoprotection of the photosynthetic apparatus by quenching reactive chlorophyll states, scavenging reactive oxygen species, and converting excess energy into harmless heat (Britton, 2008, Telfer et al., 2008). In order to fulfill these tasks, carotenoids need to be localized in the reaction center of protein complexes, which drive the primary light reaction of photosynthesis. These reaction centers are located at specific internal chloroplast membranes, the so-called thylakoids. In the extensively studied photosystem II (PSII), the reaction centers are surrounded by light harvesting (or *syn.* antenna) complexes, containing most of the sunlight-absorbing pigments of the entire photosystem. They contain the chlorophylls *a* and *b* as well as the carotenoids lutein, violaxanthin, and neoxanthin. The harvested energy is first transferred from the carotenoids to the chlorophylls, from chlorophyll *b* to *a*, and from a network of connected chlorophyll *a* molecules to the reaction center complexes (Croce and van Amerongen, 2011). The proposed structure of the PSII reaction center from plants is remarkably similar to that of cyanobacteria, containing several molecules of chlorophylls *a*, pheophytins *a*, β-carotenes, and other co-factors per monomer (Croce and van Amerongen, 2011). Since the chloroplasts and photosystems of all higher plants share the same evolutionary cyanobacterial origin, the composition of the described pigment-protein complexes is widely similar in edible green tissues of higher land plants as well. Approximately one carotenoid molecule per 3-4 chlorophylls is found (Telfer et al., 2008), and the profile and relative proportions of the main carotenoids are astonishingly similar, being composed of 20-25% β-carotene, 40-45% lutein, 10-15% violaxanthin, and 10-15% neoxanthin (Britton, 2008). Despite conserved concentration ratios,

their total carotenoid content is highly variable. High total carotenoid levels are directly related to a higher density of chloroplasts and, hence, green plant foods with high carotenoid concentrations (> 2 mg/100 g of FW) are consequently of a dark-green color, such as kale, broccoli, spinach, and other dark-green leafy vegetables (Britton and Khachik, 2009).

A transmission electron micrograph of a typical chloroplast (from green tomato fruit tissue) is shown in Figure 3. Besides the above-mentioned thylakoids, which contain the antenna complexes and reactions centers of the photosystems, further carotenoid-bearing structures are the so-called plastoglobules (Figure 3, pg). Plastoglobules represent a lipid-rich environment for the biosynthesis and deposition of many lipophilic constituents such as carotenoids, chlorophylls, and tocopherols. Previously, the plastoglobules were shown to be closely attached to the thylakoids, and an extensive exchange of lipophilic compounds between them was proposed (Austin et al., 2006). A schematic representation of a chloroplast is shown in Figure 4A.

Although most green plant foods are leafy vegetables, some fruits with green mesocarp are consumed, such as kiwi fruit (*Actinidia* spp.) and avocado (*Persea americana* Mill.). As expected, immature green kiwi fruits contain typical chloroplasts similar to those of leaves. Fruits of the kiwi species *A. deliciosa* Liang & Ferguson retain their green color and typical chloroplasts until full maturity and harvest. Consequently, major carotenoids in green kiwi fruit were found to be typical chloroplast-pigments like lutein, violaxanthin, neoxanthin, and β-carotene (Montefiori et al., 2009). In contrast to *A. deliciosa*, many kiwi genotypes of *A. chinensis* Planch. change their flesh color to yellow at full maturity. In these fruits, the thylakoids and grana of the chloroplasts disappear, and chlorophylls and chloroplast-specific carotenoids are simultaneously degraded. At the same time, esterified carotenoids and globular chromoplasts

appear and, consequently, fruit color changes from green to yellow (Montefiori et al., 2009). The conversion of chloroplasts to a broad variety of morphologically and compositionally different chromoplasts during fruit ripening is well described in numerous other fruits and vegetables, such as, e.g., bell pepper (Camara and Brangeon, 1981, Spurr and Harris, 1968) and tomato (Harris and Spurr, 1969b). The resulting chromoplast types are described in detail below (Section 2.3). The ripening-induced transition from chloroplast to chromoplast was reported to be altered in some geno- and phenotypes of pepper and tomato, yielding "stay-green" fruits of a brownish red color. In the chromoplasts of these fruits, both chromoplastidal elements appear and co-exist next to typical chloroplastidal elements. The resulting plastid was called a "chlorochromoplast". Interestingly, the carotenoid profile is dominated by those carotenoids present in the red-fleshed fruits, lacking chloroplast-specific carotenoids like lutein (Roca et al., 2006, Barry et al., 2008, Hornero-Méndez et al., 2002).

An interesting example of carotenoid deposition in green plant tissues is the edible mesocarp of avocado fruits. Due to its high lipid content of ca. 20% on fresh weight basis (Platt-Aloia and Thomson, 1981), a lipid-dissolved form would be expected. However, most of the fruit's lipids occur in droplets and lipid bodies in the cytoplasm of parenchyma cells, i.e. outside the plastids and, thus, well separated from the carotenoids. Two different plastid types were found in avocado fruits. The outer green layers of the ripe flesh contain typical chloroplasts with thylakoids and large grana stacks, while the plastids of the inner yellow part are devoid of thylakoids and grana, instead containing so-called prolamellar bodies (Platt-Aloia and Thomson, 1981). The latter consist of "a uniformly curved lattice of tubular membranes" (Park et al., 2002) and represent a typical element of so-called etioplasts (Figure 4B). Etioplasts (from French

étioler = to fade away) are commonly found in plants grown in the dark or kept out of light, which then have a pale yellowish color. When exposed to light, etioplasts rapidly convert into chloroplasts, and are therefore considered to be precursors of chloroplasts (Figure 4A). Thylakoids are assumed to emerge from prolamellar bodies due to microscopic observations and their thylakoid-like biochemical composition, containing lutein, violaxanthin, and protochlorophyllides. Consequently, the green outer and the yellow inner layers of avocado both contain chloroplast-specific carotenoids like lutein and β-carotene, although substantially higher concentrations are present in the outer green parts (Ashton et al., 2006).

In brief summary, carotenoid molecules within green plant tissues are deeply embedded into several hierarchical substructures. They are located in different and highly functional pigment-protein super-complexes, which themselves are embedded in the single thylakoid. The thylakoid is part of a grana stack, i.e. an association of multiple thylakoids. Multiple grana stacks are located inside a single chloroplast. Numerous chloroplasts are found in the cytoplasm of the plant cell, being surrounded by often rigid and stable cell walls. Consequently, the bioavailability of carotenoids deposited in such deeply embedded protein-complexes is expectedly poor as discussed below.

2.3 Yellow, orange, and red carotenoid-rich plant foods

Common carotenoid profiles

In contrast to the conserved carotenoid pattern of green plant tissues, the qualitative carotenoid composition widely varies in yellow, orange, and red colored plant parts. Noteworthy, these colors in plants are not exclusively imparted by carotenoids, as anthocyanins, betalains, and other

plant pigments may also be present. Despite the enormous variety of different carotenoids in non-photosynthetic (non-green) plant tissues, Britton and Khachik (2009) roughly related five distinctive compound pattern to the color of the plant tissue*: "1. large amounts of the acyclic carotene lycopene, as in tomatoes (red color); 2. large amounts of β-carotene and/or its hydroxyl derivatives β-cryptoxanthin and zeaxanthin (orange color); 3. as 2. but with also α-carotene and/or its hydroxyl derivatives, especially lutein (yellow-orange color); 4. large amounts of carotenoid epoxides (yellow color); and 5. carotenoids that appear to be unique to or characteristic of that species (yellow, orange, or red color), e.g. capsanthin and capsorubin in red peppers (Capsicum annum L.)."* (Britton and Khachik, 2009).

However, the carotenoid composition and, thus, the color of a certain plant organ may vary even within one plant species. For instance, both red- and yellow-fleshed papaya fruits (*Carica papaya* L.) with different carotenoid profiles are available. In agreement with the above-mentioned classification, red-fleshed types contain high amounts of lycopene, whereas β-cryptoxanthin, β-carotene, and carotenoid epoxides are predominant in yellow-fleshed fruits (Schweiggert et al., 2012b). Such differently-colored genotypes were also described for tomato (Table 1), containing either lycopene (*red tomato*), β-carotene (*orange-coloured*), δ-carotene (*orange-coloured*), (*Z*)-lycopenes (*orange-coloured*), or lutein (*yellow*) as predominant pigment (Nguyen et al., 2001). Besides fruits, some roots such as carrots and sweet potatoes contain high amounts of carotenoids. In orange carrots, α- and β-carotene consistently represent the major carotenoids, although different varieties considerably vary regarding their α-carotene proportion (5-50% of total carotenoids). By analogy to the above-mentioned tomatoes, high amounts of

lycopene and lutein were observed in red and yellow carrots, respectively (Britton and Khachik, 2009).

Britton and Khachik (2009) ranked carotenoid-rich sources according to their absolute carotenoid content in order to highlight their nutritional value, also providing a detailed food list of nutritionally important carotenoids. A concentration of 0-0.1 mg/100 g of FW is considered "low", 0.1-0.5 mg/100 g "moderate", 0.5-2 mg/100 g "high", and >2 mg/100 g "very high". This classification was applied in Table 1 for providing details about the carotenoid composition in a large variety of plant foods. However, this classification does not consider the highly variable deposition forms of the carotenoids, yet disregarding the extreme differences in carotenoid bioavailability from the different food matrices.

General aspects about chromoplasts

By analogy to carotenoid accumulation in chloroplasts of green plant tissues, carotenoids of red, orange, and yellow fruits and vegetables are also biosynthesized and deposited in a particular plastid type, so-called chromoplasts (gr. *chromos* = color). However, while chloroplast morphology is highly conserved, several subtypes of chromoplasts have been identified according to specific ultrastructural elements visualized by transmission electron microscopy (TEM). In general, all plastids are characterized by an outer envelope consisting of two separate membranes, an internal stroma matrix containing small ribosomes, and regions with slender, uranophilic filaments, presumably DNA. Plastids with a widely undifferentiated stroma and no other predominant ultrastructural elements are called proplastids (Figure 4C), since they are considered to be precursors of more complex plastids like, e.g., chromo- and chloroplasts.

Chromoplasts are subdivided into globular, tubular, membranous, or crystalloid types according to their predominant structural stroma elements (i. e. globules, tubules, membranes, or crystalloids). This classification has been introduced by the German botanist Sitte and co-workers in 1980 (Sitte et al., 1980), still being valid today. The mentioned chromoplast types are illustrated in Figures 4C-G and will be described in detail below.

Globular chromoplasts

Globular chromoplasts (Figure 4D) are lens-shaped or spheroidal and their stroma contains small lipid droplets (plastoglobules). Plastoglobule size greatly varies from 30 nm to more than one micrometer (Sitte et al., 1980). As illustrated in Figure 5A, they are composed of a core of neutral lipids such as triglycerides, surrounded by a monolayer of polar glyco- and phospholipids and proteins (Bréhélin and Kessler, 2008). While apolar carotenoids are dissolved in the lipophilic core (Sitte et al., 1980), more polar xanthophylls are located within the half-membrane of the globule (Figure 5A). Borel et al. (1996) reported the preferential localization of the xanthophyll zeaxanthin within the bipolar membrane of biological emulsion droplets, while β-carotene was shown to be located in the lipophilic core of the droplet. Globular chromoplasts are a common type of chromoplasts encountered in yellow, orange, and red colored petals and tepals of numerous plant species, such as forsythias (*Forsythia* sp.), tulips (*Tulipa* sp.), wallflower (*Erysimum cheirii* (L.) Crantz), and marigold (*Tagetes erecta* L.). They were also described in the exceptionally glossy petals of *Ranunculus* sp., the common buttercup (Weston and Pyke, 1999, Brett and Sommerard, 1986, Sitte et al., 1980).

In edible carotenoid-rich plant tissues, the sole presence of globular chromoplasts is less abundant. Hempel et al. (2014) recently reported an exclusive type of globular chromoplast in the edible mesocarp of peach palm (*Bactris gasipaes* Kunth), containing very high amounts of lipid-dissolved β-carotene, lycopene, and γ-carotene (up to 14 mg/100 g FW). At the same time, a high amount of lipid, being sufficient for dissolving the contained carotenoids, was found in the fruits. Cooperstone et al. (2015) recently found an interesting type of globular chromoplast in fruits of an orange-colored tangerine tomato. This tomato variety accumulated high amounts of apparently lipid-dissolved carotenoids, although it only contains low amounts of lipids for their dissolution. However, its major carotenoids were lycopene (*Z*)-isomers, such as 7,9,7′,9′-tetra-(*Z*)-lycopene (Cooperstone et al., 2015). Such geometrically tilted carotenoid (*Z*)-isomers were previously reported to hardly crystallize and even to form an oil in concentrated form (Ben-Amotz and Avron, 1990), being present in small lipid droplets within the tangerine tomato chromoplasts (Cooperstone et al., 2015). By analogy, Ben-Amotz and Avron (1990) reported high concentrations of dissolved (9*Z*)-β-carotene and (*all-E*)-β-carotene in small oil droplets in the microalgae *Dunaliella*. According to their report, the oily (9*Z*)-β-carotene itself might support the lipid-dissolved rather than a crystalline physical deposition state of (*all-E*)-β-carotene (Ben-Amotz and Avron, 1990).

Noteworthy, globular plastids are not necessarily colored chromoplasts, since white flowers being devoid of carotenoids were also reported to contain numerous globular plastids, which then are named leucoplasts (gr. *leukos* = colorless or white, (Pyke and Page, 1998)). Therefore, TEM micrographs always need to be complemented by visual and light microscopic observations in order to prove the colored or colorless appearance of the observed plastids.

Tubular chromoplasts

Tubular chromoplasts are characterized by internal elements of an elongated tube-shaped appearance (Figure 4E), exerting an extraordinary dichroism and positive birefringence. These highly anisotropic elements are called "tubules" in most scientific reports. They are commonly of a diameter of 20-60 nm, a length of up to 10 µm, and branched and unbranched variations were found (Sitte et al., 1980). Frequently, numerous tubules are aligned to bundles. In transverse sections, the outer tubule layer reveals a cylindrical boundary heavily contrasted by OsO_4^- or MnO_4^-, while the tubule core appears less electron-dense. After isolation and chemical analysis, Winkenbach et al. (1976) proposed a model for the fine structure of the tubules (Figure 5B). The core of the tubules is believed to contain a nematic liquid-crystalline carotenoid phase, being surrounded by a monolayer of bipolar glyco- and phospholipids and proteins (Knoth et al., 1986, Sitte, 1981). As early as in 1981, the lyotropic self-assembly of specific carotenoids was hypothesized to yield nematic mesophases identical to those of chromoplast tubules (Sitte, 1981). Indeed, Sitte's group successfully observed the lyotropic *in vitro* self-assembly of lutein diacetate into highly birefringent and thread-like structures, presumably representing the postulated nematic liquid crystals in the core of chromoplast tubules. Years later, in 2001, Zsila et al. (2001) studied these most interesting carotenoid aggregates in living petals of different plant species by UV/Vis and circular dichroism (CD) spectroscopy. Complementing the earlier hypothesis of Sitte (1981), the contained carotenoids were shown to represent supramolecular chiral aggregates of the J-type (J for Jelly, one of the pioneers in this field). In general, UV/Vis absorption spectra of J-aggregates exhibit an intense narrow bathochromic absorption band. At

the same time, the bands of the monomers are widely preserved (Köhn et al., 2008). Since both living tubular chromoplasts as well as the above-described liquid crystalline phase of lutein diacetate exhibited such absorption bands (Zsila et al., 2001), their molecular arrangement is believed to be highly similar and of the J-type, in which carotenoids are arranged in a loose head-to-tail manner of the brickwork type. The resulting supramolecular structure of these aggregates represents a nematic liquid-crystalline mesophase (Köhn et al., 2008) present within the chromoplast tubules (Figure 5B). In most tubular chromoplasts, tubules are closely associated to plastoglobules and, thus, the resulting intermediate type of chromoplast is sometimes called globular-tubular. Tubular or globular-tubular chromoplasts were previously found in numerous flowers and fruits, e.g. loquat (*Eriobotrya japonica* (Thunb.) Lindl.), mango (*Mangifera indica* L.), bell pepper (*Capsicum annum* L.), physalis (*Physalis pubescens* L. and *Physalis peruviana* L.), rowan berries (*Sorbus aucuparia* L.), rose hips (*Rosa rugosa* Thunb.), yellow-fleshed papaya, and other fruits shown in Table 1 (Vásquez-Caicedo et al., 2006, Sitte et al., 1980, Schweiggert et al., 2011, Fu et al., 2012).

Membranous chromoplasts

Membranous chromoplasts are characterized by up to 20 concentric internal double membranes (Figure 4F), and their shape is commonly spherical or ovoid. Such chromoplasts are apparently unique of flowers belonging to certain plant families, such as Amaryllidaceae (e.g., daffodil (*Narcissus* sp.)). Specific cultivars of *Capsicum annum* L. and the Golden Jubilee cultivar of yellow-fleshed tomato were also reported to contain this rare type of chromoplast (Sitte et al., 1980). A schematic illustration is depicted in Figure 4F.

Crystalloid chromoplasts

Crystalloid chromoplasts represent the fourth archetype of chromoplast (Figure 4G). Most prominent examples are the β-carotene- and lycopene-rich chromoplasts of orange carrot roots and red tomato fruits, respectively. The growing crystals often heavily distort the shape of the chromoplast, particularly when high concentrations of carotenoids are present. In many cases, the carotenoid crystals are visible by light microscopy, and impressive ribbon-like, needle-shaped, or helical forms were observed (Straus, 1961, Straus, 1950, Frey-Wyssling and Schwegler, 1965, Ben-Shaul and Naftali, 1969, Sitte et al., 1980). Submicroscopic crystallites are also known, e.g. in some *Physalis* cultivars (Sitte et al., 1980). In TEM, ordinary glutardialdehyde-OsO_4 preparation does not lead to a sufficient fixation of large carotenoid crystals. Since they do not withstand the dehydration process with acetone or ethanol, empty spaces of a white appearance (so-called crystal remnants) with internal undulating membranes, which formerly surrounded the crystal, are left over (Harris and Spurr, 1969b). A schematic example is shown in Figure 4G. Using an alternative glutardialdehyde-$KMnO_4$ fixation, the crystals appear highly electron dense without undulated structures, although then being hardly distinguishable from other plastidal elements such as thylakoid membranes in some cases. Further information about both fixation methods regarding carotenoid crystals was reported previously (Harris and Spurr, 1969b).

Surprisingly, there was much discussion about the exact physico-chemical deposition form of carotenoids in crystalloid chromoplasts in the past. In the early 1880s, many botanists were fascinated by the large anisotropic crystals in orange carrot roots and assumed them to be pure

carotene crystals without or with only very little remaining plastid substance. Later in the 1950-60s, light and electron microscopic investigations doubted this hypothesis and clearly showed that the crystalloid elements occurred within the intact double-membrane boundary of the chromoplast. Furthermore, in contrast to the assumption of "pure carotene crystals", major proportions of the carotenoids were hypothesized to be bound to repeated lamellar sheets of lipoproteins, suggesting the presence of crystalline lipochromoproteins causing the high birefringence (Frey-Wyssling and Schwegler, 1965, Straus, 1950, Straus, 1961). Following this hypothesis, several carotenoid-binding proteins were isolated and characterized from carrot roots in the early 1990s (Zhou et al., 1994, Bryant et al., 1992, Milicua et al., 1991), finally identifying the major carotenoid-carrying protein complex of about 18 kDa in carrot chromoplasts (Zhou et al., 1994). However, the evaluation of carotenoid and protein concentrations in both this complex and the whole chromoplast revealed that only minor amounts of the total carrot carotenoids were bound to protein-complexes. Consequently, the hypothesis of a major carotenoid deposition in the form of a lipochromoprotein was disproven (Zhou et al., 1994). Therefore, to date, the carotenoids in carrot chromoplasts and other crystalloid chromoplasts are believed to be deposited as relatively pure, solid-crystalline aggregates within the chromoplasts. The carotenoid crystal is considered to be surrounded by a membrane of bipolar lipids and proteins (Reiter et al., 2003), forming a crystalloid. These might occur as single crystalloids containing a bulk carotene crystal or as stacks of multiple crystalloids. Therefore, the above-mentioned "sheets of lipoprotein" as observed by (Frey-Wyssling and Schwegler, 1965) must be considered to represent stacks of such crystalloids, containing crystalline β-carotene instead of a crystalline carotenoid-protein complex. While the origin of the membrane that covers the crystals in carrot

is still unknown, Harris and Spurr (1969b) developed a hypothesis for red tomato chromoplasts. In green tomato fruits, the predominant plastids represent typical chloroplasts. Upon "colour break" during ripening, the degradation of chloroplast-specific internal structures is observed and, at the same time, lycopene deposition was shown to initiate within single and stacked thylakoids (grana). Lycopene crystals as well as the associated thylakoid membrane subsequently increase in length, while yet "*unknown forces*" between these membranes keep individual crystalloids together (Harris and Spurr, 1969b). The resulting multiple stacked crystalloids are highly similar to the above-described crystalloids in carrot (Figure 5C). However, the origin of the crystalloids in carrot must be different to those in tomato due to the lack of chloroplasts in the "unripe" colorless root. Carrot chromoplasts are assumed to originate from proplastids and amyloplasts (Frey-Wyssling and Schwegler, 1965), both of which are devoid of thylakoids. The center-to-center distance between the crystalloids in tomato was estimated to be 15 nm, while this distance was ca. 20 nm in carrot, indicating that these crystalloids are not identical (Harris and Spurr, 1969b). In addition to such stacked representatives, individual crystalloids might accumulate a bulk amount of carotenoid, then being called an "*irregular crystalloid*" (Harris and Spurr, 1969b). A significant amount of the total carotenoids might be stored in such single large crystals, since Ben-Shaul and Naftali (1969) showed that the "lycopene bodies" isolated from tomato revealed electron diffraction patterns characteristic of a large single pigment crystal.

Recently, Schweiggert et al. (2011) investigated the formation of crystalloid chromoplasts during the maturation of red-fleshed papaya fruits. In such papayas, crystalloid chromoplasts were observed when flesh color turned from white to orange, i.e. when carotenoid biosynthesis commenced. Many chromoplasts additionally contained plastoglobules and tubular elements as

described above. In yellow-fleshed papayas, chromoplasts were devoid of such crystalloids, but plastoglobules and tubules were present. Schweiggert et al. (2011) ascribed these observations to differences in the carotenoid profile, which was highly similar in both red- and yellow-fleshed types, except for the presence of high amounts of lycopene in the red type. Thus, the crystalloid elements in red-fleshed papaya are believed to contain crystalline lycopene. The formation of crystalloids was therefore suggested to highly depend on the accumulated pigment type (Schweiggert et al., 2011). Supporting this hypothesis, Cooperstone et al. (2015) observed globular chromoplasts in an orange-colored tangerine tomato, while typical crystalloid chromoplasts were observed in a red-fleshed variety. The major pigment in red tomato fruits was (*all-E*)-lycopene, while tangerine tomatoes contained several (*Z*)-isomers of lycopene, including the predominant (7*Z*,9*Z*,7′*Z*,9′*Z*)-lycopene, sometimes called prolycopene or tetra-*cis*-lycopene. As shown in Table 1, (*all-E*)-lycopene is consistently stored in crystalline form in fruits and vegetables, e.g., in watermelon (Sitte et al., 1980), red grapefruit (Purcell et al., 1963), Cara Cara oranges (Kalkan, 2013), red-fleshed papaya (Schweiggert et al., 2011), and the non-edible fruits of *Aglaonema commutatum* Schott (Knoth, 1981).

The molecular arrangement of crystalline carotenoids within these crystalloids has been scarcely studied. Marx et al. (2003) isolated crystalloid chromoplasts from carrot juice and identified their UV/Vis spectrum to be characteristic of solid-crystalline dispersions of α- and β-carotene, including a strong absorption band at ca. 535 nm, typical of J-type aggregates. Therefore, α- and β-carotene in carrots were hypothesized to be arranged in a head-to-tail manner according to the J-aggregate type (Marx et al., 2003). Unexpectedly, this head-to-tail arrangement is similar to that described for liquid-crystalline carotenoid esters in chromoplast tubules (See above).

Although studies are lacking, chromoplastidal lycopene is assumed to be deposited in an aggregate different to the J-type. While the methylated cyclohexene end groups of β-carotene and carotenoid esters are slightly twisted against the plane of the polyene chain, the lycopene molecule is fully planar and lacks this steric hindrance to aggregation due to its open-chain end groups, favoring strong π,π–stacking interactions of the polyene chains. This structural feature not only causes the poor solubility of lycopene, but also allows the formation of so-called H-aggregates. In this aggregate type, carotenoids are arranged in a tight card-pack manner (Figure 5C). Their UV/Vis absorption spectra exhibit a strong hypsochromic shift (H-aggregate for hypsochromic), while the specific absorption bands of the monomer almost completely disappear. Besides π,π stacking interactions, other "adhesive" factors like hydrogen bonds also foster the formation of H-aggregates. For instance, the dihydroxy carotenoids lutein and zeaxanthin were shown to form typical H-aggregates with parallel-oriented and closely packed molecules (distance of two lutein molecules: 5.5 Å). When the pH was increased and the consequently deprotonated hydroxyl groups were unable to form hydrogen bonds, less closely packed head-to-tail aggregates of the J-type were formed (Billsten et al., 2005, Köhn et al., 2008). Further information about carotenoid aggregates was reviewed recently (Köhn et al., 2008).

As described above, carotenoids are deposited in four different types of chromoplastidal elements. However, in many chromoplasts, more than just one type of pigment-bearing element is present. Particularly, plastoglobules represent typical elements found in all types of plastids, consequently also occurring in tubular, membranous, and crystalloid chromoplasts (Rosso, 1968,

Harris and Spurr, 1969a, Sitte et al., 1980). Crystalloid or tubular chromoplasts may contain membranous structures and, in certain cases, chromoplast classification based on fine structure becomes arbitrary. Besides the exact assignment of plant species and genotype, the investigated plant organ and the respective ripening stage should also be specified in detail, since different types of chromoplasts may occur in different tissues and different ripening stages of a plant. Regarding the plant organ, for instance, the chromoplasts of tomato fruits are crystalloid, while those of the flower petals are globular (Sitte et al., 1980). In brief conclusion, carotenoid deposition in yellow, orange, and red plant organs may substantially differ, and implications on their bioavailability are to be expected as described below.

Chromoplast ontogeny

Chromoplasts are believed to develop from other plastid types, such as proplastids, chloroplasts, and amyloplasts. Proplastids with a widely undifferentiated stroma were frequently found in white-fleshed tissues of unripe fruits, for example in unripe papaya fruits and berries of *Palisota barteri* Hook. When fruit flesh color turns yellow, orange or red during fruit maturation, progressing accumulation of carotenoid-bearing elements in the stroma is observed, leading to the typical chromoplasts of the ripe colored tissue (Knoth et al., 1986, Schweiggert et al., 2011). Besides proplastids, colorless globular leucoplasts were often concomitantly found in white plant tissues, also being considered to be chromoplast precursors (Pyke and Page, 1998, Schweiggert et al., 2011).

Furthermore, starchy fruits and vegetables contain large numbers of amyloplasts, which were also discussed to represent chromoplast precursors. The resulting chromoplasts often retain some

starch grains, then being called amylochromoplasts (Hempel et al., 2014). Amylochromoplasts with globular and crystalloid elements were described in peach palm fruit (Hempel et al., 2014), mango fruit (Vásquez-Caicedo et al., 2006), and sweet potato (Jeffery et al., 2012, Purcell et al., 1969), respectively. As summarized in Table 1, the ultrastructural elements of amylochromoplasts in the so-called Golden rice, maize, potato, and various wheat flours were not yet characterized in detail (Howitt and Pogson, 2006, Wurtzel, 2004, Lopez et al., 2008).

In fruits and vegetables that turn their color from green to yellow, orange, or red, chloroplasts represent the precursor of chromoplasts. When the color starts turning ("color break"), thylakoids and grana stacks disintegrate, and chromoplastidal substructures simultaneously appear. Such chromoplasts were reported to often contain thylakoid remnants. The transformation of chloroplasts into chromoplasts was investigated in detail in tomato and bell pepper (Rosso, 1968, Frey-Wyssling and Kreutzer, 1958). Further details concerning the interconversion of plastids have been reported elsewhere (Pyke and Page, 1998, Knoth et al., 1986, Lopez-Juez and Pyke, 2005).

2.4 Animal-derived foods

Although being unable to biosynthesize carotenoids, numerous animal species accumulate and metabolize carotenoids from their feed. An obvious and prominent example for the accumulation of carotenoids in animals is the vivid coloration of the plumage of birds, such as flamingos (*Phoenicopterus ruber*) and Atlantic canaries (*Serinus canaria domestica*). However, carotenoids were widely found throughout all phyla of the animal kingdom. In many species, externally

displayed carotenoids often serve for signaling purposes such as species recognition, sexual attraction, crypsis, and warning (Blount and McGraw, 2008). Moreover, carotenoids exert a variety of important biological functions identical or similar to those described for carotenoids in humans. This section is limited to a few typical examples of carotenoid-rich animal foods, being part of the human diet.

Egg yolk

A highly specific deposition of carotenoids was described for egg yolks of birds, including domesticated chicken (*Gallus gallus domesticus)*. Xanthophylls are commonly responsible for their coloration, depending on the used feed. Main xanthophylls found in commercially available eggs are lutein, zeaxanthin, capsanthin, canthaxanthin, 8′-apo-β-caroten-8′-al, 8′-apo-β-caroten-8′-oic acid, citranaxanthin, and β-cryptoxanthin. Astaxanthin feed supplementation leads to a pinkish egg yolk, which is currently undesired by most consumers (Breithaupt, 2008). Recently, Nimalaratne et al. (2012) investigated carotenoid levels in commercial egg yolks including geometric isomers. Raw egg yolk contained total carotenoid levels of about 2.5 mg/100 g of FW, comprising of lutein, zeaxanthin, β-apo-8′-cartenoic acid ethyl ester, and canthaxanthin in a ratio of ca. 20:10:7:3. Similar proportions of their corresponding (*Z*)-isomers were determined, representing about 10-14% of the total concentration of the respective carotenoids. Hammershoj et al. (2010) fed differently colored carrots along with a standard feed mix to hens observing an up to 100-fold increase of β-carotene in the respective egg yolks (Hammershøj et al., 2010).

Generally, major components of egg yolk are 52% (w/w) water, 16% protein, 27% total lipids, and 4% carbohydrates (U.S. Department of Agriculture, 2012). The complex structure of yolk

may be classified into two major compartments: (i) granules containing non-soluble protein aggregates and (ii) a clear yellow fluid, the plasma, containing low-density lipoproteins (LDLs) and soluble proteins. The granules account for 19-23% of yolk dry matter, including 50% of total yolk proteins and 7% of yolk lipids. The plasma contains about 77-81% of yolk dry matter, which consists of 85% LDLs and 15% livetin proteins. Most importantly, ca. 90% of yolk lipids and nearly all yolk carotenoids are found in the plasma fraction and, thus, mostly in the LDLs. In agreement, the LDL fraction is characterized by a strong orange color after its separation (Anton, 2007). The LDLs have a diameter of ca. 17-60 nm being composed of a lipid core of hydrophobic lipids, such as triglyerides and cholesterol esters, surrounded by a monolayer of phospholipids and proteins as shown in Figure 6A (Anton, 2013). The major carotenoids of common egg yolk, lutein and zeaxanthin, might be expected at the surface of the LDL complexes, since the preferential distribution of xanthophylls at the surface of biological emulsions has been shown previously (Borel et al., 1996). Although the origin of egg yolk LDLs and the above-described plastoglobules of plant chromoplasts are quite different, carotenoid deposition and distribution is interestingly similar (Figure 5A and 6A). Yolk carotenoids occur in a lipid-dissolved state and, possibly a minor proportion, loosely-associated with large membrane lipoproteins. The naturally high proportion of carotenoid (*Z*)-isomers of 10-14% of total carotenoids supports this hypothesis, since lipid-dissolved carotenoids are more prone to isomerization than crystalline and protein-associated carotenoids. In addition, (*Z*)-isomer concentrations rapidly increased after thermal treatment (Nimalaratne et al., 2012), being typical of lipid-dissolved carotenoids. This deposition state is important for understanding the high bioavailability of carotenoids from egg yolk as described below.

Milk and dairy products

A physical deposition state similar to egg yolk is encountered in dairy products. In contrast to the xanthophyll-rich egg yolk, ca. 90% of total carotenoids in bovine milk and derived products is represented by β-carotene (Hulshof et al., 2006). This carotene was found to be lipid-dissolved within the core of the milk fat globules, which vary in size from 0.2 to 15 µm. The apolar core mostly consists of triacylglycerides being surrounded by a monolayer of polar lipids, e.g., phospholipids and sterols. This internal fat globule itself is again enveloped by a double membrane, representing the former apical cell membrane of the glandular cell secreting the globule. Although some residual cytoplasm of these cells is sometimes encountered in the milk fat globule, they are often described to be surrounded by a trilayer membrane (MacGibbon and Taylor, 2006). Figure 6B depicts a schematic model of a milk fat globule.

The carotenoid concentration in milk widely depends on the feed and the cow breed. While high carotenoid levels are present in fresh grass-based feeds in summer, normal winter feedstuff is based on hay and concentrates, which are commonly low in β-carotene. Consequently, high β-carotene levels of up to 8 and 13 µg/g milk fat were reported in Friesian and Jersey cow milk after feeding fresh spring grass, respectively. Grazing on mature summer pasture significantly lower in carotenoids, the β-carotene levels decreased by half to ca. 4 and 7 µg/g, respectively. Therefore, the high variability of β-carotene concentrations reported in butter (2.5 to 12.5 µg/g fat) is not unexpected. In addition to β-carotene, milk and derived dairy products contain significant amounts of vitamin A. Dairy vitamin A is mostly represented by esterified (*all-E*)-retinol at concentrations from 8.0 to 12.0 µg/g fat (MacGibbon and Taylor, 2006).

The mammary gland derives free retinol from the liver and, after esterification, outputs it to the milk (Baldi and Pinotti, 2008). Interestingly, milk of goats and ewes is devoid of β-carotene, although containing vitamin A and minor amounts of xanthophylls. Therefore, small ruminant dairy products such as goat cheese appear whiter than comparable bovine products. These differences are partly due to the species-related superior conversion rate of provitamin A carotenoids into retinol within their enterocytes. Thus, carotenoid levels in serum, liver, and subcutaneous fat of small ruminants are considerably lower than those of cows. Consequently, due to their highly efficient formation of retinol, its concentration is about two-fold higher in dairy products from goat milk than in those from cow milk (Nozière et al., 2006).

The acceptance of the yellow-colored, i.e., carotenoid-rich dairy products by customers widely depends on the specific target market. For instance, in Europe, the yellow color of many cheese varieties is generally perceived positive, while it is often seen negative in the Middle East. As mentioned above, the feed and species largely impacts carotenoid levels, while only minimum losses occur during processing into cheese and butter. For instance, during cheese-making, about 95% of the β-carotene in the original milk is retained in the curd and, interestingly, only insignificant losses were observed during cheese ripening and storage during up to one year. In contrast, lower recovery rates for retinol and xanthophylls were observed (66 and 64%, respectively). Confirming the instability of retinol during cheese-making, high proportions of the (13*Z*)-isomer of retinyl palmitate (14-26% of total retinol) were detected in cheese, but not in milk. In addition, a substantial loss of these compounds into the whey has been observed during cheese-making and, by analogy, during the butter-making process (Nozière et al., 2006). Lucas et al. (2006) related the higher loss of retinol and xanthophylls as compared to β-carotene to their

localization in the membranes of the fat globules. Compounds at the surface of the lipid droplets might be spontaneously transferred to the aqueous phase without triglyceride lipolysis, whereas carotenoids like β-carotene are localized in the core of the droplet and, thus, less prone to spontaneous losses (Lucas et al., 2006).

In brief summary, carotenoids and fat-soluble vitamins in milk and dairy products prevail in a lipid-dissolved physical state within the milk fat globules. Their high levels in preformed vitamin A as well as the remarkable stability of the carotenoids during processing and storage make dairy products a nutritionally most interesting source of fat-soluble micronutrients. Their lipid-dissolved deposition state implies a high bioavailability, which is of particular importance for newborns and infants, since milk is their sole food. During the first days after giving birth, most mammals and humans produce a special type of milk, the colostrum, supplying even higher amounts of readily bioavailable micronutrients to meet the need of the growing offspring (Baldi and Pinotti, 2008). For instance, carotenoid levels in colostral human milk were up to 5-fold higher than in mature breast milk (Sommerburg et al., 2000). The potential importance for infant development was most recently highlighted be reports about the predominant occurrence of lutein in the infant brain (Vishwanathan et al., 2014).

Salmonide fish

Consumers expect salmon and rainbow trout flesh to be pink to red colored, relating a more intense color with better flavor and higher quality. Free-living fish derive carotenoids from ingesting Crustaceans and microorganisms, mostly algae. In aquaculture, synthetic astaxanthin and canthaxanthin are widely used, although the yeast *Xanthophyllomyces rhodorhous* and the

alga *Haematococcus pluvialis* represent upcoming natural sources (Bjerkeng, 2008). The carotenoid feed formulation largely influences their bioavailability in fish, following similar mechanisms as described below for humans. For instance, higher co-ingested fat-levels of 39% of the diet increased astaxanthin concentrations in salmon (*Salmo salar*) flesh muscle, yielding a more saturated flesh color as compared to 32% dietary fat in the feed (Bjerkeng et al., 1997). After absorption, carotenoids are distributed in the muscle by binding to the actomyosin complex *via* unspecific hydrophobic bonds. Matthews et al. (2006) found α-actinin to be the only myofibrillar protein that efficiently binds astaxanthin, reporting a molar binding ratio of astaxanthin to α-actinin of 1.11:1.00. A schematic representation of a sarcomeric Z-disc in muscle tissue is shown in Figure 6C, according to previously published data (Sjöblom et al., 2008). Naturally, α-actinin is also present in white-fleshed teleost fish, but only representatives of the Salmonidae accumulate significant amounts of astaxanthin and canthaxanthin in their muscles. According to Matthews et al. (2006), the limiting factor for carotenoid deposition is not α-actinin, but rather their efficient absorption, transport, and specifically their release to the target tissue of the Salmonidae. In agreement, extremely high concentrations in plasma and flesh can be reached by injecting high carotenoid doses into the intraperitoneal tissue of the fish (Ytrestøyl and Bjerkeng, 2007, Maltby et al., 2003). However, the clear mechanism of carotenoid deposition in the salmonid muscle remains unknown to date (Chimsung et al., 2012).

In raw salmon, Osawa et al. (2014) reported less than 5% (*Z*)-isomers of total carotenoids, while heating treatments like steaming, grilling, and microwave heating increased the proportion to 14-32% (Osawa et al., 2014). Therefore, the natural association of carotenoids to the protein matrix apparently prevents isomerization at ambient temperatures. Upon heat exposure, the denatured

protein complexes release the carotenoids into the lipid-rich compartments of the tissue, which are then prone to isomerization, degradation, and drip loss. In agreement, severe shrinkage and disintegration of the myofibres was observed in heated salmon filets, and the widening intermyofibrillar space progressively filled with liquid and lipids (Ofstad et al., 1996). The release of carotenoids from their myo-protein complex into the lipid-rich compartments of the muscle may have important consequences for their relative bioavailability.

Eggs from salmonide fish were also reported to contain carotenoids. Eggs from aquaculture-raised Chinook salmon (*Oncorhynchus tshawytscha)* contained moderate amounts of astaxanthin (0.4 mg/100 g FW) as well as minor amounts of lutein and canthaxanthin (Li et al., 2005). Slightly higher concentrations of up to 0.8 mg total carotenoids per 100 g FW were reported in Masu salmon eggs. Xanthophylls like astaxanthin, lutein, zeaxanthin, and canthaxanthin were most frequently observed in salmonide eggs (Ando and Hatano, 1991). However, by analogy to their muscles, carotenoid concentrations in fish eggs depend on the amount ingested with the feed. Regarding their physical deposition form, a large fraction of ca. 50% of the carotenoids was found in the oily lipoprotein particle fraction (Lubzens et al., 2003), thus being similarly lipid-dissolved as in chicken egg yolk (Figure 6A). The residual equal amount was bound to the yolk protein lipovitellin as reported by Lubzens et al. (2003), who provided a more detailed overview on levels of carotenoids and their deposition in fish ovaries and eggs.

Crustaceans

The worldwide capture and aquacultural production of Crustaceans has increased from 8.4 Mio. tons to 12.7 Mio tons from 2002 to 2012 (FAO, 2012). Despite their growing economic

importance, the occurrence of carotenoids in the exoskeleton and muscle of shrimp, crabs, crayfish, lobster and other Crustaceans will only be discussed briefly, since their relevance as dietary source of bioavailable carotenoids for humans is comparably limited. However, most carotenoids in Crustaceans are part of highly interesting protein complexes. The interaction of several carotenoid molecules and the protein leads to impressive grey, black, brown, or blue colors of the living animal. Cooking leads to the release of these carotenoids from the protein complex, resulting in a color change to vivid orange or red hues. Britton and Helliwell (2008) have compiled a detailed overview on the most intensively studied carotenoproteins, such as the astaxanthin-containing crustacyanin of the carapace of lobsters.

Noteworthy, carotenoids such as astaxanthin, canthaxanthin, and β-carotene also occur in the muscle flesh of numerous Crustaceans. For instance, a moderate concentration of up to 0.5 mg total carotenoids per 100 g of FW was determined in the muscles of some crayfish species, e.g., *Orconectes limosus* and *Astacus leptodactylus* (Czeczuga and Czeczuga-Semeniuk, 1999). More detailed information about carotenoids in Crustaceans may be found elsewhere (Bjerkeng, 2008, Britton and Helliwell, 2008).

3 Carotenoid bioavailability from plant and animal foods

3.1 General aspects of carotenoid absorption and distribution in humans

The absorption of carotenoids widely follows the absorption pathway of dietary fat. Since the hydrophobic carotenoids are embedded in the above-described natural matrices, dynamic and

complex mechanisms for their liberation and solubilization are required prior to any enzymatic and metabolic action (Da Costa, 2003, Deming and Erdman Jr., 1999).

The first step in carotenoid "digestion" is often the mechanical, thermal, and chemical disintegration of the food during kitchen preparation or technological processing. Due to the highly different deposition forms of carotenoids in food (lipid-dissolved, crystalline, liquid crystalline, membrane-associated, protein-complexed), the applied processing and preparation steps have an outstanding impact on the carotenoid bioavailability as described below. The actual "digestion" initiates with the mastication of the prepared food, including the action of salivary α-amylase and lingual lipase (Schweiggert et al., 2012a, Da Costa, 2003). After swallowing, gastric motility and several digestive enzymes such as pepsin and gastric lipase continue the physico-chemical disintegration of the food. Although lingual and gastric lipases may hydrolyze some specific fatty acid triacylglycerides (TAGs), their contribution to total TAG cleavage has been estimated to be marginal when compared to the intestinal lipolytic enzymes. Similarly, the hydrolysis of carotenoid esters by gastric lipase was previously demonstrated to be insignificant (Breithaupt et al., 2002). By analogy, gastric proteases only partly contribute to the overall protein hydrolysis when compared to intestinal proteases (Freeman et al., 1983). Nevertheless, thermal processing of protein-rich foods was shown to improve protein digestibility as catalyzed by pepsin (Rehman and Shah, 2005, Sun et al., 2014), being potentially important for the release of carotenoids from protein-complexes or protein-rich matrices. If liberated from the matrix, the low pH of the stomach may cause the degradation of acid-labile carotenoids, such as, e.g., of 5,6-epoxy carotenoids. Despite their high concentrations of up to 4.8 mg/100 g of FW in a spinach

test meal, the epoxy carotenoids violaxanthin and neoxanthin were undetectable in the digesta after completion of the gastric phase of a simulated digestion (Biehler et al., 2011).

Although some lipid release and degradation may occur in the stomach, the most important phase of lipid digestion takes place in the small intestine. Dietary lipids trigger the secretion of bile acids from the gall bladder and efficient lipases from the pancreas. Lipids aggregate in droplets and form an emulsion with bile salts, phospholipids, and monoacylglycerols generated by intragastric lipolysis. Subsequently, the pancreatic lipase and colipase complex binds to the interphase of the emulsified droplets, catalyzing an efficient hydrolysis of TAGs (Da Costa, 2003, Deming and Erdman Jr., 1999). While the pancreatic lipase was found to cleave carotenoid esters only inefficiently, a pancreatic cholesterol esterase was shown to rapidly hydrolyze carotenoid esters. The latter enzyme similarly cleaves dietary cholesteryl and retinyl esters (vitamin A) (Breithaupt et al., 2002). The intestinal motility and the presence of bile acids enhances the formation of mixed micelles. These are water-soluble and take up free fatty acids, monoacylglycerols, and carotenoids (Da Costa, 2003, Erdman Jr. et al., 1993). Approaching the aqueous interface of the epithelial cell surface ("unstirred water layer") in the duodenum, the micelles dissociate at the apical enterocyte membrane to release water-insoluble compounds, which then may enter the enterocyte by free diffusion (Da Costa, 2003). Passive diffusion has been believed to be the only mechanism for the uptake of carotenoids by the enterocyte, but, in 2002, carotenoid absorption was shown to be saturable, indicating a transporter-facilitated uptake (During et al., 2002). At the same time, the class B scavenger receptors SR-BI and CD36 were shown to mediate the cellular carotenoid uptake in specific tissues of *Drosophila* (Kiefer et al., 2002). Subsequently, a direct role of SR-BI in the cellular uptake of β-carotene, lutein, and

lycopene could be demonstrated with human epithelial colorectal adenocarcinoma cells (Harrison, 2012). The receptor-mediated absorption of β-carotene gave rise to investigations about its regulation and, recently, transcriptional repressors for SR-BI and the enzyme converting provitamin A carotenoids to vitamin A (β-carotene-15,15′-dioxygenase, BCO) were identified. Vitamin A deficiency enhanced the expression of both proteins, ultimately allowing the absorption and conversion of even small amounts of β-carotene. The absorption and conversion was suppressed when sufficient vitamin A was available in order to avoid excess vitamin A production, and thus toxicity. Additionally, various polymorphisms of SR-BI and BCO1 have been discovered, possibly contributing to the large variability in β-carotene absorption and metabolism among human individuals (Von Lintig, 2010). Indeed, so-called "low responder" and "low converter" phenotypes were frequently observed during intervention studies with healthy individuals (Borel et al., 1998). The large inter-individual variability of absorption and conversion might be explained by the above-mentioned feedback regulation and genetic polymorphisms (Von Lintig, 2010). Besides playing a role in the regulation of the intestinal absorption of carotenoids, such transporter proteins may also be important regarding the distribution of carotenoids within the organism (Kiefer et al., 2002). Some carotenoids, e. g. lutein and zeaxanthin, are very specifically deposited in the *Macula lutea*, while the prostate is possibly a specific deposition site of various lycopene isomers (Britton, 2008, Clinton et al., 1996). A zeaxanthin-binding protein was proposed to serve for this purpose in the *Macula lutea* (Bhosale et al., 2004).

Prior to their distribution in the human organism, absorbed carotenoids are partly metabolized and packaged into chylomicrons (CMs) and very low density lipoproteins (VLDL) in the

intestinal cells. CMs and other lipoprotein particles are released to the lymph and transported to the thoracic duct, which empties into the left subclavian vein and enters the main circulation (Schweiggert, 2014). Further details about the transport and distribution of carotenoids (Erdman Jr. et al., 1993, Reboul, 2013, Canene-Adams and Erdman Jr., 2009) as well as the numerous health benefits and rare adverse effects (Britton et al., 2009a) were reviewed previously.

Compiling knowledge about the impact of different carotenoid deposition forms on carotenoid bioavailability, the following sections will review current human intervention trials and, if clinical data is lacking, so-called bioaccessibility studies. In carotenoid research, the term bioaccessibility (BA) is defined as "*the fraction of a compound that is released from its matrix in the gastrointestinal (GI) tract and thus becomes available for intestinal absorption"* (Fernández-García et al., 2009), whereas bioavailability generally refers to "*the fraction of an oral dose of a parent compound or active metabolite from a particular preparation that reaches the systemic circulation"* (Fernández-García et al., 2009). While human studies are required for the ultimate evaluation of carotenoid bioavailability in humans, bioaccessibility can be estimated using fast and comparatively inexpensive *in vitro* digestion models, often being coupled to Caco-2 cell absorption assays. The obtained results have a highly tentative character, but are most valuable for the subsequent design of costly human clinical trials.

3.2 Carotenoid bioavailability from green plant foods

Carotenoids in green plant foods such as broccoli, spinach, and other green leafy vegetables are embedded in protein complexes in the thylakoids of chloroplasts (Figure 5D). As a consequence,

carotenoid bioavailability from raw and unprocessed green vegetables consumed without any additional lipid is extremely poor, and even their overall value for providing vitamin A has been questioned in the past. However, their bioavailability can be modulated by technological and dietary measures, such as mechanical disintegration (e.g., mincing and pureeing), enzymatic maceration of matrix compounds, the addition of lipids, and thermal treatments. All these measures either aim at 1) degrading the often rigid cell walls as well as chloroplast and thylakoid membranes, 2) denaturation and degradation of the protein-pigment complexes, and 3) improving the dissolution of liberated carotenoids in dietary lipids during food preparation and digestion.

Simple mechanical measures were shown to improve carotenoid bioavailability from green plant foods. Castenmiller et al. (1999) observed a slightly higher blood serum response of β-carotene and lutein after the consumption of minced spinach as compared to whole leaf spinach. In a similar study (Van Het Hof et al., 1999), the plasma response of lutein was shown to be about 14% higher when chopped spinach instead of whole leaf spinach was consumed (Van Het Hof et al., 1999). Beyond mechanical disintegration of the food matrix, a further increase was observed after enzymatic liquefaction of the above-mentioned minced spinach leaves using a technical pectinase, hemicellulase, and cellulase preparation. When dietary fibers were re-substituted to the liquefied spinach meal to compensate for the enzymatic "loss" of fibers, the expected change of the β-carotene serum response was not observed (Castenmiller et al., 1999). Apparently, the degradation of the complex food matrix, i.e. the embedment of the carotenoids in the protein-pigment complexes in the thylakoids, cannot be reversed by the simple addition of dietary fiber. This was partly unexpected, since a previous study with 7 participants (Riedl et al., 1999) and

another study with 6 participants (Rock and Swendseid, 1992) had observed a slightly suppressed bioavailability from a carotenoid supplement co-consumed with additional dietary fiber. However, when considering more complex foods instead of supplements, the total amount of intrinsic dietary fibers seems to be less important than the integrity of the complex plastidal substructures. For instance, Schweiggert et al. (2014) compared the absorption of equivalent doses of β-carotene from papaya, tomato, and carrot. Despite the highest content of dietary fiber, bioavailability was greatest from the papaya test meal. However, the plastidal deposition form of the carotenoids in papaya chromoplasts was highly different to that in tomato and carrot, being proposed by the authors to be responsible for surpassing the effect of increased dietary fiber (Schweiggert et al., 2014). In agreement, further studies in which the effect of dietary fibers was ruled out by other factors are available (Unlu et al., 2005, Cooperstone et al., 2015).

While the effect of added dietary fiber may be controversially discussed, dietary lipids were consistently shown to enhance carotenoid bioavailability. For instance, when mixed salads consisting of fresh spinach, romaine lettuce, carrots, and cherry tomatoes were ingested without any dietary lipids, the absorption of the monitored carotenes was negligible. The addition of canola oil (6 g) substantially increased their bioavailability, which was further enhanced when 28 g oil per meal were ingested (Brown et al., 2004). In agreement, Unlu et al. (2005) observed a significantly boosted bioavailability of lutein, α-carotene, and β-carotene when a carotenoid-rich salad (spinach, lettuce, carrots) was co-consumed with lipid-rich avocado. An identical boost in bioavailability was observed when pure avocado oil was used instead of avocado fruit as the lipid source, although the avocado fruit added considerable amount of dietary fiber. Besides the total amount of lipids, the degree of saturation as well as the chain length of the triglycerides co-

consumed is controversially discussed. For instance, unsaturated lipids from canola and soybean oils increased intestinal micellization and carotenoid uptake by Caco-2 cells. Thus, unsaturated TAGs were proposed to enhance the bioavailability more than saturated fats such as butter (Failla et al., 2014). However, in a study with Mongolian gerbils (Conlon et al., 2012), saturated coconut oil mostly consisting of lauric (12:0) and myristic (14:0) acid enhanced tissue uptake of tomato carotenoids more than safflower oil, containing mostly unsaturated fatty acids, i.e. oleic (18:1) and linoleic (18:2) acid.

The minimum amount of dietary fat for achieving "sufficient" carotenoid bioavailability has been subject to controversial discussions, and values of 3-5 g per meal have often been discussed (Unlu et al., 2005). Nevertheless, the above-mentioned study showed that bioavailability was still improved when lipids were increased from 6 to 28 g/meal. Thus, it appears unlikely that an exact optimum amount should be deduced and generalized to a wide array of food sources without at least some classification.

By analogy to lipid addition, heat treatments are often believed to generally increase carotenoid bioavailability. However, controversial data was obtained from *in vitro* and *in vivo* studies. Using *in vitro*-digestion models coupled to Caco-2 cell absorption assays, one study reported an equal (lutein) or lower (β-carotene) intestinal micellization of carotenoids from heated spinach (Ferruzzi et al., 2001), while an increased micellization was reported elsewhere (O'Sullivan et al., 2008). In both studies, the subsequent uptake of the micellized carotenoid by Caco-2 cells was lower from heated spinach. According to O'Sullivan et al. (2008), the reasons for these observations remain unknown. Results from clinical studies are also not as clear as expected. A slightly enhanced bioavailability from thermally processed spinach and carrots was found when

comparing to the respective unheated test meal containing an equivalent dose of carotenoid. However, the authors stated that the effect was much smaller than initially anticipated (Rock et al., 1998). While this study fed carotenoids from both carrots and spinach, another trial (Tassi and Amaya-Farfan, 2008) implemented raw and cooked (5 min, 97°C) arugula leaves (syn. rocket = *Eruca sativa* L.) as the only carotenoid source. Differences in the post-prandial bioavailability of β-carotene and lutein were insignificant, although the carotenoid dose provided with the cooked arugula was even slightly higher than in the uncooked meal. Noteworthy, the β-carotene responses from both test meals were substantially lower than those obtained after the ingestion of an equally-dosed β-carotene supplement (Tassi and Amaya-Farfan, 2008).

In 1990s, the low bioavailability of β-carotene from green plant foods had given rise to doubts of their overall nutritional value as vitamin A source. For instance, the daily consumption of broccoli (300 g) over 6 weeks led to an increase in the plasma levels of lutein, but not of β-carotene (Micozzi et al., 1992). Similar findings were obtained after the consumption of whole leaf and chopped spinach (Van Het Hof et al., 1999). In 1995, De Pee et al. (1995) directly questioned the nutritional value of dark-green leafy vegetables as a vitamin A source. A mix of different green leafy vegetables containing 3.5 mg β-carotene and 5.5 mg lutein and zeaxanthin was fed to 57 breastfeeding Indonesian women for 5 days per week. After 12 weeks, β-carotene and retinol (vitamin A) serum levels remained unchanged and even lutein as well as zeaxanthin levels only slightly increased. In contrast, β-carotene and retinol levels in a group receiving a supplement with lipid-dissolved β-carotene significantly increased. De Pee et al. (1995) associated the poor bioavailability from the green leafy vegetables with the difficult liberation of

carotenoids from the protein-complexes in chloroplasts, although the leaves were stir-fried and fed with lipids (7.8 g). In a very similar study in 2004, test meals containing spinach with ca. 2.9 mg β-carotene and 5.3 mg lutein were fed to Bangladeshi men for 60 days (Haskell et al., 2004). This time, significant increases in plasma retinol, β-carotene, and lutein levels were observed. In contrast to the above-mentioned study, the spinach was additionally steamed (10 min) and pureed prior to stir-frying in corn oil (6.8 g/serving). The authors (Haskell et al., 2004) assumed that their additional heat and mechanical treatment resulted in the better liberation and absorption of carotenoids from their test meal when compared to the earlier Indonesian study (De Pee et al., 1995). In addition, they suggested that the low bioavailability in the Indonesian study was caused by the high rate of *Ascaris* infection among the study population (ca. 60%). Consequently, highly different vitamin A equivalence factors (β-carotene:retinol, wt:wt) for β-carotene from green leafy vegetables were deduced from the former (26:1) and the later study (10:1), respectively.

A further study investigated the equivalence factor of β-carotene from spinach and carrots grown in hydroponic cultures using deuterium oxide for the intrinsic labeling of β-carotene (Tang et al., 2005). The vegetables were chopped, steamed for 5 min, pureed, frozen, thawed, and re-heated in a microwave oven for 2 min prior to ingestion by 7 participants. After applying a single dose of ca. 11 mg β-carotene from either 300 g spinach or 100 g carrots, the resulting vitamin A equivalence factors were 21:1 and 15:1 for spinach and carrots, respectively. Consequently, the liberation of β-carotene from the chloroplasts in spinach was assumed to be lower than from the crystalline β-carotene in carrot chromoplasts during food preparation and the subsequent digestion (Tang et al., 2005). Noteworthy, the current vitamin A equivalence factors published

by the U.S. Institute of Medicine, also called retinol activity equivalents (RAE), were set at 12:1 for β-carotene from food without further differentiation (Institute of Medicine, 2001).

In conclusion, green leafy vegetables and other green plant foods represent an important source of dietary β-carotene and lutein, particularly when food preparation measures are applied to enhance their bioavailability. Meanwhile, further studies demonstrated a positive effect on the vitamin A status in children, particularly when dietary lipids were co-consumed (Jayarajan et al., 1980, Takyi, 1999, Ribaya-Mercado et al., 2007). While the addition of lipids and, to a minor extent, mechanical and enzymatic treatments were found to boost carotenoid bioavailability from green plant foods, sole heat treatments are considered to be less effective.

3.3 Carotenoid bioavailability from yellow, orange, and red-colored plant foods

The conclusions on the effect of technological and dietary measures to improve the bioavailability of carotenoids from green plant foods can be widely transferred to yellow, orange, and red plant foods. However, their overall bioavailability, particularly among raw untreated foods, substantially differs due to their highly different forms of carotenoid deposition (Figure 5A-C).

Foods with crystalloid chromoplasts

Most carotenoid bioavailability studies on non-green carotenoid-rich plant foods were conducted with common carrots and red tomatoes. While the commonly consumed orange-colored carrots are rich in the provitamin A carotenoids α- and β-carotene, usual red-colored tomato cultivars contain very high amounts of the non-provitamin A carotenoid lycopene, whose potential health

benefits were discussed elsewhere (Erdman Jr. et al., 2009, Ford and Erdman Jr., 2012, Wei and Giovannucci, 2012, Böhm, 2012). In both vegetables, these carotenoids are deposited in crystalloid chromoplasts with large, often up to 15 µm long crystals (Schweiggert et al., 2011). Their crystalline state was previously associated with their poor bioavailability in an animal model (Zhou et al., 1996). Regarding subsequent human interventions, the bioavailability of both α- and β-carotene from a mixed salad containing raw carrots was negligible when consumed without dietary lipid (Brown et al., 2004, Unlu et al., 2005). Although the salad also contained green leafy vegetables, monitoring the α-carotene response allowed separate conclusions about its bioavailability from carrots, since relevant amounts were exclusively present in carrots. The bioavailability of α-carotene sequentially increased with higher amounts of added lipid in form of pure oil or avocado, in accordance with all other monitored carotenoids (Brown et al., 2004, Unlu et al., 2005). By analogy, Kopec et al. (2014) showed that α- and β-carotene from raw carrots was 4.8- and 6.6-fold more bioavailable, respectively, when avocado was co-consumed with a meal only containing raw petite baby carrots. The same authors described a 2.4-fold increase in β-carotene bioavailability when avocado was added to a thermally treated sauce made from high-β-carotene tomatoes (Kopec et al., 2014), which also contain crystalline carotenoids (Table 1). The added dietary lipid is required for the dissolution of crystalline carotenoids, which is a prerequisite for their incorporation into mixed micelles and, thus, their efficient absorption to the enterocyte (Erdman Jr. et al., 1993). Beyond this, Kopec et al. (2014) also observed a higher post-prandial response of retinyl esters in the chylomicron-rich plasma fraction, postulating that the added dietary lipid might have enhanced the conversion rate of β-carotene to vitamin A in the

enterocyte. A similar hypothesis for a lipid-enhanced hepatic conversion of β-carotene was previously raised (Deming et al., 2000).

Besides adding dietary lipids, carotenoid bioavailability from crystalline sources like carrot and tomato can be modulated by thermal processing. *In vitro*-bioaccessibility studies have suggested an enhanced liberation of lycopene from the food matrix into the intestinal fluid after more intense heating (Colle et al., 2010, Svelander et al., 2010), and several *in vivo* studies have shown a higher bioavailability of lycopene from processed tomatoes as compared to fresh tomatoes (Böhm and Bitsch, 1999, Porrini et al., 1998, Stahl and Sies, 1992, Gartner et al., 1997). The better bioavailability was mostly associated with the intense break-down of cellular and plastidal structures, particularly the disintegration of lycopene aggregates and their dissolution in dietary lipids. In most studies on the effect of heating, harsh mechanical treatments were simultaneously used during processing and, thus, it was often difficult to separate the effect of mechanical and heat processing in the above-mentioned studies. Edwards et al. (2003) fed different doses of unheated lycopene-rich watermelon juices to 24 participants over 6 weeks. They observed a significant but apparently dose-independent lycopene response in human plasma, although watermelon similarly contains crystalline lycopene aggregates (Table 1). However, since the meals were consumed "in the presence of ample fat" (34% of total energy) and no comparison to fresh fruit was made (Edwards et al., 2003), drawing conclusions about the sole effect of the mechanical treatment is impossible from this study.

In the above-mentioned trial of Kopec et al. (2014), the β-carotene response after consuming raw petite baby carrots without lipids was marginal (AUC median [25^{th}, 75^{th} percentile] = 88 [24, 125] nmol*h/L plasma). In contrast, the consumption of the mechanically- and thermally-treated

sauce made from high β-carotene tomatoes with crystalline β-carotene led to a substantially greater absorption even when no extra dietary lipid was consumed (AUC = 202 [111, 273] nmol*h/L plasma), indicating the enhancing effect of mechanical and thermal processing.

Rock et al. (1998) fed vegetable diets containing either raw or heated carrots to eight female participants over 4 weeks. They observed a higher plasma response for α- and β-carotene in the group consuming heated carrots, although not reaching statistical significance. A current study using intrinsically labelled carrots reported a significant increase in carotenoid bioavailability from blanched and pureed carrots (100 g) when applying an additional heat treatment (stir-frying). Noteworthy, additional dietary lipids (10.5 mL groundnut oil) were also added at the same time (Ghavami et al., 2012).

While heat treatments might accelerate the dissolution of crystalline carotenoids in dietary lipids, the simultaneous softening of the plant tissue facilitates the breakdown of the food matrix during mastication and subsequent digestion, being particularly important for foods with rigid cell walls, such as carrots. For example, Lemmens et al. (2010) showed a strong positive correlation of a more intense mastication with an enhanced *in vitro* carotenoid bioaccessibility from raw, fresh carrots. After rigorous cooking of the carrots, the specific dependency of β-carotene bioaccessibility on mastication of the carrot tissue disappeared. The softer tissue of the cooked carrots could be ruptured more easily by the motile GI tract and, therefore, carotenoid liberation was independent of mastication (Lemmens et al., 2010). To the best of our knowledge, evidence from human trials for this observation is lacking. In agreement with Lemmens et al. (2010), Netzel et al. (2011) reported the highest release of carotenes from cooked carrots when compared

to blanched and raw carrots, furthermore observing the highest carotenoid uptake by Caco-2 cells from cooked carrots (Netzel et al., 2011).

In brief summary, several human studies clearly demonstrated the enhancing effect of dietary lipids on carotenoid bioavailability. Less clear and often indirect evidence has been provided for the enhancing effect of mechanical and thermal treatments. Nevertheless, a positive effect may be deduced when the currently available information is considered as a whole. Such treatments aid the disintegration of carotenoid crystals and their dissolution in present lipids, but they cannot overcome the need for adding dietary lipids when aiming at maximum absorption of crystalline carotenoids.

Foods with globular and tubular chromoplasts

In comparison to food with crystalloid chromoplasts, much less studies are available on fruits and vegetables with globular and tubular chromoplasts, such as mango, papaya, and pumpkin (Table 1). These "orange fruits" were previously shown to be more effective than dark-green leafy vegetables in increasing serum β-carotene and retinol concentrations in Indonesian schoolchildren. Interestingly, the "green vegetable" diet even contained higher amounts of carotenoids (4.1 mg β-carotene/d, 5.9 mg lutein/d, 684 retinol equivalents (RE) in total) as compared to the "orange fruit" diet (2.6 mg β-carotene/d, 1.3 mg β-cryptoxanthin/d, 535 RE/d). Noteworthy, the green leafy vegetable diet also contained minor amounts of carrot. As outlined above, the low bioavailability from green leafy vegetables and carrot was explained by the deposition of carotenoids in protein-pigment complexes and crystalline aggregates, respectively. Furthermore, the authors assumed that the carotenoids in the used fruits were deposited in

"chromoplasts […] where β-carotene is dissolved in oil droplets" (de Pee et al., 1998). These findings, i.e. the preferable absorption of β-carotene from fruits like mango and papaya, were confirmed by a later study with breastfeeding women (Khan et al., 2007). Meanwhile, the chromoplast types of these fruits have been elucidated to be of the globular-tubular type, containing partly lipid-dissolved but mostly liquid-crystalline carotenoids (Table 1). Consequently, the globular-tubular deposition form might be more favorable than the protein-complexed and solid-crystalline forms, respectively. In full agreement with de Pee et al. (1998) and Khan et al.´s (2007) findings, a recent study demonstrated that the post-prandial absorption of β-carotene to the TRL fraction of human plasma was 3-fold higher after the consumption of papaya as compared to an equivalent dose from carrots and tomatoes (Schweiggert et al., 2014). The same authors found the *in vitro* bioaccessibility of β-carotene from mango and papaya to be significantly higher than that from carrots and tomatoes (Schweiggert et al., 2012a). Besides their different deposition within the plant plastids, other food-matrix characteristics may also have contributed to these observations, such as, e.g., the higher firmness of raw carrots when compared to papaya fruit. However, in the study reported by Schweiggert et al. (2014), the firmness of the used tomatoes was as low as that of the papaya fruits, and the bioavailability of both β-carotene and lycopene was still significantly higher from papaya. At the same time, the bioavailability of crystalline β-carotene from both carrots and tomatoes was low and highly similar (P = 0.86). Eliminating such cross-species food matrix effects, Cooperstone et al. (2015) investigated the bioavailability of lycopene from tangerine tomatoes (globular chromoplasts) and common red tomatoes (crystalloid chromoplasts). The post-prandial response of (*all-E*)-lycopene was ca. 2-fold higher from tangerine tomatoes than from red tomatoes. Moreover, an 8.5-fold

higher bioavailability of total lycopene from tangerine tomatoes (globular chromoplasts) was observed. Since a major proportion of tangerine tomato carotenoids were (*Z*)-isomers, the extreme differences in total lycopene absorption may also be partly explained by their preferential liberation and absorption (Cooperstone et al., 2015).

Nevertheless, the currently available information indicates a high bioavailability of carotenoids from globular and tubular chromoplasts, being important for estimating the basic vitamin A potential of raw and untreated fruits and vegetables.

3.4 Bioavailability from animal foods

In contrast to numerous studies regarding carotenoid bioavailability from feed in animals, clinical trials on the bioavailability of carotenoids from animal foods are less abundant. Regarding salmonide fish, Rüfer et al. (2008) studied the absorption of astaxanthin from wild (*Oncoryhnchus* spp.) and aquacultured (*Salmo* salar) salmon in 28 healthy men, consuming the same dose of astaxanthin from 250 g wild or aquacultured salmon daily for 28 days. Astaxanthin plasma levels were higher in those men consuming aquacultured salmon during the days 3 to 14. On day 28, differences in the plasma levels of both groups were insignificant. The higher total lipid content of aquacultured salmon (17.3% w/w) as compared to that of wild salmon (6.5% w/w) might have been responsible for the enhanced astaxanthin bioavailability. However, the authors stated that the fat content in the wild salmon (16.3 g/meal) should have sufficed to ensure maximum astaxanthin bioavailability, suggesting that the presence of different astaxanthin isomers in the two salmon types might have further influenced their bioavailability. The different distribution of astaxanthin isomers in the plasma samples of both study groups resembled that of

the isomers in the different test meals. For instance, the (3*R*,3′*S*)-form represented the major astaxanthin isomer in the non-organically aquacultured salmon due to its predominant occurrence in the feed. Noteworthy, the exact preparation procedure of the daily portion of salmon was left to the participants and thus remains unknown (Rüfer et al., 2008).

By analogy to salmonide fish, most bioavailability studies on poultry deal with the improvement of carotenoid bioavailability from feed to the animals. Fewer reports about the bioavailability from eggs are available. The consumption of 1.3 cooked egg yolks (= 380 μg lutein and 280 μg zeaxanthin) per day over 4.5 weeks resulted in enhanced plasma lutein and zeaxanthin levels in a study with 11 participants (Handelman et al., 1999). Since similar amounts of zeaxanthin (300 μg) fed from 60 g spinach or from 150 g cooked corn did not result in a significant plasma increase in a previous study (Hammond et al., 1997), the authors concluded that carotenoid bioavailability from egg yolk is comparably high. Their deposition in lipid droplets within the egg yolk supports this hypothesis (Figure 6A). Testing this hypothesis in a cross-over study, Chung et al. (2004) fed an equivalent dose of lutein (6 mg/d) from eggs, spinach, and two different supplements to ten healthy men over 10 days. While the post-prandial lutein response in the TRL fraction was similar after the consumption of a single dose of the test meals, the serum lutein response was significantly higher when subjects consumed eggs for 10 days as compared to the other test foods (Chung et al., 2004). Particularly, the consumption of zeaxanthin from egg yolk was shown to effectively raise its serum levels by various further studies previously (Wenzel et al., 2006, Kelly et al., 2014, Blesso et al., 2013).

While the high bioavailability of vitamin A from dairy products and their significance as an important vehicle for this micronutrient is widely accepted (Weinberg et al., 2004, Dror and

Allen, 2014), studies regarding the bioavailability of carotenoids from dairy foods are scarce. Due to the importance for infant nutrition, some investigators focused on carotenoid bioavailability from breast milk. Bettler et al. (2010) observed higher bioavailability of lutein from breast milk than from lutein-fortified infant formula, postulating that 4-fold more lutein is needed in infant formulas to reach bioequivalence (Bettler et al., 2010). Lutein might be of particular importance for infants, since it represents ca. 60% of the total carotenoids in their brain and its levels were found to be depleted in preterm infants (Vishwanathan et al., 2014).

Cow milk has been proposed as a vehicle to be fortified for the dietary delivery of carotenoids. For instance, Benzie et al. described an enhanced bioavailability of zeaxanthin from a milk-based formulation of wolfberry when compared to a water-based formulation (Benzie et al., 2006). Similarly, Granado-Lorencio et al. (2010) described fermented milk to be a suitable carrier for lutein esters. Although more lutein was absorbed after fortification with higher amounts of lutein, the percentage of the absorbed lutein remained similar (Granado-Lorencio et al., 2010). A similar relative absorption of vitamin A was previously described from vitamin A-fortified milk (Herrero-Barbudo et al., 2006). The fortification of staple food may represent a powerful tool to diminish vitamin A deficiencies. Further details about food fortification to reduce vitamin A deficiency may be found elsewhere (Dary and Mora, 2002).

In brief summary, carotenoid bioavailability from animal foods, including fortified dairy products, is generally considered to be high.

4 Concluding Remarks

According to the current state of the art, carotenoid deposition in plant and animal foods as well as their bioavailability is extremely variable, and only a few attempts were made for their rough categorization. The established vitamin A equivalence factors for provitamin A carotenoids were proposed to be rather low due to the poor bioavailability of carotenoids when compared to vitamin A. Regarding β-carotene, generalized equivalence factors of 6:1 and 12:1 were proposed by the FAO/WHO (2002) and the U.S. Institute of Medicine (2001), respectively. As summarized by Khan et al. (2007), these conversion factors for estimating the vitamin A equivalency of β-carotene and other provitamin A carotenoids from foods might be much lower as previously assumed. Khan et al. (2007) proposed a further differentiation of the above-mentioned vitamin A equivalency factors, suggesting factors of 12:1 for β-carotene from "fruits" and 28:1 for β-carotene from "dark-green leafy vegetables". Ultimately, these factors would result in an average conversion factor of 21:1 for dietary β-carotene from a mixed Western diet (ratio of vegetables to fruit = 4:1, (Khan et al., 2007)).

In this review, we aimed at highlighting the natural differences of carotenoid deposition in the most common carotenoid-rich foods. In our opinion, this intrinsic factor is of utmost importance for assessing and, possibly, categorizing carotenoid bioavailability and its enhancement. While carotenoid bioavailability from animal foods such as egg yolk and dairy products with their mostly lipid-dissolved carotenoids was shown to be comparably high, the bioavailability from plant foods will be more difficult to categorize due to the large natural diversity of carotenoid deposition. There is growing evidence that the chromoplast morphology, an intrinsic

"unchangeable" factor of a specific plant food, is highly decisive for the basic bioavailability and, furthermore, for the design of enhancing measures. A further differentiation of conversion factors for provitamin A carotenoids from plant foods beyond the classification into "fruits" and "dark-green leafy vegetables" might only be achievable, if the deposition state of the carotenoids in the chromoplasts was considered. Further investigations should carefully aim at comparing carotenoid bioavailability from foods with different deposition forms - a decisive factor that should not be ignored when aiming at a further classification of carotenoid bioavailability from foods.

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Table 1. Major nutritionally-relevant carotenoids and predominant plastid types in plant foods. βC: β-carotene, βCRP: β-cryptoxanthin, LUT: lutein, ZEA: zeaxanthin, LYC: lycopene, CAR-esters: carotenoid esters. Carotenoid levels: low: < 0.1 mg/100 g FW, mod.: from 0.1-0.5 mg/100 g FW, high: 0.5-2.0 mg/100 g FW, very high: 2.0 mg/100 g FW.

Commodity	**Lat. name**	**βC**	**βCRP**	**LUT**	**ZEA**	**LYC**	**CAR-esters**	**Irregular Carotenoids**	***Carotenoid Ref.***	**Predominant plastid type**	***Plastid Ref.***
Avocado fruit	*Persea americana* Mill.	low		low-mod.					*(Ashton et al., 2006)*	Chloroplasts (green parts), Etioplasts (yellow parts)	*(Platt-Aloia, 1980, Platt-Aloia and Thomson, 1981, Cran and Possingham, 1973)*
Apricot	*Prunus armeniaca* L.	high - very high				low	mod.		*(Britton and Khachik, 2009, Breithaupt*	Unknown type	-

					and Bamedi, 2001)		
Broccoli	*Brassica oleracea* var. *italica* Plenck	very high	very high		*(Britton and Khachik, 2009)*	Chloroplasts	*deduced by color*
Carrot, orange	*Daucus carota* subsp. *carota* (Hoffm.) Schübl. & G.Martens	very high			*(Britton and Khachik, 2009)*	Crystalloid chromoplast	(Schweiggert et al., 2011)
Cauliflower, Or mutant	*Brassica oleracea* var. Botrytis L.	mod.			*(Li et al., 2001, Paolillo Jr. et al., 2004)*	Crystalloid chromoplast	*(Paolillo Jr. et al., 2004)*
Chinese wolfberry	*Lycium barbarum* L.		very high	very high	*(Breithaupt and Bamedi,*	Unknown type	-

								2001)		
Corn	*Zea mays* L.	mod.	mod.	mod.	mod. - high			*(Britton and Khachik, 2009, De Oliveira and Rodriguez-Amaya, 2007)*	Amylochromoplast	*(Wurtzel, 2004)*
Garden nasturtium	*Tropaeolum majus* L.					high - very high		*(Winkenbach et al., 1976)*	Tubular chromoplast	*(Winkenbach et al., 1976)*
Guava	*Psidium guajava* L.	mod.			high			*(Britton and Khachik, 2009)*	Unknown type	-
Golden Rice	*Oryza sativa* L.	mod. -						*(Tang et al., 2009, Paine et*	Amylochromoplasts assumed due	-

		very high							*al., 2005)*	to carotenoid localization in endosperm	
Grapefruit	*Citrus paradisi* Macfad.	low-mod.							*(Britton and Khachik, 2009)*	Unknown type	-

Commodity	**Lat. name**	**βC**	**βCRP**	**LUT**	**ZEA**	**LYC**	**CAR-esters**	**Irregular Carotenoids**	***Carotenoid Ref.***	**Predominant plastid type**	***Plastid Ref.***
Grapefruit, pink	*Citrus paradisi* Macfad.					mod.-high			*(Britton and Khachik, 2009)*	Crystalloid chromoplast	*(Purcell et al., 1963)*
Green leafy vegetables	Various species	very high		very high					*(Britton and Khachik,*	Chloroplast	*(Britton and Khachik, 2009)*

						2009)		
Kiwifruit, green flesh	*Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson	mod.	low	high		*(Montefiori et al., 2009)*	Chloroplasts	*(Montefiori et al., 2009)*
Kiwifruit, yellow flesh	*Actinidia chinensis* Planch.	mod. - high	low	mod. - high	low-mod.	*(Montefiori et al., 2009)*	Globular chromoplast	*(Montefiori et al., 2009)*
Loquat, yellow-peel, white flesh	*Eriobotrya japonica* (Thunb.) Lindl.	high	low	mod.		*(Fu et al., 2012)*	globular-tubular chromoplast	*(Fu et al., 2012)*
Loquat, red-peel, orange flesh	*Eriobotrya japonica* (Thunb.) Lindl.	very high	low-mod.	high	high	*(Fu et al., 2012, Breithaupt and Bamedi,*	globular-tubular chromoplast with some crystalloids	*(Fu et al., 2012)*

				2001)		
Mango	*Mangifera indica* L.	high - very high	mod.	*(Britton and Khachik, 2009, Breithaupt and Bamedi, 2001)*	Globular-tubular chromoplast	*(RW.ERROR - Unable to find reference:568)*
Marigold flowers	*Tagetes erecta* L.		high - very high	*(Gregory et al., 1986)*	Globular and Globular-tubular chromoplasts	*(Sitte et al., 1980, Vanegas-Espinoza et al., 2011)*
Orange, flesh	*Citrus sinensis* (L.) Osbeck	low-mod.	high	*(Britton and Khachik, 2009, Breithaupt and Bamedi,*	Unknown type	

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						2001)		
Orange, Cara Cara	*Citrus sinensis* (L.) Osbeck "Cara Cara"	low-mod.		very high		*(Kalkan, 2013)*	Crystalloid chromoplast	*(Kalkan, 2013)*
Papaya, red-fleshed	*Carica papaya* L.	mod.	mod. - high	mod. - high	high - very high	*(Schweiggert et al., 2011, Schweiggert et al., 2012b, Britton and Khachik, 2009)*	Crystalloid (lycopene) globular-tubular (rest) chromoplasts	*(Schweiggert et al., 2011)*
Papaya, yellow-fleshed	*Carica papaya* L.	mod.	mod. - high		high - very high	*(Schweiggert et al., 2012b, Schweiggert et al., 2011)*	Globular-tubular chromoplast	*(Schweiggert et al., 2011)*

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Commodity	**Lat. name**	**βC**	**βCRP**	**LUT**	**ZEA**	**LYC**	**CAR-esters**	**Irregular Carotenoids**	***Carotenoid Ref.***	**Predominant plastid type**	***Plastid Ref.***
Peach	*Prunus persica* (L.) Batsch	high					high		*(Britton and Khachik, 2009, Breithaupt and Bamedi, 2001)*	Unknown tyüe	-
Peach palm	*Bactris gasipaes* Kunth	very high				high		carotenoid (Z)-isomers:	*(Hempel et al., 2014)*	Globular chromoplast	*(Hempel et al., 2014)*

					very high			
Pepper, red	*Capsicum annuum* L.	high	mod.	very high	very high	*(Britton and Khachik, 2009, Breithaupt and Bamedi, 2001)*	Globular-tubular chromoplast	(Sitte et al., 1980, Frey-Wyssling and Kreutzer, 1958, Spurr and Harris, 1968)
Pepper, orange	*Capsicum annuum* L.	high	mod.	very high	very high	*(Britton and Khachik, 2009, Breithaupt and Bamedi, 2001)*	Globular-tubular chromoplast	*(Sitte et al., 1980)*
Pepper, yellow	*Capsicum*			very	very	*(Britton and*	Globular-tubular	*(Spurr and Harris,*

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	annuum L.			high		high	*Khachik, 2009, Breithaupt and Bamedi, 2001)*	chromoplast	*1968)*
Pepper, green	*Capsicum annuum* L.	high		very high			*(Britton and Khachik, 2009)*	Chloroplast	*(Camara and Brangeon, 1981)*
Pepper, brownish red („stay-green“)	*Capsicum annuum* L.	very high	very high		very high	n.a.	*(Roca et al., 2006, Hornero-Méndez et al., 2002)*	Chlorochromoplast	*(Roca et al., 2006)*
Persimmon	*Diospyros kaki* Thunb.		high		mod.	low-high	*(Britton and Khachik, 2009,*	Unknown type	-

								Breithaupt and Bamedi, 2001)		
Physalis	*Physalis* sp.	very high	mod.			mod.		*(Pintea et al., 2005a, Breithaupt and Bamedi, 2001)*	Tubular chromoplast	*(Sitte et al., 1980)*
Potato, white	*Solanum tuberosum* L.	low	low	low	low-mod.	low-mod.		*(Breithaupt and Bamedi, 2002)*	Uncharacterized Amylochromoplast assumed	(Lopez et al., 2008)
Potato, yellow	*Solanum tuberosum* L.	low	low	low	low	low-mod.		*(Breithaupt and Bamedi, 2002)*	Uncharacterized Amylochromoplast assumed	(Lopez et al., 2008)
Rowan berries	*Sorbus aucuparia* L.						"highly hydroph	*(Emter et al., 1990)*	Tubular chromoplast	*(Emter et al., 1990)*

								obic pigments"			
Dog rose hips	*Rosa canina* L.							"highly hydrophobic pigments"	*(Emter et al., 1990)*	Tubular chromoplast	*(Emter et al., 1990)*
Commodity	**Lat. name**	**βC**	**βCRP**	**LUT**	**ZEA**	**LYC**	**CAR-esters**	**Irregular Carotenoids**	***Carotenoid Ref.***	**Predominant plastid type**	***Plastid Ref.***
Rugosa rose	*Rosa rugosa*							"highly	*(Emter et al.,*	Tubular	*(Emter et al., 1990)*

hips	Thunb.							hydrophobic pigments"	*1990)*	chromoplast	
Saffron	*Crocus sativus* L.							crocetin esters: very high	*(Anastasaki et al., 2010)*	Tubular chromoplast	*(Grilli Caiola and Canini, 2004)*
Sea buckthorn	*Hippophae rhamnoides* L.	high	low	low-mod.	high	high	very high		*(Andersson et al., 2009, Pintea et al., 2005b)*	Unknown type	-
Squash	*Cucurbita pepo* L.	low-high	mod.-high	mod.-very	mod.		mod.		*(Britton and Khachik, 2009,*	Globular chromoplast	*(Devidé and Ljubešic, 1972)*

				high		*Breithaupt and Bamedi, 2001)*		
Squash	*Cucurbita maxima* Duch.	high	low	mod. - very high	high - very high	*(Khachik and Beecher, 1988)*	Tubular chromoplast	*(Ljubešić, 1977)*
Squash (butternut squash)	*Cucurbita moschata* (Duchesne ex Lam.) Duchesne ex Poir	mod.		low	very high	*(Jeffery et al., 2012, Chitchumroon chokchai and Failla, 2006)*	Globular-tubular chromoplast	*(Jeffery et al., 2012)*
Sweet potato	*Ipomoea batatas* (L.) Lam.	very high				*(Britton and Khachik, 2009)*	Crystalloid amylochromoplast	*(Jeffery et al., 2012, Purcell et al., 1969)*

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Tomato, red	*Lycopersicon esculentum* L.	mod. - high	high - very high		*(Britton and Khachik, 2009, Schweiggert et al., 2011)*	Crystalloid chromoplast	*(Schweiggert et al., 2011, Harris and Spurr, 1969b)*
Tomato, high-beta	*Lycopersicon esculentum* L.	very high			*(Kopec et al., 2014)*	Crystalloid chromoplast	*(Harris and Spurr, 1969a)*
Tomate, tangerine	*Lycopersicon esculentum* L.		low	carotenoid (Z)-isomers: very high	*(Cooperstone et al., 2015)*	Globular chromoplast	*(Cooperstone et al., 2015)*
Tomato, delta-mutant	*Lycopersicon esculentum* L.			δ-carotene:	*(Britton and Khachik, 2009)*	Crystalloid chromoplast	*(Harris, 1970)*

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								high			
Tomato, low pigment	*Lycopersicon esculentum* L.	low		low		low			*(Cooperstone et al., 2012)*	Globular chromoplast	*(Harris and Spurr, 1969a)*

Commodity	**Lat. name**	**βC**	**βCRP**	**LUT**	**ZEA**	**LYC**	**CAR-esters**	**Irregular Carotenoids**	***Carotenoid Ref.***	**Predominant plastid type**	***Plastid Ref.***
Tomato, green flesh mutant	*Lycopersicon esculentum* L.	mod.				very high			*(Ramirez and Tomes, 1964)*	Chlorochromoplast	*(Cheung et al. 1993)*
Watermelon, red flesh	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	low-mod.		low		high - very high			*(Yoo et al., 2012)*	Crystalloid chromoplast	*(Sitte et al., 1980)*

Wheat flour	*Triticum* sp.	low-mod	*(Panfili et al., 2004, Ziegler et al., 2015)*	Amylochromoplasts in endosperm	*(Howitt and Pogson, 2006)*

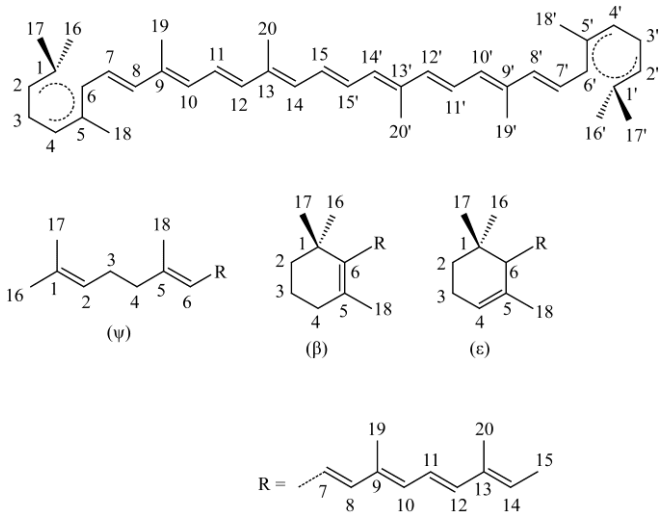

Figure 1 Basic carotenoid structure and terminal groups of the most common nutritionally-relevant carotenoids (Weedon and Moss, 1995).

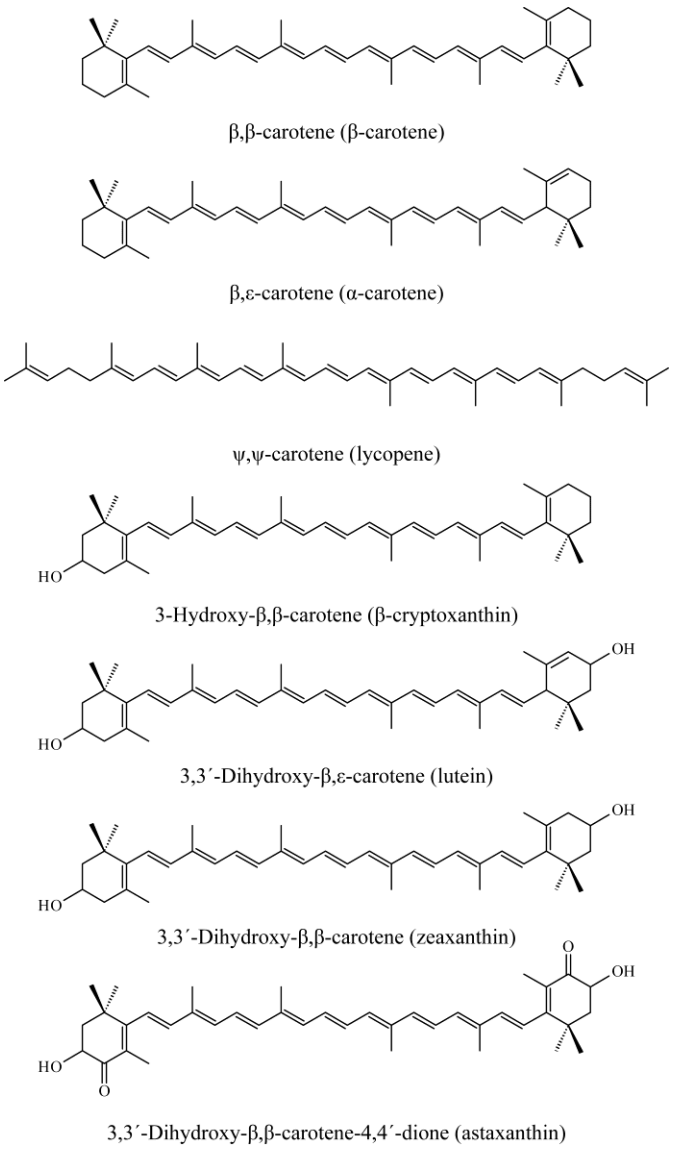

Figure 2 Most common nutritionally-relevant carotenoids in plant and animal foods.

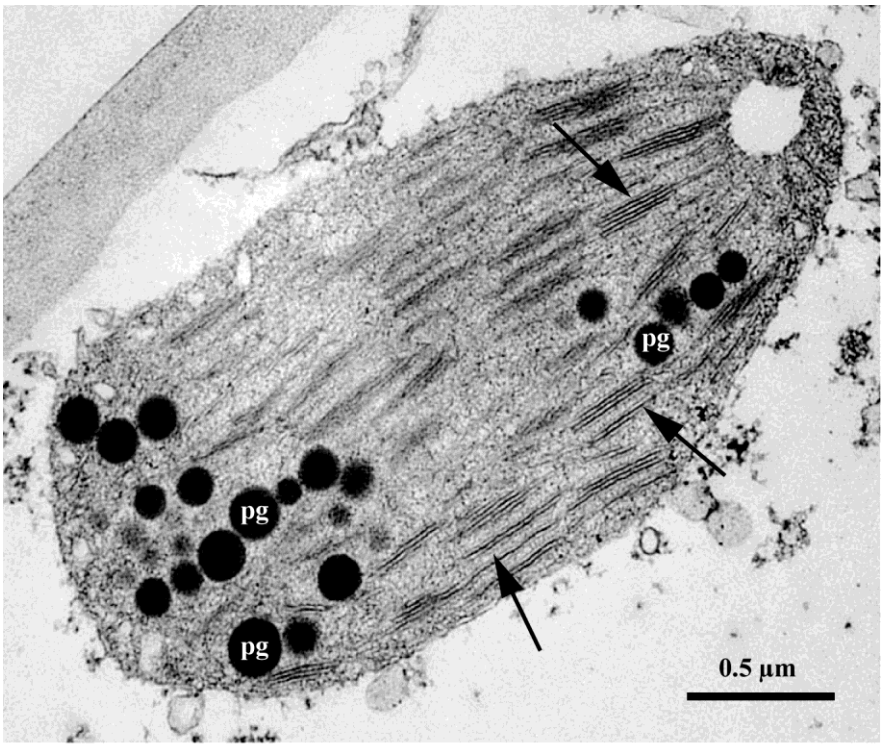

Figure 3 Transmission electron micrograph of a chloroplast from green tomato fruit tissue (with permission from (Schweiggert, 2014). *Arrows:* grana thylakoids (thylakoid stacks), *pg*: plastoglobules.

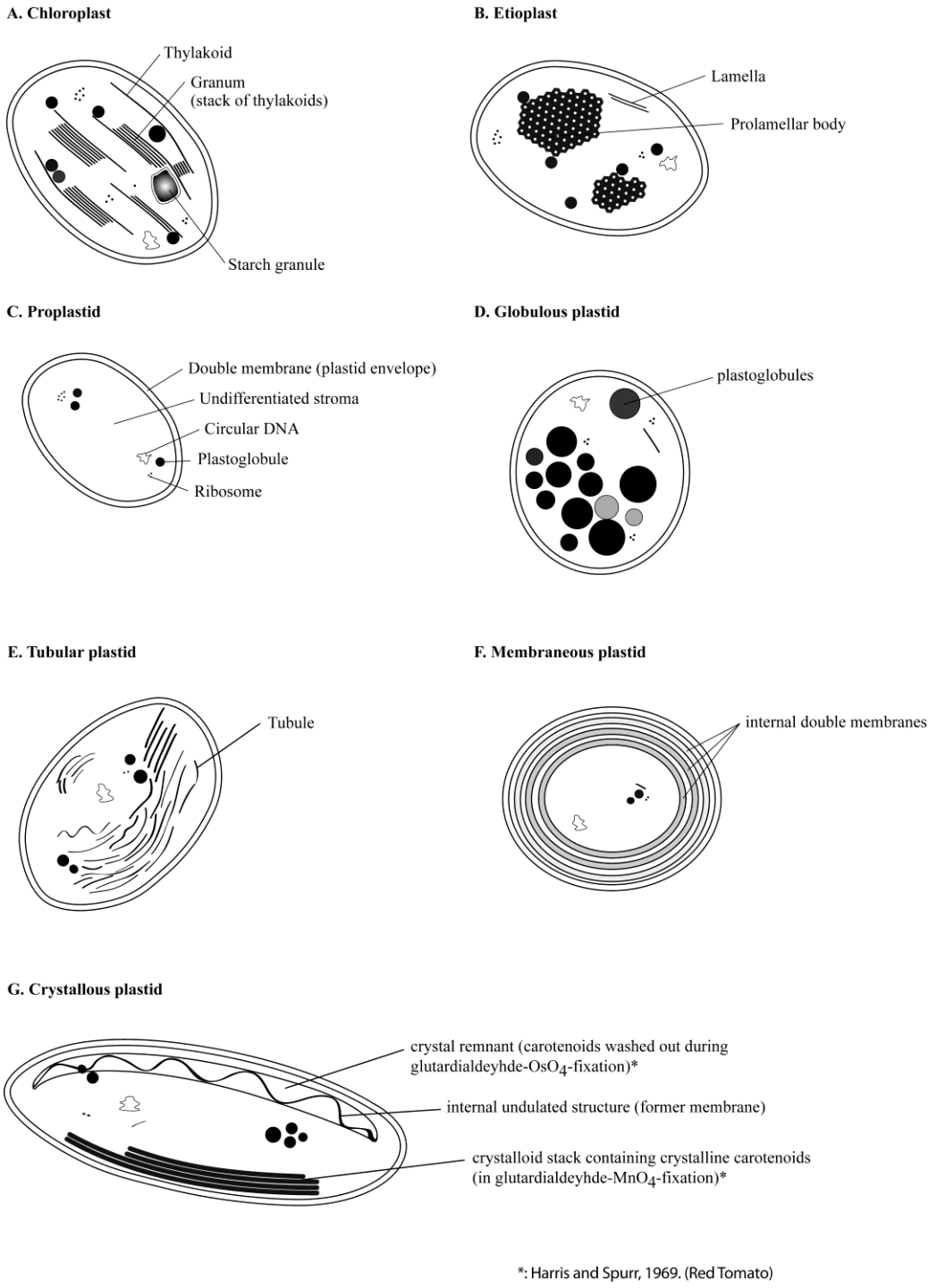

Figure 4 Schematic representation of major plastid types found in plant foods.

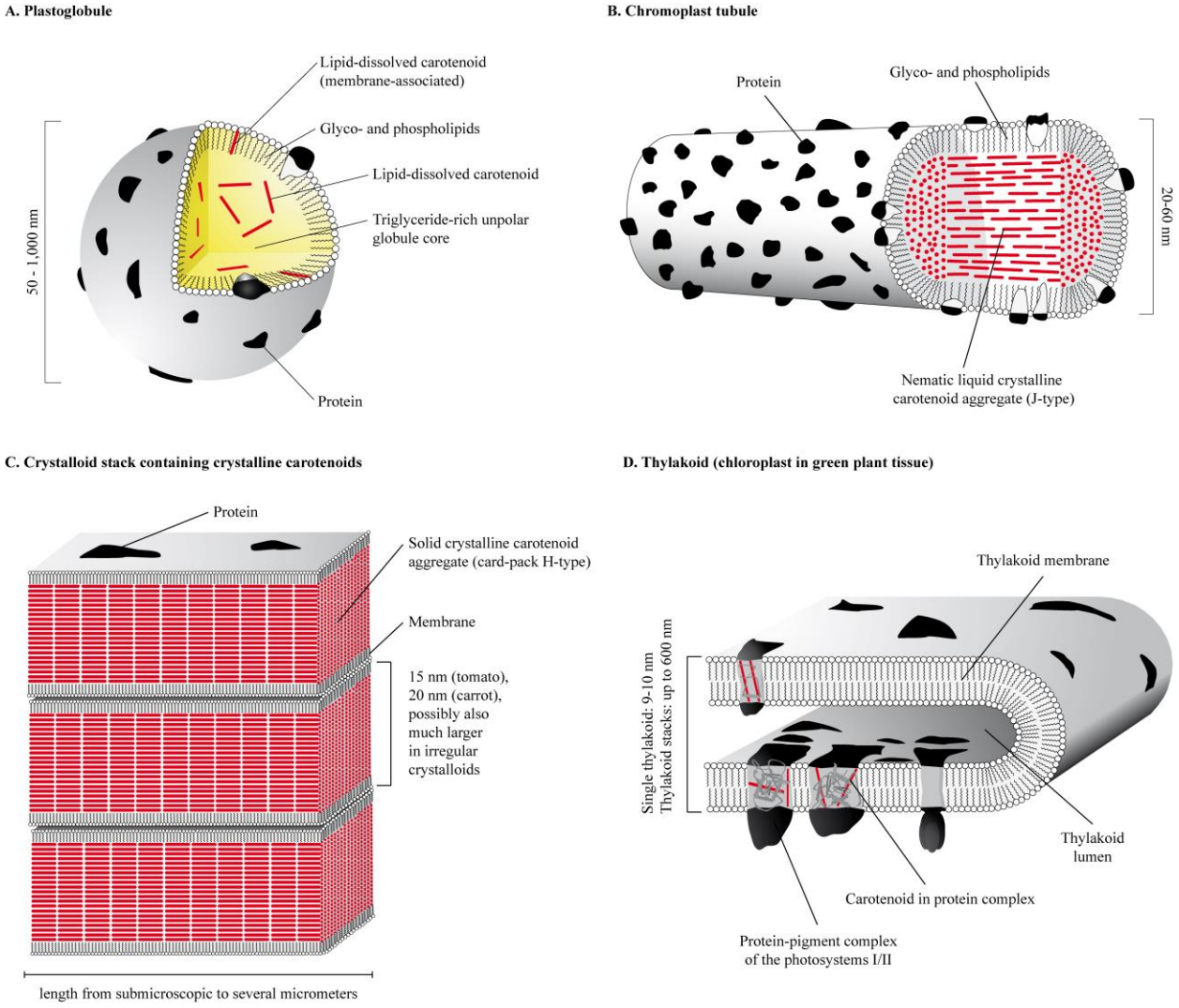

Figure 5 Carotenoid deposition forms in plant foods. Graphical reproduction of content reported by Sitte et al. (1980), Sitte (1981), and Harris and Spurr (1969b).

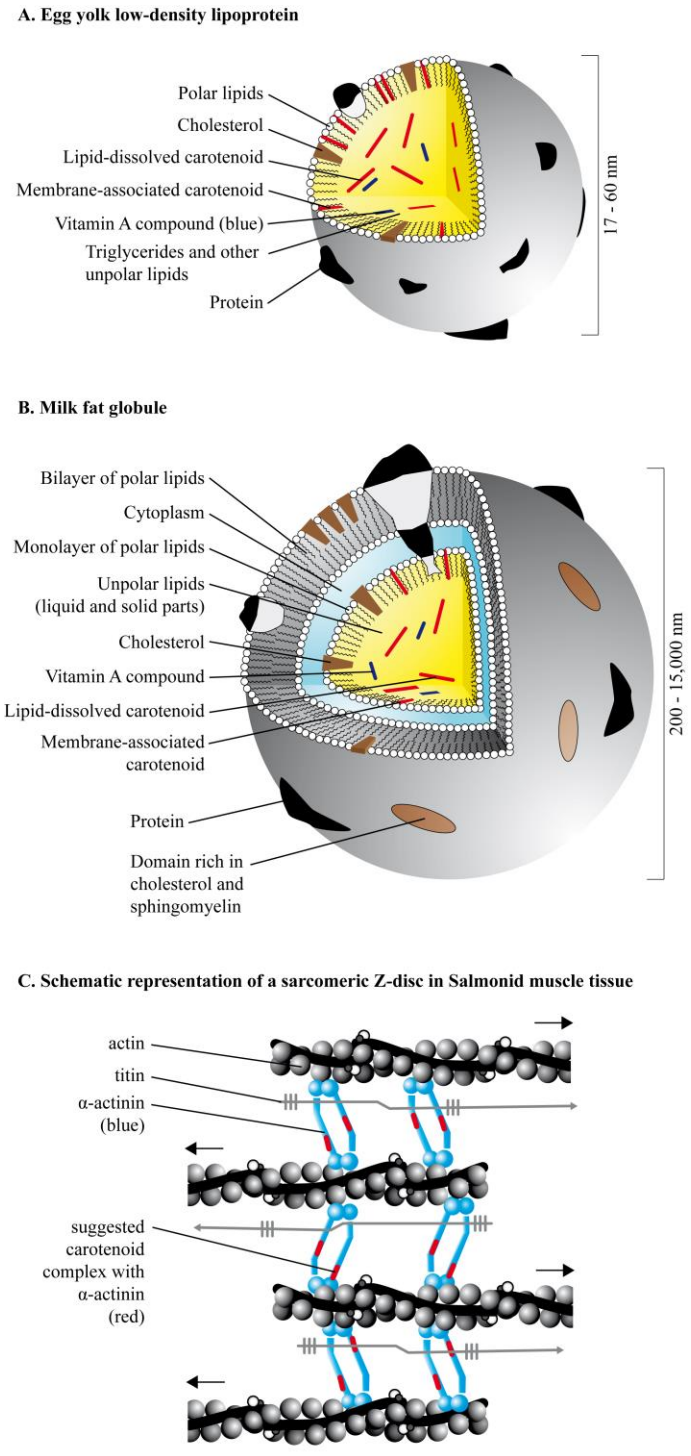

Figure 6 Carotenoid deposition forms in animal foods. Graphical reproduction of content reported previously (Sjöblom et al., 2008, Keenan and Mather, 2006, Anton, 2013)).