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Nutritionally important carotenoids as consumer products

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Abstract Carotenoids are nutritionally-beneficial organic tetraterpenoid pigments synthesized mainly by plants, bacteria and fungi. Although research has focused on the production of carotenoids in staple crops to improve nutritional welfare in developing countries, there is also an enormous market for carotenoids in the industrialized world, where they are produced both as commodities and luxury goods targeted at the pharmaceutical, nutraceutical, food/feed additive, cosmetics and fine chemicals sectors. Carotenoids are economically valuable because they have diverse bioactive and chemical properties. Some are essential nutrients (e.g. β-carotene), others are antioxidants with specific roles (e.g. lutein and zeaxanthin) or general health-promoting roles that reduce the risk or progression of diseases associated with oxidative stress (e.g. lycopene), and still others are natural pigments (e.g. astaxanthin, which is added to fish feed to impart a desirable pink flesh color). Even carotenoid degradation products, such as damascones and damascenones, are used as fragrances in the perfumes industry. Here we discuss the importance of carotenoids in different market sectors, review current methods for commercial production and its regulation, summarize the most relevant patents and consider evidence supporting the health claims made by different industry sectors, focusing on case studies representing the most commercially valuable carotenoids on the market: β-carotene, lycopene, lutein, zeaxanthin and astaxanthin.

Keywords Health claims · Intellectual property · Market production · Nutraceuticals · Regulation

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Abbreviations

CAGR	Compound annual growth rate
CBFD	Carotenoid β-ring 4-dehydrogenase
CRTISO	Carotenoid isomerase
DMAPP	Dimethylallyl diphosphate
DSHEA	Dietary supplement health and education act
EFSA	European food safety authority
FDA	Food and drug administration
GGPP	Geranyl geranyl diphosphate
GGPPS	Geranyl geranlyl diphosphate synthase
HBFD	4-Hydroxy-β-ring 4-dehydrogenase
HYDB	β-Carotene hydroxylase
IPP	Isopentenyl diphosphate
LYCB	Lycopene β-cyclase
LYCE	Lycopene ε-cyclase
MEP	Methylerythritol 4-phosphate
PDS	Pytoene desaturase
PSY	Phytoene synthase
USPTO	United States patent and trademark office
VAD	Vitamin A deficiency
ZDS	ζ-Carotene desaturase
Z-ISO	ζ-Carotene isomerase

Introduction

Structure and classification of carotenoids

Carotenoids are natural pigments that are synthesized in the plastids of plants and in some other photosynthetic organisms such as algae, bacteria and fungi (Zhu et al. 2010). Humans and most animals cannot synthesize carotenoids, and must obtain them from dietary sources. The notable exceptions are the red pea aphid (*Acyrthosiphon pisum*) and the two-spotted spider mite (*Tetranychus urticae*), which have acquired the ability to produce carotenoids from fungi by horizontal gene transfer (Moran and Jarvik 2010; Altincicek et al. 2012).

Hydrocarbon-only carotenoids are known as carotenes (e.g. α-carotene, β-carotene and lycopene), whereas the more complex xanthophylls contain oxygen in hydroxyl groups (e.g. lutein and zeaxanthin), keto/oxo groups (e.g. echinenone and canthaxanthin) epoxide groups (e.g. violaxanthin, antheraxanthin and neoxanthin) and/or methoxy groups (e.g. spirilloxanthin). Most carotenoids are tetraterpenoids, with 40 carbon atoms derived from the condensation of eight isoprene precursors. All carotenoids have a polyisoprenoid structure comprising a long conjugated chain rich in double bonds and with near symmetry around the central double bond. This basic acyclic structure can be modified by the cyclization of the end groups or the introduction of oxygen-rich functional groups, to yield a large family of >800 compounds, not including *cis*/*trans* isomers (Britton et al. 2004). Because of their polyene structure, carotenoids are efficient free-radical scavengers with singlet oxygen quenching properties and the ability to trap peroxyl radicals (Krinsky 1998; Rice-Evans et al. 1997).

Uses of carotenoids

Carotenoid which contain at least one un-substituted β-ionone ring, (β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin) can be converted into retinal by humans and animals, and are therefore classified as pro-vitamin A carotenoids (Bai et al. 2011). Vitamin A deficiency (VAD) weakens the immune system, causes the deterioration of light-sensitive rod cells required for low-light vision, and in extreme cases can lead to an irreversible form of blindness called xerophthalmia (Bai et al. 2011; Farré et al. 2011). Lutein and zeaxanthin accumulate in the macula of the eye protecting the retina from damaging blue and near-ultraviolet light (Landrum and Bone 2001). Therefore, individuals with a carotenoid-rich diet may be protected against age-related macular degeneration (Fraser and Bramley 2004; Hammond et al. 1997), a disease that affects 30 % of people over 75 years of age (Mozaffarieh et al. 2003). Lycopene is a potent antioxidant and reduces the risk of coronary heart disease and certain cancers (Fraser and Bramley 2004; Knekt et al. 1994).

The beneficial properties of carotenoids reflect the observed correlation between carotenoid-rich diets and protection against chronic illnesses, also suggesting that the most potent effects are conferred by multiple carotenoids in combination (Diplock et al. 1998; Van Poppel 1996). The consumption of raw tomato (*Solanum lycopersicum*) fruits protects against cancers of the oral cavity, pharynx, esophagus, stomach, colon and rectum, with the most potent effects seen with stomach neoplasia (Franceschi et al. 1994). However, inverse relationships have been reported between lycopene intake or serum lycopene values and the risk of cancer of the prostate, pancreas and stomach (Giovannucci et al. 1995; Mills et al. 1989). Combinations of natural carotenoids present in orange (*Citrus* × *sinensis*) juice also lower the risk of liver cancer (Nishino et al. 2009).

Ketocarotenoids, such as astaxanthin, have important applications in the nutraceutical, cosmetics and feed industries (Fassett and Coombes 2005; Zhu et al. 2009), reflecting their anti-inflammatory properties, ability to inhibit the oxidation of low-density lipoprotein and to produce animal pigmentation (Iwamoto et al. 2000). They also protect against cancer (Tanaka et al. 1994; Chew et al. 1999) and enhance the immune response (Jyonouchi et al. 1995). Astaxanthin is regarded as a potential novel treatment for oxidative stress and inflammation in cardiovascular diseases (Pashkow et al. 2008; Fassett and Coombes 2012).

Certain apocarotenoids resulting from carotenoid degradation are used as fragrances, e.g. β-cyclocitral, β-ionone, geranial, geranial acetone, theaspirone, α-damascenone and β-damascenone (Auldridge et al. 2006). Others are used as colorants. The yellow/orange compound bixin (in *Bixa orellana*) is widely used in cosmetics. It is also used in dairy foods, such as orange cheeses (e.g. Cheddar) to ensure color consistency (Kang et al. 2010).

The flavoring and coloring properties of saffron, a spice derived from stigmata of crocus plants (*Crocus sativus*), is due to the presence of the three major apocarotenoids crocin, crocetin and picrocrocin. The saffron apocarotenoids are only found in the red stigmata of crocus flowers (Bouvier et al. 2003). Saffron is also a medicinal product that confers protection against coronary heart disease and cancer (Bathaie and Mousavi 2010), and it is used as herbal remedy for cramps, asthma/bronchospasms, menstrual pain, liver disease and digestive disorders (Abdullaev 2002; Hensel and Rösing 2003).

Natural sources of carotenoids

Carotenoids are present in many fruits and vegetables. For example, β-carotene accumulates to high levels in many yellow-orange fruits and yellow vegetables such as carrots (*Daucus carota*), squash (*Cucurbita* spp.) and sweet potato (*Solanum tuberosum*), whereas β-cryptoxanthin is enriched in peach (*Prunus persica*), papaya (*Carica papaya)* and citrus fruits, particularly the Satsuma mandarin (*Citrus unshiu* MARC) (Farré et al. 2010; Bai et al. 2011). Lycopene is the red fruit pigment in tomato, watermelon (*Citrullus lanatus)*, pink grapefruit (*Citrus* × *paradisi*) and guava (*Psidium guajava*) (Bramley 2000), although the highest levels of lycopene are found in gac fruits (*Momordica cochinchinensis*) (Aoki et al. 2002). Lutein is the most abundant carotenoid in all green vegetables, often representing 50 % of the total carotenoid pool. In contrast, zeaxanthin is present in minute quantities in most foods (Sommerburg et al. 1998) although some varieties of yellow corn (*Zea mays*) and yellow and tabasco pepper (*Capsicum* spp.) provide adequate amounts (Quackenbush et al. 1963; Minguez-Mosquera and Hornero-Mendez 1994).

Astaxanthin is found in microalgae, yeast, salmon (*Salmo salar*), trout (*Salmo trutta*), krill, shrimp (*Peneaus* spp.), crayfish, crustaceans and the feathers of some birds. It confers the pink color of salmon flesh and the pink-red color of cooked shellfish (Zhu et al. 2009). The oil-soluble apocarotenoid bixin and its water-soluble analog 9′-*cis*-norbixin account for 80 % of the total carotenoids present in achiote seeds (*B. orellana*) (Rivera-Madrid et al. 2006).

The carotenoid biosynthesis pathway

Terpenoid biosynthesis begins with the condensation of three molecules of isopentenyl diphosphate (IPP) with one molecule of dimethylallyl diphosphate (DMAPP) to produce the C20 compound geranylgeranyl diphosphate (GGPP). In plants, this reaction is catalyzed by GGPP synthase (GGPPS) in the plastids (Chappell 1995) and the equivalent enzyme in bacteria is CrtE (Fig. 1). The isomeric precursors IPP and DMAPP are derived predominantly from the plastidial methylerythritol 4-phosphate (MEP) pathway although the same precursors are formed by the cytosolic mevalonic acid (MVA) pathway, with which there may be some cross-talk (Rodriguez-Concepcion 2006).

The first committed step in plant carotenoid biosynthesis is the condensation of two GGPP molecules into 15-*cis*-phytoene by the enzyme phytoene synthase (PSY) (Misawa et al. 1994) and the equivalent enzyme in bacteria is CrtB. This intermediate then undergoes a two-step desaturation reaction in plants catalyzed by phytoene desaturase (PDS) to generate 9,15-*cis*-phytofluene and then 9,15,9′-tri-*cis*-

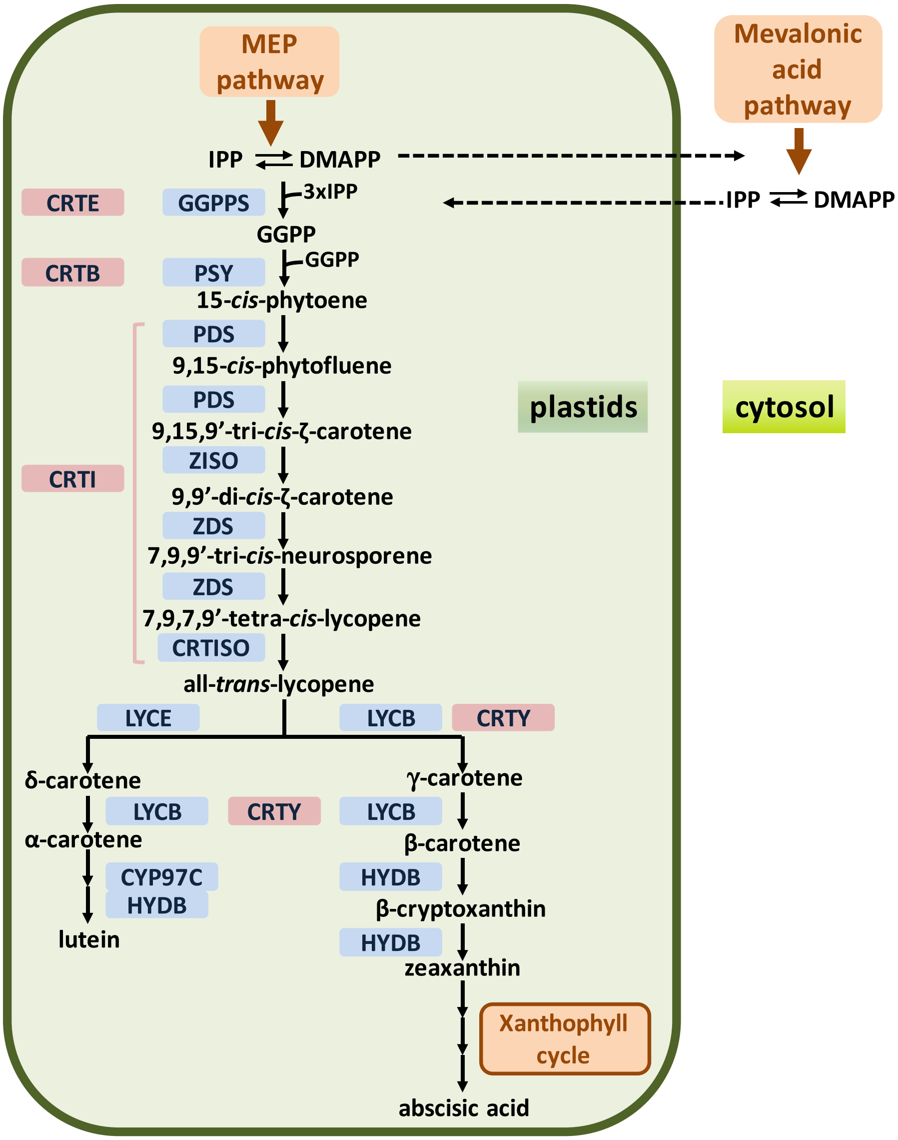

Fig. 1 The carotenoid biosynthesis pathway in plants (*blue*) and equivalent steps in bacteria (*red*). *IPP* Isopentenyl diphosphate, *DMAPP* dimethylallyl diphosphate, *GGPP* geranylgeranyl diphosphate, *GGPPS* GGPP synthase, *PSY* phytoene synthase, *PDS* phytoene desaturase, *ZISO* ζ-carotene isomerase, *ZDS* ζ-carotene desaturase, *CRTISO* carotenoid isomerase, *LYCB* lycopene β-cyclase, *LYCE* lycopene ε-cyclase, *CYP97C* carotene ε-ring hydroxylase, *HYDB* β-carotene hydroxylase, *CRTE* bacterial GGPP synthase, *CRTB* bacterial phytoene synthase, *CRTI* bacterial phytoene desaturase/isomerase, *CRTY* bacterial lycopene cyclase, *CRTZ* bacterial β-carotene hydroxylase, *CRTW* bacterial β-carotene ketolase (modified from Breitenbach and Sandmann 2005 and Cunningham et al. 1996). (Color figure online)

ζ-carotene. This is isomerized by light and/or ζ-carotene isomerase (Z-ISO) to yield 9,9′-di-*cis*-ζ-carotene, which is converted by ζ-carotene desaturase (ZDS) into 7,9,9′-tri-*cis*-neurosporene and then 7,9,7′9′-tetra-*cis*-lycopene (Li et al. 2007; Chen et al. 2010). The end product of the desaturation reactions is converted to all-*trans*-lycopene by carotenoid isomerase (CRTISO) in non-green tissue, and by light and chlorophyll (acting as a sensitizer) in green tissue (Breitenbach and Sandmann 2005; Isaacson et al. 2004; Li et al. 2010). In non-photosynthetic bacteria, the single enzyme CrtI accomplishes all the above steps and produces all-*trans*-lycopene directly from 15-*cis*-phytoene (Fig. 1).

Lycopene is an important branch point in the carotenoid pathway because it acts as the substrate for two competing enzymes: lycopene β-cyclase (LYCB), and lycopene ε-cyclase (LYCE) (Cunningham et al. 1996). Both enzymes cyclize the linear backbone to generate terminal α- or β-ionone rings, differing by the 4,5- or 5,6-position of the double bond. The addition of one β-ring by LYCB generates γ-carotene, and the addition of a second β-ring to the free end by the same enzyme produces β-carotene. This reaction is rapid, so γ-carotene tends not to accumulate. In bacteria, this reaction is carried out by CrtY. Alternatively, the addition of one ε-ring to lycopene by LYCE generates δ-carotene. This is a poor substrate for LYCE so it is unusual for the second ε-cyclization to take place, but it is a good substrate for LYCB, which adds a β-ring to the free end to produce α-carotene or zeinoxanthin depending on the ring where the reaction takes place. In the presence of the enzyme β-carotene hydroxylase (HYDB) (Fig. 1), both α-carotene and β-carotene can be converted into more complex downstream carotenoids. In the case of α-carotene, this downstream product is lutein, and in the case of β-carotene the downstream product is zeaxanthin, although the reactions involve the intermediates α-cryptoxanthin and β-cryptoxanthin, respectively. A single hydroxylase is required to produce zeaxanthin but two different hydroxylases are essential for the synthesis of lutein (Kim et al. 2009). In bacteria, a functionally similar enzyme is CrtZ. Whereas lutein represents the natural end point of the α-carotene branch, zeaxanthin can be further converted to 5,6-epoxy derivatives, which are part of the xanthophyll cycle. This cycle involves the enzymatic removal of epoxy groups from violaxanthin, antheraxanthin and zeaxanthin which play a critical role in stimulating energy dissipation in photosystem II. At the end of the pathway these products can be converted through a number of additional steps into the important plant hormone abscisic acid (Seo and Koshiba 2002) (Fig. 1).

An alternative pathway is found in *Adonis aestivalis*, which synthesizes the important ketocarotenoid astaxanthin from β-carotene. Carbon 4 in the β-ring is activated by a carotenoid β-ring 4-dehydrogenase (CBFD), allowing further dehydrogenation to yield a carbonyl group in a reaction catalyzed by a carotenoid 4-hydroxy-β-ring 4-dehydrogenase (HBFD) and the addition of a hydroxyl group to carbon 3 in a reaction catalyzed by CBFD (Cunningham and Gantt 2011). In *Paracoccus* sp. N81106, β-carotene is converted to astaxanthin by a β-carotene ketolase (CrtW) and a β-carotene hydroxylase (CrtZ) (Misawa et al. 1995).

Commercial production of carotenoids

Carotenoid production by industrial fermentation

Carotenoids can be produced by industrial fermentation, usually comprising a growth phase to increase microbial biomass followed by a production phase where the biomass remains constant but carotenoid synthesis is increased. For example, cultures of *Blakeslea trispora* produce up to 44.5 mg β-carotene per g biomass after 4 days growth and 4 days production initially at pH 11.0 (Nanou et al. 2012). Similarly, heterotrophic fed-batch cultures of *Chlorella protothecoides* grown under nitrogen-limiting conditions produce 225 μg lutein per ml culture volume after 10 days (Shi et al. 2002) and *Dunaliella salina* cultures produce up to 6 mg zeaxanthin per g of biomass (Jin et al. 2003).

The yields of astaxanthin are much lower in fungi [0.40 % dry weight (DW)] than in algae such as *Haematococcus* spp. (up to 3.0 % DW), and production can be induced by unfavorable growth conditions or environmental stresses such as phosphate or nitrogen starvation, salinity, or high light intensity (Boussiba and Vonshak 1991). High-density fed-batch fermentation of *Haematococcus pluvialis* has produced up to 64.4 mg astaxanthin per/l but only low cell density can be achieved with traditional media (Zhang et al. 1999). More efficient scale-up is therefore required in order to compete with the production of synthetic astaxanthin (Bhosale and Bernstein 2005). Recently a mutant of *Xanthophyllomyces dendrorhous* has been reported to accumulate up to 9.7 mg astaxanthin per g DW in a fermenter culture (Gassel et al. 2013).

Carotenoid extraction from plant sources

As the demand for carotenoids increased, research shifted from chemical synthesis to natural products from plants and microorganisms as biological sources. Carotenoids are isolated mainly by solvent extraction, solid phase extraction (SPE), supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) (Gua et al. 2008; Rodriguez-Amaya et al. 2008; Simkin et al. 2008; Mustafa et al. 2012). Solvent extraction is applicable to all carotenoids because of its simplicity and low cost. Several chemicals are then added to precipitate carotenoids, including calcium chloride, calcium hydroxide, calcium lactate or calcium gluconate. The carotenoid-enriched solid precipitate is then separated from the carotenoid-depleted liquid. Typical carotenoid sources include carrots (*D. carota*), palm oil (*Arecaceae* spp.) and alfalfa (*Medicago sativa*) for β-carotene, marigold (*Tagetes erecta*) for lutein, yellow maize (*Z. mays*) for lutein and/or zeaxanthin (depending on the maize variety), and tomato (*S. lanum lycopersicum*) for lycopene. Lutein and zeaxanthin are often added to chicken feed to improve egg yolk color. This is also the case for paprika powder with red capsanthin as coloring pigment.

Chemical synthesis of carotenoids

Chemical synthesis is often used to manufacture carotenoids, particularly β-carotene and astaxanthin. A method was developed in the 1950s to synthesize β-carotene by assembling the C40 backbone in either a symmetrical manner (e.g. C20 + C20) or an asymmetrical manner (e.g. C20 + C6 + C6 + C3 + C3 + C2) (Dawson 2009; Ernst 2002). The principal industrial method involves the Wittig reaction (Pommer and Thieme 1983) in which an aldehyde or ketone reacts with a triphenyl phosphonium ylide nucleophile to produce an alkene and triphenylphosphine oxide. A three-stage procedure was later developed by BASF (Germany), involving the production of vinyl-β-ionol from β-ionone, followed by condensing the phosphonium chloride salt of vinyl-β-ionol with two molecules of a symmetrical C10 dialdehyde (Pommer and Thieme 1983).

Astaxanthin synthesis also involves a Wittig reaction between two equivalents of a C15-phosphonium salt with a C10-dialdehyde. This may also be achieved through a symmetrical C10 + C20 + C10 reaction involving dienol ether condensation (Rüttimann 1999). Partial chemical synthesis can also be used to manufacture astaxanthin, including the hydroxylation of canthaxanthin (Bernhard et al. 1984). Although chemical synthesis produces a mixture of stereoisomers with limited applications (Scaife et al. 2012), 97 % of commercial astaxanthin is produced in this manner because it is nearly four times more expensive to extract astaxanthin from natural sources (Schmidt et al. 2011).

Partial chemical synthesis may also be used to increase the aqueous solubility of xanthophylls because the natural compounds are only sparingly soluble. Hawaii Biotech, Inc. and others have been able to increase the solubility of carotenoid derivatives by chemical modification (Nadolski et al. 2006). For example, the phosphorylation of lutein hydroxyl groups followed by deprotection to yield free phosphates allows the formation of a lutein diphosphate sodium salt, which has an aqueous solubility of 29.7 mg/ml. This is easier to absorb in the human gut and there is no loss of free radical scavenging or singlet oxygen quenching activity (Nadolski et al. 2006).

Commercial sources of carotenoids

Chemically-synthesized β-carotene accounts for 90 % of the market, most produced by DSM (Heerlen, Netherlands) or by BASF (Raja et al. 2007). The remaining 10 % is sourced naturally, e.g. *B. trispora* is used by DSM and by its Spanish subsidiary, Vitatene; *Sphingomonas* spp. is used by Biotrend in Portugal; and the marine alga *D. salina* is used by Aquacarotene Ltd and Cognis Australia Pty Ltd (a BASF subsidiary) and Nature Beta Technologies Eilat at some of the world's largest algal farms in Australia and Israel, respectively. Under appropriate culture conditions, this algal species accumulates up to 10 % of its biomass as carotenoids (Lamers et al. 2008) mostly as β-carotene (Ben-Amotz et al. 1982).

The major commercial sources of lycopene are red tomatoes and the fungus *B. trispora* (Garcia and Barrett 2006; Soroka et al. 2012). However, synthetic lycopene can be produced at 96 % purity, typically containing the same proportions of 9-*cis*- and 13-*cis* isomers as found in raw tomatoes, and the same proportion of 5-*cis* lycopene as found in cooked tomatoes and human blood plasma (Rao et al. 2003).

Although many fruits and vegetables contain lutein, the best commercial source is marigold flowers (*T. erecta*) which typically contain 0.6–2.5 % by DW of xanthophylls, up to 92 % of which is lutein (Tsao et al. 2004).

Astaxanthin is manufactured predominantly by chemical synthesis. Biological production systems have also been developed using the yeast *X. dendrorhous* (formerly *Phaffia rhodozyma*) and the green alga *H. pluvialis*, which is the major natural source because it can accumulate more than 30 mg astaxanthin per g DW (Johnson and An 1991; Suseela and Toppo 2006).

Market supply and demand

The global market for carotenoids was US$1.2 billion in 2010 and is projected to grow to US$1.4 billion by 2018 with a compound annual growth rate (CAGR) of 2.3 % (BCC Research 2011). The carotenoid market can be broken down by compound, representing 10 submarkets for β-carotene, lutein, astaxanthin, capsanthin, annatto, canthaxanthin, lycopene, β-apo-8-carotenal, zeaxanthin and β-apo-8-carotenal-ester. The largest market for individual carotenoids is β-carotene (US$261 million in 2010, projected to grow to $334 million by 2018, CAGR 3.1 %) whereas the fastest growing segment is lutein (US$233 million in 2010, projected to grow to US$309 million in 2018, CAGR 3.6 %).

Carotenoids produced by chemical synthesis dominate the global market, although the natural carotenoids sector is growing rapidly (Global Industry Analysts 2011). The United States of America (USA), Japan and Europe are the major consumers, but there is also increasing demand in emerging economies such as China, India and Malaysia. The animal feed sector is the largest end-use sector, primarily reflecting the demand for canthaxanthin and astaxanthin in aquaculture. The human use of carotenoids for nutrition, pharmaceuticals and cosmetics is another large end-use sector, and the market share in Europe for lycopene, β-carotene and lutein is shown in Supplementary Table 1. These end-use sectors have blurred boundaries, as exemplified by the fast growing cosmeceuticals and nutricosmetics sectors, both of which combine the properties of cosmetics and pharmaceuticals to promote skin health, with the former applied topically and the latter ingested (Anunciato and da Rocha Filho 2012; Draelos 2010).

Health claims

Health claims such as '…promotes cardiovascular, prostate and skin health…' are present on some carotenoid-derived supplements and carotenoid-containing processed foods in the USA, and under the Dietary Supplement Health and Education Act of 1994 (DSHEA) they are treated as a category of food rather than drugs and must carry the following qualification:

"These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease."

In the European Union (EU), health claims made in relation to food products require authorization under Regulation (EC) 1924/2006 before they can be used in labeling or marketing, and the claims must be substantiated by the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA). In this context, it is permitted within the EU to claim that β-carotene promotes the maintenance of normal vision, skin and mucosa, because of its relationship with vitamin A (EFSA 2010). However, it is not permitted to claim health benefits for other carotenoids, such as the need for lutein and zeaxanthin to maintain normal vision (EFSA 2011a), the ability of lycopene to protect DNA, proteins and lipids from oxidative damage, protect the skin from UV-induced damage, contribute to normal cardiac function and maintain normal vision (EFSA 2011b), or the ability of astaxanthin to protect the skin from UV-induced damage (EFSA 2011c).

In the EU, the use of health claims to promote products containing protein, carbohydrate, fiber, sodium, vitamins and minerals was harmonized in 2006 (Regulation 1924/2006) but this only covers β-carotene and not the other carotenoids. All the carotenoids discussed in this article can be used as food additives (Commission Regulation 231/2012), food supplements (Commission Regulation 1170/2009) and cosmetics (Regulation 1223/2009) in Europe, but not as pharmaceutical compounds because the heath claims have yet to be assessed in clinical trials. The Food and Drug Administration (FDA) has also dismissed health claims for carotenoids, rejecting for example applications that lycopene reduces the risk of cancer, and that lutein esters reduce susceptibility to age-related macular degeneration and cataracts (FDA 2009).

The period of authorization for novel compounds is normally 10 years, but this can be extended either for a fixed period or indefinitely, as is the case for β-carotene and canthaxanthin (Commission Regulation

880/2004). Each time the same compound is produced using a different platform it must be evaluated and regulated as a new product to confirm its safety. Metabolic engineering to increase the production of antioxidants can contribute to better health and nutrition (Pérez-Massot et al. 2013; Zhu et al. 2013; Berman et al. 2013). However, this strategy would not be encouraged by the current industry in the EU because it would reduce the margins of the major players and they would need to embrace genetic engineering in plants which currently has a negative public and political perception and an excessive, onerous and costly regulatory framework.

Case studies

The carotenoid value chain involves a small number of manufacturers that supply raw materials to a larger number of intermediate companies producing formulated supplements and/or finished products for distribution to consumers or to larger companies in different sectors. The number of intermediate steps in the chain varies, and the involvement of one or more distributors and resellers can inflate the final consumer price. Some case studies are provided below to demonstrate the structure of the value chain for particular carotenoids, focusing on the key manufacturers and production platforms, market segmentation, and the principal regulatory and intellectual property (IP) constraints.

β-Carotene

In the EU, β-carotene is defined as a food additive (E160a) according to Commission Regulation (EU) 231/2012 and may be chemically synthesized, extracted from plants, or produced by the cultivation of *B. trispora* or *D. salina*. Commission Regulation (EC) 1170/2009 defines β-carotene as a vitamin A formulation and it can be added to foods, including food supplements, for this purpose. It is also

Table 1 Current commercial products based on β-carotene

Product	Receptor	Content	Formulation	Uses	Source	Production method	Company
CaroCare®	Humans	Variable from 1 to 30 %	Powder	Food colorant, food fortification, dietary supplements	Fungus (*Blakeslea trispora*)	Fermentation	DSM
ROVIMIX® β-carotene	Farm animals and pets	10 %	Powder	Animal nutrition in premixes and compound feeds	Fungus (*Blakeslea trispora*)	Fermentation	DSM
Lucarotin® Dispersions	Humans	Variable from 1 to 30 %	Powder	Food colorant and food fortification	Synthetic	Chemical synthesis	BASF
Beta-carotene	Humans	Variable from 10 to 20 %	Powder	Dietary supplement	Synthetic	Chemical synthesis	BASF
Beta-carotene 20 % CWD/R	Humans	20 %	Powder	Food colorant and food fortification	Synthetic	Chemical synthesis	BASF
Lyc-*O*-Beta	Humans	Variable from 1 to 30 %	Liquid and oil suspension, powder	Food colorant, food fortification, dietary supplements	Fungus (*Blakeslea trispora*)	Fermentation	LycoRed
Betacote	Humans	Variable from 2 to 30 %	Powder	Food colorant and food fortification	Synthetic	Chemical synthesis	LycoRed
BetaBeads®	Humans	7.50 and 15 %	Capsules	Dietary supplements	Fungus (*Blakeslea trispora*)	Fermentation	LycoRed

Source DSM, BASF and LycoRed (www.dsm.com, www.basf.com, www.lycored.com; accessed online 22 October 2013) authorized as a feed additive with no dosage specifications in Commission Regulation (EC) 880/2004.

Three major manufacturers (DSM, BASF and LycoRed) currently produce eight food/feed supplements containing 1–30 % β-carotene. Four of these products are produced by chemical synthesis and the other four are produced by fermentation in *B. trispora* (Table 1). The United States Patent and Trademark Office (USPTO) lists nearly 6000 patents that contain the term ‘beta-carotene’, and an exhaustive search of those in which the term is found in the abstract revealed 191 relevant patents and 173 focusing on feed or human use applications, production in algae, fungi, bacteria or plants, chemical synthesis, DNA sequences/enzymes related to β-carotene synthesis, and extraction methods (Supplementary Table 2). More of 50 % of these patents relate to human use applications, including pharmaceuticals, nutraceuticals, food supplements and cosmetics (Fig. 2).

Lycopene

In the EU, lycopene is defined as a food additive (E160d) according to Commission Regulation (EU) 231/2012, and may be chemically synthesized, extracted from red tomatoes or produced by cultivation in *B. trispora*. Regulation (EC) 1223/2009 authorizes lycopene for use in cosmetics. In 2003, the Spanish company Vitatene (now owned by DSM) applied to the United Kingdom competent regulatory authorities for permission to market lycopene produced in *B. trispora* as a novel food ingredient. The European Commission (EC) granted permission in 2006 according to Decision OJEC L 296/13 under Regulation (EC) 258/97, although maximum doses were specified for different foods with none exceeding 0.7 mg per 100 g. In 2009, the EC approved requests to consider synthetic lycopene produced by BASF and DSM, as well as natural lycopene from *B. trispora* (Vitatene) and tomatoes (LycoRed) as novel ingredients (OJEC 2009a, b, c, d: L 111/31; L 110/54; L 109/47; L 106/55). Lycopene can be added as an ingredient in fruit/vegetable juices, sports drinks, foods intended for use in energy-restricted diets for weight reduction, breakfast cereals, fats and dressings, soups other than tomato soups and bread. Lycopene can also be included in special medical diets to achieve particular nutritional requirements, and is added to food supplements with a maximum recommended intake of 15 mg/day.

Three major manufacturers (DSM, BASF and LycoRed) currently produce five lycopene-based food supplements containing 2–20 % of the active ingredient. Four of these are produced by fermentation in the

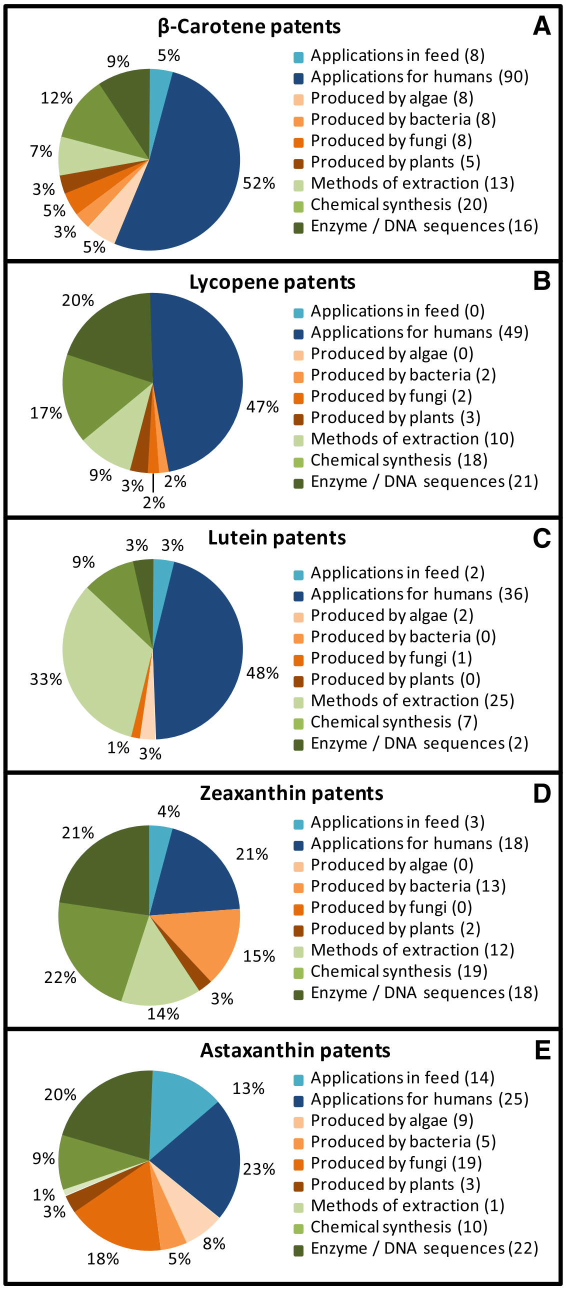

Fig. 2 Percentage of β-carotene (**a**), lycopene (**b**), lutein (**c**), zeaxanthin (**d**) and astaxanthin (**e**) patents per sector. In *brackets* total number of patents per sector. *Source*: supplementary Tables 2–6

Table 2 Current commercial products based on lycopene

Product	Receptor	Concentration	Formulation	Uses	Source	Production method	Company
Redivivo®	Humans	Variable from 5 to 10 %	Liquid suspension and powder	Food colorant, food fortification, dietary supplements	Tomato	Extraction	DSM
LycoVit®	Humans	Variable, from 10 to 20 %	Powder, oily dispersion	Processed food, beverages, confectionery, dairy products fortification, dietary supplements	Synthetic	Chemical synthesis	BASF
Lyc-O-Mato®	Humans	Between 4.5 and 15 %	Crystalline, insoluble in water	Food supplementation and colorant	Tomato	Extraction	LycoRed
LycoBeads	Humans	Variable from 5 to 20 %	Tablet-grade	Dietary supplements	Tomato	Extraction	LycoRed
Tomat-O-Red®	Humans	Variable from 2 to 10 %	Liquid and powder	Dietary supplements	Tomato	Extraction	LycoRed

Source DSM, BASF, LycoRed (www.dsm.com, www.basf.com, www.lycored.com; accessed online 22 October 2013)

Table 3 Current commercial products based on lutein

Product	Receptor	Concentration	Formulation	Uses	Source	Production method	Company
EZ Eyes	Humans	Variable from 0.5 to 1 %	Capsules and oil suspension	Dietary supplement	*Tagetes erecta* flower	Extraction	Chrysantis
Lutemax	Humans	Variable from 5 to 25 %	Capsules, oil suspensions and powder	Dietary supplement	*Tagetes erecta* flower	Extraction	OmniActive
Floraglo	Humans	Between 6 mg and 25 mg/portion	From oil suspensions to dry microcapsules	Dietary supplement	*Tagetes erecta* flower	Extraction	DSM
Lyc-O-lutein	Humans	Variable from 2 to 20 %	Oil suspension, capsules, cold water dispensable powder, liquid suspension	Processed food, beverages, confectionery, dairy products fortification, dietary supplements	*Tagetes erecta* flower	Extraction	LycoRed

Source DSM, OmniActive, Chrysantis and LycoRed (www.dsm.com, www.lycored.com, www.omniactives.com, www.chrysantis.com; accessed online 22 October 2013)

fungus *B. trispora* and the other by chemical synthesis (Table 2). USPTO lists 2,068 patents containing the word lycopene, including 122 with lycopene in the abstract. There are 105 relevant patents describing human use applications of lycopene, extraction methods, production in fungi, bacteria and plants, chemical synthesis, and DNA sequences/enzymes related to lycopene synthesis (Supplementary Table 3). Nearly 50 % of the patents describe human use applications, including pharmaceuticals, nutraceuticals, food supplements and cosmetics (Fig. 2).

Lutein and zeaxanthin

In the EU, lutein is defined as a food additive (E161b) according to Commission Regulation (EU) 231/2012, and is extracted from edible fruits, grass, lucerne (*Medicago falcata*) and marigold. Commission Regulation 1129/2011 lists the foods to which lutein can be added, i.e. processed cheese, jam jellies and marmalades and sweetened chestnut purée, fruit or vegetable spreads and processed fish. The maximum concentration varies but cannot exceed 100 mg/kg.

Table 4 Current commercial products based on zeaxanthin

Product	Receptor	Concentration	Formulation	Uses	Source	Production method	Company
EZ Eyes	Humans	Variable from 5 to 10 %	Capsules and oil suspension	Dietary supplements	*Tagetes erecta* flower	Extraction	Chrysantis
OmniXan	Humans	5 and 20 %	Capsules and oil suspension	Dietary supplements	*Capsicum*	Extraction	OmniActive
ZeaGold	Humans	Variable from 3 to 10 %	Oil suspension and cold water soluble beadlet	Coloring, dietary supplements and flavor masking	*Capsicum*	Extraction	Kalsec
OptiSHARP	Humans	Variable from 20 to 25 %	From oil suspensions to dry microcapsules	Dietary supplements	Synthetic	Chemical synthesis	DSM

Source DSM, Kalsec, OmniActive and Chrysantis (www.dsm.com, www.omniactives.com, www.chrysantis.com, www.kalsec.com; accessed online 22 October 2013)

Zeaxanthin can be present in algal-derived carotenoid preparations (E160a) according to Commission Regulation (EU) 231/2012 but there are no further specifications for its use.

DSM, LycoRed, Chrysantis and OmniActive manufacture four products containing 0.5–20 % lutein, focusing on human use applications such as food additives, supplements and fortification. All the available lutein products are extracted from *T. erecta* (Table 3). DSM, Kalsec, Chrysantis and OmniActive manufacture four products containing 1–25 % zeaxanthin as human food supplements. Three of these products are extracted (two from *T. erecta* and the other from *Capsicum annuum*) and the last is produced by chemical synthesis (Table 4). USPTO lists 1,301 patents containing the term lutein and 1,001 containing the term zeaxanthin, including 102 and 108 respectively listing these terms in the abstract. Seventy-five patents for lutein and 85 patents for zeaxanthin related to feed and human use applications, production in algae and fungi, extraction methods, chemical synthesis methods and relevant DNA sequences/enzymes (Supplementary Tables 4 and 5). Almost 50 % of lutein patents represent human use applications, whereas most of the zeaxanthin patents relate to chemical synthesis (Fig. 2).

Astaxanthin

Astaxanthin was first regulated in 1988 by Directive 87/552/EC for use in salmon and trout feed at a maximum concentration of 100 mg/kg in combination with canthaxanthin. In 2004, herbal capsules containing astaxanthin-rich oleoresin from *H. pluvialis* were approved as a novel food ingredient under Regulation (EC) 258/97 with a maximum concentration of 4 mg per capsule and marketed by Herbal Science International (Loughton, UK). Similar food supplements derived from *H. pluvialis* (Cyanotech Corporation, Kailua-Kona, USA), astaxanthin-rich oleoresin extracted from *H. pluvialis* (AstaReal, Gustavsberg, Sweden) and astaxanthin-rich extracts of *H. pluvialis* (Alga Technologies, Hevel Eliot, Israel) were later approved under the same regulation.

According to Commission Regulation 1288/2004 (EC), additive E161z is astaxanthin produced by *P. rhodozyma* and can be used in salmon and trout feed at a maximum concentration of 100 mg/kg of the complete feed, whereas Commission Regulation (EC) 393/2008 lists E161j as astaxanthin dimethylsuccinate (Carophyll® Stay-pink) with the same purpose. Astaxanthin can also be produced in *Paracoccus carotinifaciens* and added to feed at a maximum concentration of 100 mg/kg of complete feed with a moisture content of 12 %, in combination with adonirubin and canthaxanthin (Commission Regulation 721/2008).

In this context, up to nine different products are manufactured by DSM, BASF, LycoRed, Naturxan, Cyanotech and Fuji Chemical Industry, containing 0.77–10 % astaxanthin. These products focus mainly on the human dietary supplements and cosmetics markets, but also include aquaculture feed. Six of these

Table 5 Current commercial products based on astaxanthin

Product	Receptor	Concentration	Formulation	Uses	Source	Production method	Company
Carophyll-pink®	Aquaculture	10 %	Capsules	Colorants—substances which, when fed to animals, add colors to food of animal origin	Synthetic	Chemical synthesis	DSM
Lucantin® pink	Aquaculture	10 %	Capsules	Colorants—Substances which, when fed to animals, add colors to food of animal origin	Synthetic	Chemical synthesis	BASF
Aquasta®	Aquaculture	1 %	Capsules	Colorants—Substances which, when fed to animals, add colors to food of animal origin	*Phaffia rhodozyma*	Fermentation	Naturxan
AstaReal®	Humans	Variable from 0.75 to 10 %	Tablet-grade	Dietary supplements	*Haematococcus pluvialis*	Extraction	Fuji Chemical Industry
AstaTROL	Humans	5 %	Oil	Personal care and cosmetic product	*Haematococcus pluvialis*	Extraction and distillation	Fuji Chemical Industry
Novasta	Animals	4.5 % min	Powder (insoluble in water)	For further processing into feeds	*Haematococcus pluvialis*	Extraction	Fuji Chemical Industry
BioAstin	Humans	4 mg to 12 mg/capsule	Capsules	Dietary supplements	*Haematococcus pluvialis*	Fermentation	Cyanotech
Lyc-*O*-Asta	Humans	2 %	Capsules (without porcin gelatin)	Dietary supplements	*Haematococcus pluvialis*	Fermentation	LycoRed
Astaxanthinbeads	Humans	2 %	Capsules (with porcin gelatin)	Dietary supplements	*Haematococcus pluvialis*	Fermentation	LycoRed

Source DSM, BASF, Naturxan, Fuji Chemical Industry, Cyanotech and LycoRed (www.dsm.com, www.basf.com, www.igene.com, www.lycored.com, www.fujichemical.co.jp/english, www.cyanotech.com; accessed online 22 October 2013)

products are derived from *H. pluvialis* (three by fermentation and three by extraction from natural populations), another is produced by fermentation in *P. rhodozyma* and the last two are chemically synthesized (Table 5). USPTO lists 956 patents containing the term ‘astaxanthin’ including 129 patents with the term listed in the abstract. More in depth analysis narrowed this number to 108 patents related to feed and human use applications, production in algae, yeast, bacteria and plants, methods of extraction, chemical synthesis and DNA sequences/enzymes relevant to production (Supplementary Table 6). Most of the patents describe DNA sequences/enzymes, human use applications and production in fungi (Fig. 2).

Biofortified crops to increase carotenoid intake

Plants, particularly fruits and vegetables, are the most important source of carotenoids in the human diet. A diverse and balanced diet provides adequate amounts of these beneficial compounds, but many developing-country populations lack access to fruits and vegetables so risk malnutrition. Even in well-nourished populations, many people do not gain the full benefits of carotenoids, such as zeaxanthin, which are only present in a small number of food products. Conventional breeding and genetic engineering can be used to enhance the carotenoid content of edible crops and thus increase the total carotenoid intake of the population (Capell and Christou 2004; Sanahuja et al. 2013) (Supplementary Table 7). For example, tomatoes contain 43.8 µg lycopene per g fresh weight (FW) (Jaswir et al. 2011) but this concentration has been increased to 169.0 µg/g FW by conventional breeding (Frusciante et al. 2007) and to 313.2 µg/g FW by overexpressing *Pantoea ananatis* CrtB under the control of a fruit-specific promoter (Fraser et al. 2002). Although there is no clear dose recommendation for lycopene, most commercial formulations contain 10–40 mg/dose which can be also achieved by eating 118 g of the best conventionally bred tomato, or 64 g of transgenic tomato (Supplementary Table 7). The best natural source of β-carotene is carrot (79.8 µg/g FW) (Jaswir et al. 2011), but genetic engineering in canola has produced seeds containing up to 949.0 µg/g FW (Shewmaker et al. 1999).

Genetic engineering can not only increase carotenoid levels in plants that already produce these compounds in substantial amounts, but can also boost production in plants such as rice that produce negligible amounts in the grain. Although rice endosperm lacks of β-carotene, Golden Rice engineered with PSY and CrtI accumulated 31.0 µg/g DW of β-carotene in the endosperm (Paine et al. 2005). Transgenic maize lines have been produced that provide 6 mg of β-carotene in 100 g of dry kernels (Naqvi et al. 2009), which compares well with commercial formulations of β-carotene providing 6–15 mg/day. Similarly, commercial formulations of lutein provide 5–20 mg/day, which can be obtained by eating 100 g of fresh spinach, the best natural source of lutein (62 µg/g FW) (Jaswir et al. 2011) Transgenic canola seeds have been engineered to accumulate up to 76.2 µg/g FW lutein (Yu et al. 2008). A cross between an inbred maize line (A639) and a transgenic line expressing maize PSY, bacterial CrtI and gentian LYCB, accumulated up to 56.5 µg/g DW zeaxanthin (Naqvi et al. 2011). Therefore, 100 g of dry maize kernels provide more than 40 % of the zeaxanthin in commercial supplements (1–10 mg/dose). Genetic engineering has also enabled the production of 91.6 µg/g FW astaxanthin in transgenic carrots, which could never be achieved by conventional breeding because *Adonis aestivalis* is the only plant species known to produce this compound (Jayaraj et al. 2008). Only 22 g of transgenic carrot is required to provide the same amount of astaxanthin found in commercial formulations (2–4 mg/dose). Even better results have been recently achieved in tomato (16 mg/g DW) where <400 mg FW are required to ingest the same astaxanthin amount than in a commercial dose (Huang et al. 2013) (Supplementary Table 7).

Although the genetic engineering approaches discussed above have focused on individual carotenoids, there are often collateral benefits in the production of additional carotenoid molecules because the metabolic flux is distributed throughout the biosynthesis pathway. For example, transgenic maize plants have been engineered to produce large amounts of β-carotene by overexpressing all the early pathway enzymes up to and including LYCB, but these plants not only accumulate β-carotene but also produce higher levels of lutein, zeaxanthin and other carotenoids (Naqvi et al. 2011; Zhu et al. 2008; 2009; Farré et al. 2013). Even if deliberate steps are taken to avoid the synthesis of these other carotenoids by inhibiting LYCE and/or HYBD, there is always some leakage which allows the accumulation of nutritionally adequate levels of lutein and zeaxanthin (Farré et al. 2011).

Although the intake of carotenoids can be increased by consuming foods containing higher levels of these compounds, or commercial supplements, it is important to determine whether the total amount ingested can be absorbed by the body and utilized for metabolic processes. In this context, bioavailability and bioaccessibility are important concepts. Nutrient bioaccessibility is defined as the fraction of the ingested nutrients that is released from the food matrix and is available for intestinal absorption from the lumen, whereas nutrient bioavailability includes additionally nutrient absorption, tissue distribution and metabolism (Lemmens et al. 2011).

Outlook

There is a high demand for carotenoids across multiple industry sectors and this is increasing in line of public awareness of health-promoting benefits of these molecules. Although the global market demand is currently met by a combination of chemical synthesis, fermentation in microbes and extraction from natural sources, the costs of production ensure that many of the world's poorest people cannot access these nutritionally-beneficial molecules. Research has focused on the production of carotenoids in staple crops to improve nutritional welfare in developing countries, which should not disrupt the market for carotenoids in the industrialized world where they represent both commodities and luxury goods. Metabolic engineering in plants provides a suitable strategy to provide abundant carotenoids in the diet even where the diet comprises staples such as rice and/or maize which generally contain insufficient carotenoid levels for nutritional fulfillment. However, the production of carotenoids in plants by metabolic engineering will require a change in political and public attitudes to the benefits of genetically engineered crops.

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References

Abdullaev FI (2002) Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus*). Exp Biol Med 227:20–25

Altincicek B, Kovacs JL, Gerardo NM (2012) Horizontally transferred fungal carotenoid genes in the two-spotted spider mite *Tetranychus urticae*. Biol Lett 8:253–257

Anunciato TP, da Rocha Filho PA (2012) Carotenoids and polyphenols in nutricosmetics, nutraceuticals, and cosmeceuticals. J Cosmet Dermatol 11:51–54

Aoki H, Kieu NT, Kuze N et al (2002) Carotenoid pigments in GAC fruit (*Momordica cochinchinensis* SPRENG). Biosci Biotechnol Biochem 66:2479–2482

Auldridge ME, McCarty DR, Klee HJ (2006) Plant carotenoid cleavage oxygenases and their apocarotenoid products. Curr Opin Plant Biol 9:315–321

Bai C, Twyman RM, Farré G et al (2011) A golden era—provitamin A enhancement in diverse crops. In vitro Cell Dev Biol Plant 47:205–221

Bathaie SZ, Mousavi SZ (2010) New applications and mechanisms of action of saffron and its important ingredients. Crit Rev Food Sci Nutr 8:761–786

BCC Research (2011) http://www.bccresearch.com/report/carotenoids-global-market-fod025d.html

Ben-Amotz A, Katz A, Avron M (1982) Accumulation of β-carotene in halotolerant algae: purification and characterization of β-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). J Phycol 18:529–537

Berman J, Zhu C, Pérez-Massot E et al (2013) Can the world afford to ignore biotechnology solutions that address food insecurity? Plant Mol Biol 83:5–19

Bernhard K, Müller RK, Spruijtenburg R (1984) Process for the preparation of astaxanthin and intermediates in the astaxanthin synthesis. Eur Patent EP0101597

Bhosale P, Bernstein PS (2005) Microbial xanthophylls. Appl Microbiol Biotechnol 68:445–455

Boussiba S, Vonshak A (1991) Astaxanthin accumulation in the green alga *Haematococcus pluvialis*. Plant Cell Physiol 32:1077–1082

Bouvier F, Suire C, Mutterer J et al (2003) Oxidative remodeling of chromoplast carotenoids: identification of the carotenoid dioxygenase CsCCD and CsZCD genes involved in crocus secondary metabolite biogenesis. Plant Cell 15:47–62

Bramley PM (2000) Is lycopene beneficial to human health? Phytochemistry 54:233–236

Breitenbach J, Sandmann G (2005) ζ-Carotene cis isomers as products and substrates in the plant poly-cis carotenoid biosynthetic pathway to lycopene. Planta 220:785–793

Britton G, Liaaen Jensen S, Pfander H (2004) Carotenoids handbook. Birkauser, Basel

Capell T, Christou P (2004) Progress in plant metabolic engineering. Curr Opin Biotechnol 15:148–154

Chappell J (1995) Biochemistry and molecular biology of the isoprenoid biosynthetic pathway in plants. Ann Rev Plant Physiol Plant Mol Biol 46:521–547

Chen Y, Li F, Wurtzel ET (2010) Isolation and characterization of the ZISO gene encoding a missing component of carotenoid biosynthesis in plants. Plant Physiol 153:66–79

Chew BP, Park JS, Wong MW et al (1999) A comparison of the anticancer activities of dietary β-carotene, canthaxanthin and astaxanthin in mice in vivo. Anticancer Res 19:1849–1853

Commission Directive 87/552/EECC amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs (2004). Official J EEUU, 30.04.2004

Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and mineral and their forms that can be added to foods, including food supplements. Official J EEUU, 01.12.2009

Commission Regulation (EC) No 1288/2004 of 12 July 2004 concerning the permanent authorization of certain additives and the provisional authorization of a new use of an additive already authorized in feedingstuffs. Official J EEUU, 14 July 2004

Commission Regulation (EC) No 393/2008 of 30 April 2008 concerning the authorization of astaxanthin dimethylsdisuccinate as a feed additive. Official J EEUU, 01.05.2008

Commission Regulation (EC) No 721/2008 of 25 July 2008 concerning the authorization of a preparation of red carotenoid-rich bacterium Paracoccus carotinifaciens as a feed additive. Official J EEUU, 26.07.2008

Commission Regulation (EC) No 880/2004 of 29 April 2004 authorising without time limit the use of beta-carotene and canthaxanthin as additives in feeding-stuffs belonging to the group of colouring matters including pigments. Official J EEUU, 30.04.2004

Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annexx II to Regulation (EC) No

1333/2008 of the European Parliament and of the Council. Official J EEUU, 12.11.2011

Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. Official J EEUU, 22.03.2012

Cunningham FX, Gantt E (2011) Elucidation of the pathway to astaxanthin in the flowers of *Adonis aestivalis*. Plant Cell 23:3055–3069

Cunningham FX, Pogson B, Sun Z, McDonald KA, DellaPenna D, Gantt E (1996) Functional analysis of the β and ε lycopene cyclase enzymes of Arabidopsis reveals a mechanism for control of cyclic carotenoid formation. Plant Cell 8:1613–1626

Dawson TL (2009) Biosynthesis and synthesis of natural colours. Color Technol 125:61–73

Diplock AT, Charleux JL, Crozier-Willi G et al (1998) Functional food science and defence against reactive oxidative species. Br J Nutr 80:S77–S112

Draelos ZD (2010) Nutrition and enhancing youthful-appearing skin. Clin Dermatol 28:400–408

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2010) Scientific Opinion on the substantiation of health claims related to vitamin A (including β-carotene) and maintenance of normal vision (ID 4239, 4701), maintenance of normal skin and mucous membranes (ID 4660, 4702), and maintenance of normal hair (ID 4660) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 8:1754–1767

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2011a) Scientific Opinion on the substantiation of a health claim related to lutein in combination with zeaxanthin, and maintenance of normal vision (ID 1606) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 9:2039–2053

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2011b) Scientific Opinion on the substantiation of health claims related to lycopene and protection of DNA, proteins and lipids from oxidative damage (ID 1608, 1609, 1611, 1662, 1663(1664), 1899, 1942. 2081, 2082, 2142, 2374), protection of the skin from UV-induced (including photo-oxidative) damage (ID 1259, 1607, 1665, 2143, 2262, 2373), contribution to normal cardiac function (ID 1610, 2372), and maintenance of normal vision (ID 1827) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 9:2031–2059

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2011c) Scientific Opinion on the substantiation of health claims related to astaxanthin and protection of the skin from UV-induced damage (ID 1687, 1979), defence against *Helicobacter pylori* (ID 1686), contribution to normal spermatogenesis (ID 1688), contribution to normal muscle function (ID 1685), and “immune system” (ID 1689, 1919, 1980) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 9:2206–2224

Ernst H (2002) Recent advances in industrial carotenoid synthesis. Pure Appl Chem 74:2213–2226

Farré G, Sanahuja G, Naqvi S et al (2010) Travel advice on the road to carotenoids in plants. Plant Sci 179(28):48

Farré G, Bai C, Twyman RM et al (2011) Nutritious crops producing multiple carotenoids—a metabolic balancing act. Trends Plant Sci 16:532–540

Farré G, Rivera SM, Alves R et al (2013) Targeted transcriptomic and metabolic profiling reveals temporal bottlenecks in the maize carotenoid pathway that may be addressed by multigene engineering. Plant J 75:441–455

Fassett RG, Coombes JS (2005) Astaxanthin: a potential therapeutic agent in cardiovascular disease. Mar Drugs 9:447–465

Fassett RG, Coombes JS (2012) Astaxanthin in cardiovascular health and disease. Molecules 17:2030–2048

FDA (2009) Guidance for industry: a food labeling guide (11. Appendix C: health claims) http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm064919.htm

Franceschi S, Bidoli E, La Vecchia C et al (1994) Tomatoes and risk of digestive-tract cancers. Int J Cancer 59:181–184

Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 43:228–265

Fraser PD, Romer S, Shipton CA et al (2002) Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. Proc Natl Acad Sci USA 99:1092–1097

Frusciante L, Carli P, ERcolano MR et al (2007) Antioxidant nutritional quality of tomato. Mol Nutr Food Res 51:609–617

Garcia E, Barrett DM (2006) Assessing lycopene content in California processing tomatoes. J Food Process Preserv 30:56–70

Gassel S, Schewe H, Schmidt I et al (2013) Multiple improvement of astaxanthin biosynthesis in *Xanthophyllomyces dendrorhous* by a combination of conventional mutagenesis and metabolic pathway engineering. Biotechnol Lett 35:565–569

Giovannucci E, Ascherio A, Rimm EB et al (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 87:1767–1776

Gua ZX, Chen DM, Han YB et al (2008) Optimization of carotenoids extraction from *Rhodobacter sphaeroides*. LWT-Food Sci Technol 41:1082–1088

Hammond BR Jr, Johnson EJ, Russel RM et al (1997) Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 38:1795–1801

Hensel A, Rösing M (2003) Crocus. In: Blaschek W, Ebel S, Hackenthal E, Holzgrabe U, Keller K, Reichling J (eds) HagerRom: Hagers Handbuch der Drogen und Arzneistoffe. Springer Electronic Media, Heidelberg

Huang JC, Zhong YJ, Liu J, Sandmann G et al (2013) Metabolic engineering of tomato for high-yield production of astaxanthin. Metab Eng 17:59–67

Isaacson T, Ohad I, Beyer P et al (2004) Analysis in vitro of the enzyme CRTISO establishes a poly-cis-carotenoid biosynthesis pathway in plants. Plant Physiol 136:4246–4255

Iwamoto T, Hosoda K, Hirano R et al (2000) Inhibition of low-density lipoprotein oxidation by astaxanthin. J Atheroscler Thromb 7:216–222

Jaswir I, Noviendri D, Hasrini RF et al (2011) Carotenoids: sources, medicinal properties and their application in food and nutraceutical industry. J Med Plants Res 5:7119–7131

Jayaraj J, Devlin R, Punja Z (2008) Metabolic engineering of novel ketocarotenoid production in carrot plants. Transgenic Res 17:489–501

Jin E, Feth B, Melis A (2003) A mutant of the green alga *Dunaliella salina* constitutively accumulates zeaxanthin under all growth conditions. Biotechnol Bioeng 8:115–124

Johnson EA, An GH (1991) Astaxanthin from microbial sources. Crit Rev Biotechnol 11:297–326

Jyonouchi H, Sun S, Tomita Y et al (1995) Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and suboptimal doses of antigen. J Nutr 125:2483–2492

Kang EK, Campbell RE, Bastian E et al (2010) Invited review: annatto usage and bleaching in dairy foods. J Dairy Sci 93:3891–3901

Kim J, Smith JJ, Tian L et al (2009) The evolution and function of carotenoid hydroxylases in Arabidopsis. Plant Cell Physiol 50:463–479

Knekt P, Reunanen A, Jarvinen R et al (1994) Antioxidant vitamin intake and coronary mortality in a longitudinal population study. Am J Epidemiol 139:1180–1189

Krinsky NI (1998) Overview of lycopene, carotenoids, and disease prevention. Proc Soc Exp Biol Med 218:95–97

Lamers PP, Janssen M, De Vos RC et al (2008) Exploring and exploiting carotenoid accumulation in *Dunaliella salina* for cell-factory applications. Trends Biotechnol 26:631–638

Landrum JT, Bone RA (2001) Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 385:28–40

Lemmens L, Colle IJ, Van Buggenhout S et al (2011) Quantifying the influence of thermal process parameters on in vitro β-carotene bioaccessibility: a case study on carrots. J Agric Food Chem 59:3162–3167

Li F, Murillo C, Wurtzel ET (2007) Maize Y9 encodes a product essential for 15-cis-zeta-carotene isomerization. Plant Physiol 144:1181–1189

Li Q, Farré G, Naqvi S et al (2010) Cloning and functional characterization of the maize carotenoid isomerase and β-carotene hydroxylase genes and their regulation during endosperm maturation. Transgenic Res 19:1053–1068

Mills PK, Beeson WL, Phillips RL et al (1989) Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 64:598–604

Minguez-Mosquera MI, Hornero-Mendez D (1994) Comparative study of the effect of paprika processing on the carotenoids in pepper (*Capsicum annuum*) of the Bola and Agridulce varieties. Food Chem 42:1555–1560

Misawa N, Truesdale MR, Sandmann G et al (1994) Expression of a tomato cDNA coding for phytoene synthase in *Escherichia coli*, phytoene formation in vivo and in vitro, and functional analysis of the various truncated gene products. J Biochem 116:980–985

Misawa N, Satomi Y, Kondo K et al (1995) Structure and functional analysis of a marine bacterial carotenoid biosynthesis gene cluster and astaxanthin biosynthetic pathway proposed at the gene level. J Bacteriol 177:6575–6584

Moran NA, Jarvik T (2010) Lateral transfer of genes from fungi underlies carotenoid production in aphids. Science 328:624–627

Mozaffarieh M, Sacu S, Wedrich A (2003) The role of the carotenoids, lutein and zeaxanthin, in protecting against age-related macular degeneration: a review based on controversial evidence. Nutr J 11:20–28

Mustafa A, Trevino ML, Turner C (2012) Pressurized hot ethanol extraction of carotenoids from carrot by-products. Molecules 17:1809–1818

Nadolski G, Cardounel AJ, Zweier JL, Lockwood SF (2006) The synthesis and aqueous superoxide anion scavenging of water-dispersible lutein esters. Bioorg Med Chem Lett 16:775–781

Nanou K, Roukas T, Papadakis E (2012) Improved production of carotenes from synthetic medium by *Blakeslea trispora* in a bubble column reactor. Biochem Eng J 67:203–207

Naqvi S, Zhu C, Farré G et al (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci USA 106:7762–7767

Naqvi S, Zhu C, Farré G et al (2011) Synergistic metabolism in hybrid corn indicates bottlenecks in the carotenoid pathway and leads to the accumulation of extraordinary levels of the nutritionally important carotenoid zeaxanthin. Plant Biotechnol J 9:384–393

Nishino H, Murakoshi M, Tokuda H et al (2009) Cancer prevention by carotenoids. Arch Biochem Biophys 483: 165–168

OJEC (2006) Official Journal of the European Commision of 23 October 2006 authorising the placing on the market of lycopene from *Blakeslea trispora* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. L 296/13

OJEC (2009a) Official Journal of the European Commision of 23 April 2009 authorising the placing on the market of lycopene as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. L 106/55

OJEC (2009b) Official Journal of the European Commision of 28 April 2009 authorising the placing on the market of lycopene from *Blakeslea trispora* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. L 111/31

OJEC (2009c) Official Journal of the European Commision of 28 April 2009 authorising the placing on the market of lycopene oleoresin from tomatoes as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. L 109/47

OJEC (2009d) Official Journal of the European Commision of 30 April October 2009 authorising the placing on the market of lycopene as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. L 110/54

Paine JA, Shipton CA, Chaggar S et al (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23:482–487

Pashkow FJ, Watumull DG, Campbell CL (2008) Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. Am J Cardiol 101:58D–68D

Pérez-Massot E, Banakar R, Gómez-Galera S et al (2013) The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. Genes Nutr 8:29–41

Pommer H, Thieme PC (1983) Industrial applications of the Wittig reaction. Top Curr Chem 109(165–188):166

PRweb: Global Carotenoids Mark.et to Reach US$1.3 Billion by 2017, According to a New Report by Global Industry Analysts, Inc. http://www.prweb.com/releases/carotenoids/astaxanthin_beta_carotene/prweb8849957.htm

Quackenbush FW, Firch JG, Brunson AM et al (1963) Carotenoid, oil, and tocopherol content of corn inbreds. Cereal Chem 40:250–253

Raja R, Hemaiswarya S, Rengasamy R (2007) Exploitation of *Dunaliella* for beta-carotene production. Appl Microbiol Biotechnol 74:517–523

Rao LG, Guns E, Rao AV (2003) Lycopene: its role in human health and disease. AGROFood Industry Hi-Tech

Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Official J EEUU, 22.12.2009

Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official J EEUU, 30.12.2006

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official J EEUU, 14.07.1997

Rice-Evans CA, Sampson J, Bramley PM et al (1997) Why do we expect carotenoids to be antioxidants in vivo? Free Radic Res 26:381–398

Rivera-Madrid R, Escobedo-Medrano RM, Balam-Galera E et al (2006) Preliminary studies toward genetic improvement of annatto (*Bixa orellana* L.). Sci Hortic 109:165–172

Rodriguez-Amaya DB, Kimura M, Godoy HT et al (2008) Updated Brazilian database on food carotenoids: factors affecting carotenoid composition. J Food Compos Anal 21:445–463

Rodriguez-Concepcion M (2006) Early steps in isoprenoid biosynthesis: multilevel regulation of the supply of common precursors in plant cells. Phytochem Rev 5:1–15

Rüttimann A (1999) Dienolether condensations—a powerful tool in carotenoid synthesis. Pure Appl Chem 71:2285–2293

Sanahuja G, Farré G, Berman J et al (2013) A question of balance—achieving appropriate nutrient levels in biofortified staple crops. Nutr Res Rev 26:235–245

Scaife MA, Ma CA, Ninlayarn T et al (2012) Comparative analysis of β-carotene hydroxylase genes for astaxanthin biosynthesis. J Nat Prod 22:1117–1124

Schmidt I, Schewe H, Gassel G et al (2011) Biotechnological production of astaxanthin with *Phaffia rhodozyma/Xanthophyllomyces dendrorhous*. Appl Microbiol Biotechnol 89:555–571

Seo M, Koshiba T (2002) Complex regulation of ABA biosynthesis in plants. Trends Plant Sci 7:41–48

Shewmaker CK, Sheehy JA, Daley M et al (1999) Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. Plant J 20:401–412

Shi XM, Jiang Y, Chen F (2002) High-yield production of lutein by the green microalga *Chlorella protothecoides* in heterotrophic fed-batch culture. Biotechnol Prog 18:723–727

Simkin AJ, Moreau H, Kuntz M et al (2008) An investigation of carotenoid biosynthesis in *Coffea canephora* and *Coffea arabica*. J Plant Physiol 165:1087–1106

Sommerburg O, Keunen JEE, Bird AC et al (1998) Fruits and vegetables that are source of lutein and zeaxanthin: the macular pigment in human eye. Br J Ophthalmol 82:907–910

Soroka IM, Narushin VG, Turivansky YD et al (2012) Spectroscopy analysis for simultaneous determination of lycopene and β-carotene in fungal biomass of *Blakeslea trispora*. Acta Biochim Pol 59:65–69

Suseela MR, Toppo K (2006) *Haematococcus pluvialis*—a green alga, richest natural source of astaxanthin. Curr Sci 90:1602–1603

Tanaka T, Morishita Y, Suzui M et al (1994) Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin. Carcinogenesis 15:15–19

Tsao R, Yang R, Young JC et al (2004) Separation of geometric isomers of native lutein diesters in marigold (*Tagetes erecta* L.) by high-performance liquid chromatography–mass spectrometry. J Chromatogr A 1045:65–70

Van Poppel G (1996) Epidemiological evidence for beta-carotene in prevention of cancer and cardiovascular disease. Eur J Clin Nutr 50:S57–S61

Widmer E, Zell R, Broger EA et al (1981) Technische Verfahren zur Synthese von Carotenoiden und verwandten Verbindungen aus 6-oxo-isophoron. II. Ein neues Konzept für die Synthese von (3RS, 3′RS)-astaxanthin. Helv Chim Acta 64:2436–2446

Yu D, Lydiate DJ, Young LW et al (2008) Enhancing the carotenoid content of *Brassica napus* seeds by downregulating lycopene epsilon cyclase. Transgenic Res 17:573–585

Zhang XW, Gong XD, Chen F (1999) Kinetic models for astaxanthin production by high cell density mixotrophic culture of the microalga *Haematococcus pluvialis*. J Ind Microbiol Biotech 23:691–696

Zhu C, Naqvi S, Gómez-Galera S et al (2007) Transgenic Strategies for the nutritional enhancement of plants. Trends Plant Sci 12:548–555

Zhu C, Naqvi S, Breitenbach J et al (2008) Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in corn. Proc Natl Acad Sci USA 105:18232–18237

Zhu C, Naqvi S, Capell T et al (2009) Metabolic engineering of ketocarotenoid biosynthesis in higher plants. Arch Biochem Biophys 483:182–190

Zhu C, Bai C, Sanahuja G et al (2010) The regulation of carotenoid pigmentation in flowers. Arch Biochem Biophys 504:132–141

Zhu C, Sanahuja G, Yuan D et al (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11:129–141