Review

Biosynthesis and extraction of high-value carotenoid from algae

Amit Kumar Gupta¹, Kunal Seth², Kirti Maheshwari¹, Prabhat Kumar Baroliya³, Mukesh Meena¹, Ashwani Kumar¹*, Vandana Vinayak⁵, Harish¹,‡

¹Department of Botany, Mohanlal Sukhadia University, 313 001 Udaipur, Rajasthan, India, ²Department of Botany, Government Science College, Pardi, 396125 Valsad, Gujarat, India, ³Department of Chemistry, Mohanlal Sukhadia University, 313 001 Udaipur, Rajasthan, India, ⁴Metagenomics and Secretomics Research Laboratory, Department of Botany, Dr. Harisingh Gour Central University, 470003 Sagar, MP, India, ⁵Diatom Nanoengineering and Metabolism Laboratory (DNM), School of Applied Sciences, Dr. Harisingh Gour Central University, 470003 Sagar, MP, India

1. Abstract

Algae possess a considerable potential as bio-refinery for the scale-up production of high-value natural compounds like—carotenoids. Carotenoids are accessory pigments in the light-harvesting apparatus and also act as antioxidants and photo-protectors in green cells. They play important roles for humans, like—precursors of vitamin A, reduce the risk of some cancers, helps in the prevention of age-related diseases, cardiovascular diseases, improve skin health, and stimulates immunity. To date, about 850 types of natural carotenoid compounds have been reported and they have approximated 1.8 billion US$ of global market value. In comparison to land plants, there are few reports on biosynthetic pathways and molecular level regulation of algal carotenogenesis. Recent advances of algal genome sequencing, data created by high-throughput technologies and transcriptome studies, enables a better understanding of the origin and evolution of de novo carotenoid biosynthesis pathways in algae. Here in this review, we focused on, the biochemical and molecular mechanism of carotenoid biosynthesis in algae. Additionally, structural features of different carotenoids are elaborated from a chemistry point of view. Furthermore, current understandings of the techniques designed for pigment extraction from algae are reviewed. In the last section, applications of different carotenoids are elucidated and the growth potential of the global market value of carotenoids are also discussed.

2. Introduction

Carotenoids comprehend a group of naturally occurring lipophilic (fat-soluble) pigments. C₄₀ carbon atoms with varying numbers of the double bond (polyene backbone) interlink and forms the basic structure of the carotenoid molecule, resulting from the isoprenoid pathway [1, 2]. The discovery of carotenoid has far been decoded the mystery behind the prismatic and radiant colors, we observe in fruits, vegetables, flowers, and leaves. They are also responsible for the flamboyant coloration in animals like flamingos, crustaceans, shells, and fish skin as in salmon [3]. In nature, all photosynthetic organisms (cyanobacteria, algae, higher plants), as well as some non-
photosynthetic organisms such as fungi (\textit{Umbelopsis isabelлина}) and bacteria (\textit{Deinococcus-Thermus}), have the capability of carotenoid biosynthesis \cite{4,5}. The sundry shades of colors in fruits and vegetables, while they undergo ripening as well as color change during metamorphosis of crab, are also because of carotenoid transitions \cite{6}. From an aesthetic point of view, carotenoids augment the beauty of the environment by adding pigmentation to fruits and flowers, enhancing the taste of fruits, and adding aromas to the flower, which in turn fascinate pollinators and engross seed dispersal organisms.

About 850 kinds of carotenoids have been reported up to 2018 \cite{7}. They are broadly grouped into two categories either on basis of functional properties or chemical structure. Functionally, they can be either primary having a vital role in photosynthesis or secondary having a role in stress conditions \cite{8}. Based on chemical structure, carotenoids having pure carbon skeleton and are referred to as carotenes (cyclized or uncyclized, e.g., \(\alpha\)-carotene, \(\beta\)-carotene, \(\gamma\)-carotene, lycopene, phytoene) and another, the oxygenated carotenoids, are known as xanthophylls. Lutein, zeaxanthin, astaxanthin, violaxanthin, canthaxanthin, echinoene, \(\epsilon\)-cryptoxanthin, fucoxanthin, peridinin, neoxanthin are some of the well-known xanthophylls \cite{9}. The presence of xanthophyll as fatty acid esters, glycosides, sulfates, and protein complexes have been reported \cite{10}. Around 50 types of carotene and ~800 types of xanthophyll have been reported. Carotenoids with more than 45 or 50 carbon atoms are referred to as higher carotenoids, while, with less-than 40-carbon skeleton are known as apocarotenoids. About 40 types of higher carotenoids are reported in archaea and about 120 types of apocarotenoids are reported in higher plant and animals. Most carotenoids have a 40-carbon skeleton \cite{10}. Sweet potato, carrots, pumpkin, apricots, cantaloupe, spinach, and broccoli are a rich source of \(\alpha\)-carotene \cite{11}. Lycopene is abundant in tomato, watermelon, pink grape fruit \cite{12}. \(\beta\)-cryptoxanthin and zeaxanthin are found in peach, papaya, mandarin, orange, and tangerine \cite{13}. Collards, butternut, raw spinach, corn are also enriched with zeaxanthin \cite{14}. Lutein, violaxanthin, \(\beta\)-carotene, and neoxanthin are abundant in green leafy vegetables \cite{15}. Crocin, crocetin, picrocrocin are the three apocarotenoids present in stig mata of \textit{Crocus} flower which provide coloring properties to saffron \cite{16}.

Chloroplast—the green organ of photosynthetic tissue of higher plants, is not only the site of photosynthesis, but also plays an important role in biosynthesis and accumulation of carotenoid. Stanely and Yuan have reported many nuclei encoded membrane proteins, their synthesis in the cytoplasm as polypeptide precursor with amino terminus extension, directed to the chloroplast, for the biosynthesis of carotenoids \cite{17}. The carotenogenesis pathway is under strict gene control and acts as a chemotaxonomic marker \cite{18}. On the flip side, this pathway is equally prone to stress periods and affected by physical and environmental factors like salinity, temperature, irradiance, nutrition, and growth factors \cite{19}. The foremost, premier, and rate-limiting step of the biosynthetic pathway is the condensation of two GGPP (Geranyl geranyl pyrophosphate), to originate phytoene (colorless carotenoid) in presence of PSY (Phytoene synthase) enzyme \cite{20}. Subsequently, an array of sequential desaturations results in the production of all-trans lycopene. Major enzymes involved are Phytoene desaturase (PDS), \(\zeta\)-Carotene Isomerase (Z-ISO), \(\zeta\)-Carotene desaturase (ZDS), and Carotenoid isomerase (Crt-ISO). The colored sequence from phytoene is as follows: phytofluene (colorless), \(\zeta\)-carotene (green), neurosporene (orange/yellow), lycopene (red), and \(\gamma\)-carotene (orange). Lycopene then undergoes cyclization by Lycopene \(\epsilon\)-cyclase (LCY-E), and Lycopene \(\beta\)-cyclase (LCY-B), forming \(\alpha\)-carotene and \(\beta\)-carotene, respectively and this step is a critical branch point \cite{21}. \(\alpha\)- and \(\beta\)-carotene undergoes hydroxylation in presence of EHY (\(\epsilon\)-carotene hydroxylase) and BCH (\(\beta\)-carotene hydroxylase) to form lutein and zeaxanthin, respectively. \(\beta\)-Cryptoxanthin is an intermediate product during zeaxanthin formation. Antheraxanthin and violaxanthin are formed by epoxidation and de-epoxidation of zeaxanthin by ZEP (Zeaxanthin epoxidase) and VDE (Violaxanthin de-epoxidase), respectively, making up the xanthophyll cycle. In the cytoplasm, zeaxanthin may form adinoxanthin by BKT (\(\beta\)-carotene ketolase) which in turn forms astaxanthin using the same enzyme by incorporation of additional keto group. BKT can also act as an intermediate enzyme to \(\beta\)-carotene by adding a keto group to it, resulting in the formation of echinenone and canthaxanthin as an intermediate. Neoxanthin synthase enzyme converts violaxanthin into 9-cis neoxanthin \cite{22}.

Carotenoids can be stored inside or outside the chloroplast according to their functional role. Primary pigments are stored inside while secondary pigments remain outside the chloroplast in lipid globules. Green tissue conserves the accumulation of carotenoids while the levels in non-green tissues may vary according to the developmental stage. Though, cell storage capacity, catabolism, and degradation rate may alter the carotenoid profile \cite{20}. The phenomenal process of photosynthesis on the whole needs chlorophyll as a pre-dominant pigment, while carotenoids play a donative role in the overall mechanism of energy transport and conversion \cite{23}. Carotenoids majorly play a dual role, primarily; they act as accessory light-harvesting pigments in photosystem, thereby extending the range of solar radiation (wavelength) which is not absorbed by chlorophyll and hence, drive the process of photosynthesis to a greater peak. Secondly, the noteworthy role of carotenoids is photo protective by dissipating extra energy and scavenging toxic oxygen molecules. In this way, carotenoids stabilize pigment-protein complexes; and maintain the integrity of membranes necessary for cell survival and development \cite{24}. Secondary carotenoids like astaxanthin and canthaxanthin, accumulates in high amount
in cytoplasmic lipid globules under stress conditions. Begum et al. [25] have reported, the presence of characteristic pink/red color of some stressed algae due to carotenoid accumulation as a protective layer. In non-photosynthetic bacterium (Deinococcus-Thermus) and fungi, carotenoid plays a proficient photo-protective role [26]. The overall stability and functionality of the photosynthetic apparatus can be attributed to the antioxidant property these pigments own, which can prevent photo-oxidative damage [27]. Another role of carotenoids is a requirement to form prolammellar bodies (PLB’s) in etiolated seedlings to speed up photo-morphogenesis [27, 28]. Their role as a precursor of phytohormone ABA (abscisic acid) and SL (strigolactone) has been reported [29, 30]. Cazzonelli and Pogson have reported the role of β-ionone’s (catabolism product of carotenoid) in plant-insect interaction [31].

Unlike higher plants and other conventional sources, algae have a small life cycle with a speedy growth, cover less area for cultivation purposes, and are more efficient at biomass production and therefore serve as a better source for carotenoid production that too in cost-effective way. Different algae have been explored and utilized for the production of different carotenoids, which are listed in Table 1 (Ref. [31–51]). In the next few sections of this paper, we have highlighted the biosynthesis pathway for different carotenoids with special reference to algae. Further chemical aspects, extraction of synthesized carotenoid and their application and global market scenario are also discussed.

### 3. Carotenoid biosynthesis pathways in algae

The basic carotenoid biosynthesis pathway seems to be the same in algae and streptophytes. Based on genome and transcriptome-wide studies, now it is clear that the evolution of carotenoid biosynthesis pathways in algae have involve various genetic mechanism like gene duplications, gene loss, gene transfer etc. Due to these genetic events, the carotenoid biosynthesis pathways in algae became more complex than that of terrestrial plants [52]. The biochemical and molecular mechanism for carotenoid biosynthesis has been studied in detail for microorganism and higher plants. But there are fewer reported in different groups of algae. In 1997, Hirschberg et al. [53] reported some genes and enzymes involved in the carotenoid biosynthesis pathway in algae and plants. Recently, Wang et al. [52] analyses the transcriptome of 22 red algae and 19 brown algae and then combine it with the data available publicly at different databases. Based on this study, they identified some important genes of the carotenoid biosynthetic pathway in algae. In 2019, Negre et al. [54] sequenced the genome of Saccharina japonica and Cladosiphon okamuranus and have proposed the model for carotenoid biosynthesis pathway in these brown algae using genome-scale metabolic networks (GSMNs). Based on available research data, the whole carotenoid biosynthesis pathway in algae can be divided into the following six steps (Fig. 1).

#### 3.1 Biosynthesis of C5 isoprene units (IPP/DMAPP)

Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are five-carbon (C5) ubiquitous precursor metabolites, required for the biosynthesis of carotenoids. In living systems, two different pathways: (1) MVA (mevalonate) pathway and (2) MEP (methylerythritol phosphate) pathway, participate in biosynthesis of these precursor metabolites [20]. The MVA pathway was discovered in the 1950s and was considered as the sole path for the biosynthesis of IPP in all living organisms [55]. But results of MVA pathway enzyme inhibition and isotopic labeling studies on prokaryotes, algae and higher plants, indicated the absence of some key enzymes of the MVA pathway. However, IPP was well incorporated in different compounds of these organisms under study. These studies pointed out the presence of another route of IPP biosynthesis. This new route of IPP biosynthesis is called a non-mevalonate pathway or 1-deoxy-D-xylulose 5-phosphate (DXP) pathway or methylerythritol phosphate (MEP) pathway [56]. Animals and fungi use the MVA pathway for biosynthesis of IPP while the MEP pathway is identified in plants and algae. In higher plants, both MVA and MEP

![Table 1. Different algae studied for the production of different pigments.](image)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Algae</th>
<th>Pigment</th>
<th>Reference</th>
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<tr>
<td>1</td>
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<td>[31]</td>
</tr>
<tr>
<td>2</td>
<td>Chlorella vulgaris</td>
<td>Astaxanthin</td>
<td>[32]</td>
</tr>
<tr>
<td>3</td>
<td>Chlorella striolata var. multistriata</td>
<td>Astaxanthin</td>
<td>[33]</td>
</tr>
<tr>
<td>4</td>
<td>Botryococcus braunii</td>
<td>Astaxanthin</td>
<td>[31]</td>
</tr>
<tr>
<td>5</td>
<td>Chlorella zofingiensis</td>
<td>β-Carotene</td>
<td>[34]</td>
</tr>
<tr>
<td>6</td>
<td>Dunaliella salina</td>
<td>β-Carotene</td>
<td>[35, 36]</td>
</tr>
<tr>
<td>7</td>
<td>Dunaliella bardwil</td>
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<td>[37]</td>
</tr>
<tr>
<td>8</td>
<td>Coelastrella striolata var. multistriata</td>
<td>β-Carotene</td>
<td>[33]</td>
</tr>
<tr>
<td>9</td>
<td>Dunaliella salina</td>
<td>Bixin</td>
<td>[37]</td>
</tr>
<tr>
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<td>Fucoxanthin</td>
<td>[38]</td>
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<td>Fucoxanthin</td>
<td>[38]</td>
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<td>14</td>
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<td>Chlorella vulgaris</td>
<td>Lutein</td>
<td>[49]</td>
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<tr>
<td>23</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Muriellopsis sp.</td>
<td>Lutein</td>
<td>[51]</td>
</tr>
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</table>
Fig. 1. A consensus carotenoid biosynthesis pathway in algae. Different boxes represent the different steps involved in biosynthesis process as follows: (A) Mevalonate (MVA) pathway. (B) Methylerythritol phosphate (MEP) pathway. (C) Biosynthesis of geranylgeranyl diphosphate (GGPP). (D) Biosynthesis of phytoene and lycopene. (E) Biosynthesis of carotenes. (F) Biosynthesis of xanthophylls derived from α-carotene. (G) Biosynthesis of xanthophylls derived from β-carotene. Symbols of enzymes and carotenoids according to their occurrence in different group of algae are also denoted in the figure.

pathways operates simultaneously. While, in the case of algae, the MVA pathway has been lost in several groups like: Chlorophyceae, Prasinophyceae, Trebouxiophyceae and Cyanidioschyzon merolae (red alga). Therefore, the MEP pathway is the key pathway, which supplies the majority of IPP/DMAPP for carotenoid biosynthesis in these algal groups. In other groups of algae, like Glaucophyta and Heterokontophyta, both MVA and MEP pathways participate in the synthesis of IPP/DMAPP isoprene molecules [57]. In the case of red algae, Lohr et al. [55] reported that both MVA and MEP pathways participate in the biosynthesis of IPP molecules. On the other hand, Deng et al. [58] suggested that bioinformatics analyses are not able to characterize the genes for MVA pathway enzymes in red algae. Based on the existing ESTs, genome data, and phylogeny clustering analysis, Du et al. [59] also reported that green algae and red algae received their plastid by primary endosymbiotic events with cyanobacteria, while brown algae obtained their plastid via secondary endosymbiotic event with red algae.

3.1.1 Mevalonate (MVA) pathway

MVA is a specific intermediate of IPP biosynthesis. This classical MVA pathway is a multistep-cytosolic pathway, which begins with the condensations of three acetyl-CoA molecules and comes to an end with the synthesis of one IPP molecule (Fig. 1). In the initial two steps, one molecule of HMG-CoA (β-Hydroxy β-methylglutaryl-CoA) is formed by the condensation of three acetyl-CoA molecules. In the subsequent step, this HMG-CoA molecule is reduced to MVA with the consumption of two NADPH molecules. Then MVA molecule was transformed into an IPP molecule via two-time phosphorylation and one ATP coupled decarboxylation reaction [56]. A total of six enzymes AACT, HMGS, HMGR, MVK, PMK and MVD participate in this pathway [55]. An extra enzyme isopen- tenyl diphosphate isomerase (IDI) is also required for the conversion of IPP to DMAPP. Out of these, HMGR is an ER membrane-anchored protein, while others are soluble protein in nature. Enzyme 3-hydroxy-3-methylglutaryl
CoA reductase (HMGR) and mevalonate kinase (MVK) are identified as key regulators, catalyzes the committed steps: formation and phosphorylation of mevalonate, respectively. Conversion of HMG-CoA to MVA can be inhibited by the use of mevinolin, which is a highly specific inhibitor of the enzyme HMGR [60]. Most of the researchers agreed that, MVA pathway operates in the cytosol. Sapir-Mir et al. [61] have challenged this and according to them, the MVA pathway is compartmentalized to peroxisomes, as several studies indicate the peroxisomal location of enzyme AACT and IDI in plants.

3.1.2 Methylerithritol phosphate (MEP) pathway

Biological, ESTs and genomic research-based data elucidated that, MEP pathway evolved due to cyanobacterial ancestry in algal cells. That is why, this pathway operates in plastids of algal cells in contrast to the MVA pathway which operates in the cytosol [62]. D-glyceraldehyde-3-phosphate and pyruvate participate as a substrate in this pathway [63]. This pathway consists of eight steps, which start with the conversion of G3P and pyruvate to DXS (1-deoxy-D-xylulose-5-phosphate synthase) and end with the formation of IPP and DMAPP. A total of seven different enzymes (DXS, DXR—1-deoxy-D-xylulose-5-phosphate reductoisomerase, MCT—2-C-methyl-D-erythritol 4-phosphate cytidyltransferase, CMK—4- (cytidine 5’-diphospho)-2-C-methyl-D-erythritol kinase, MDS—2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, HDS—4-hydroxy-3-methylbut-2-enyl diphosphate synthase, and HDR—4-hydroxy-3-methylbut-2-enyl diphosphate reductase) participate in this pathway. These enzymes are encoded by nuclear genes and guided by N-terminal transit peptide sequences for their transport into plastids [64].

Out of these enzymes, three enzymes: DXS, DXR and HDR catalyze the rate-limiting steps of the pathway. DXS is a thiamine-dependent enzyme which catalyzes the first step of this pathway. In this step, 1-deoxy-D-xylulose-5-phosphate (DXP) is synthesized by the decarboxylation of pyruvate and subsequent condensation reaction between resultant and glyceraldehyde-3-phosphate [62]. It is a main rate-limiting enzyme, its over expression enhances the rate of carotenoid synthesis [20]. Sun and Li [65] also reported that, protein-protein interaction between DXS and PSY (phytoene synthase) enzyme also regulates the carotenogenesis. The enzyme DXR catalyzes the synthesis of 2-C-methyl-D-erythritol 4-phosphate (MEP) by rearrangement and subsequent reduction of DXP [62]. This step might be considered as the primary committed step of the MEP pathway. The activity of DXR enzyme can be inhibited by fosmidomycin. The decreased activity of DXR affects the activity of downstream enzymes like GGPS (geranylgeranyl phosphate synthase) and ultimately interrupts the biosynthesis of carotenoids in algae. Du et al. [39] cloned the cDNA of DXS and DXR genes from Pyropia haitanensis (red alga). The HDR is another key enzyme, which catalyzes the reductive dehydration reaction in the final step of the MEP pathway. As a result, HMBPP (4-Hydroxy-3-methyl-but-2-enyl pyrophosphate) is converted to C5 isoprene units. Both IPP and DMAPP are synthesized in this step; therefore, there is no need for enzyme IDI, which is required for isomerization of IPP to DMAPP (dimethylallyl diphosphate) in MVA pathway. However, it may require for the balance supply of IPP and DMAPP [56].

Ramos et al. [66] characterized the HDR gene and enzyme from green alga Dunaliella salina and reported that, it shows response to different stress conditions and played important role in regulation of carotenoid biosynthesis.

3.2 Biosynthesis of geranylgeranyl diphosphate (GGPP)

Geranylgeranyl diphosphate (GGPP) is an immediate metabolic predecessor of the carotenoid biosynthesis pathway. Formation of GGPS (geranylgeranyl phosphate synthase) is a three-step process. In the initial step, 10-carbon compound sesquiterpene is synthesized by the addition of one IPP and one DMAPP molecule. In subsequent steps, FPP is synthesized by the addition of one IPP molecule to sesquiterpene and then GGPP is formed by the addition of one more IPP molecule to FPP (farnesyl pyrophosphate) [67, 68]. This process involves three different types of enzymes: GGPS—geranyl diphosphate synthase, FPPS—farnesyl diphosphate synthase, and GGPPS—geranylgeranyl diphosphate synthase, sequentially [65]. Ruiz-Sola et al. [69] reported 12 paralogues genes for GGPPS in Arabidopsis. They also suggested that, out of different GGPPS isozymes, GGPPS11 isozyme behave like a hub isozyme and it interacts with other proteins required for the biosynthesis of carotenoids. These enzymes are rarely studied in algae in comparison to higher plants. Yang et al. [70] cloned and characterized the GGPP synthase gene (PuGGPS) in a red alga Pyropia umbilicalis (Bangiales). They reported that a polypeptide sequence of 345 amino acids with transit peptide sequence (N-terminal) is encoded by this gene. Lao et al. [67] reported that, GGPPS of an alga Haematococcus pluvialis (HpGGPPS) have tri-functional catalytic activities, which catalyzes all three steps of GGPP biosynthesis. Deng et al. [58] cloned and characterize the bgGGPPS from the red alga Bangia fuscocuprea. They also report that GGPP is the only product of this enzyme and it also interacts with psy gene, which is the rate-limiting enzyme of the carotenoid biosynthesis pathway. Deng et al. [58] also performed the phylogenesis analysis and suggest that the red algal and diatoms share a common ancestor for GGPPS but green algae and higher plants show an early divergence of GGPPS during evolution. It has been reported that, the supply of GGPP and its precursors decides the rate and subsequent flux of carotenoid biosynthesis in algae [68].
3.3 Biosynthesis of phytoene

Biosynthesis of phytoene is the first entry step reaction towards the carotenoid biosynthesis. It is the first colorless carotenoid (40-carbon) compound, which is synthesized by condensation of two GGPP molecules. This reaction is catalyzed by enzyme phytoene synthase (PSY) [65, 71]. PSY is one of the important rate-limiting and key flux controlling enzyme, which decides the pool size of carotenoids. Some studies indicate that several isoforms of PSY exist in different plant species and these are regulated by alternative splicing and protein modifications under the influence of different abiotic and biotic signals [20]. Recently, various genomic and phylogenetic studies are conducted using genomic sequences of some algae belonging to different groups like red algae, green algae, brown algae and diatoms to identify the PSY genes [52]. Tran et al. [72] reported the two orthologous copies of the PSY gene in green algae Micrornonas and Ostreococcus. These studies indicate the gene duplication events of the PSY gene during ancient evolution, which produces the two classes of PSY gene in algae. But presently, these two classes, PSY I and PSY II are only retained in members of Prasinophyceae (Chlorophyta). Green algae (other than Prasinophyceae) and higher plants have lost PSY II and reported to have only the PSY I class. In contrast to this, the members of algae belong to Rhodophyta, Heterokontophyta and Haptophyta have only the class PSY II gene [52]. Due to the major flux controlling enzyme of the carotenoid biosynthesis pathway, PSY has been recognized as a major target for metabolic engineering [73]. Recently a novel protein CPSFL1 has been identified, which is bound with phytoene and modulates the accumulation of carotenoids in the chloroplast [74].

3.4 Biosynthesis of lycopene

Biosynthesis of lycopene is a multistep process in which phytoene is converted to lycopene via sequential desaturation and isomerization reactions. This whole process includes four conserved enzymes: PDS, Z-ISO, ZDS and CrtISO [75]. Phytoene, which is synthesized in the previous step, is desaturated to ω-carotene. This occurs in two steps and both steps are catalyzed by an enzyme PDS. In the first step, phytoene is converted to 9,15-di-cis-phytofluene, and then in the next step, this phytofluene is converted to 9,15,9-tri-cis-ω-carotene. This 9,15,9-tri-cis-ω-carotene is yellow in color. The 9,15,9-tri-cis-ω-carotene is then converted to lycopene via multiple steps. In the first step, 9,15,9-tri-cis-ω-carotene is converted to 9,9-di-cis-ω-carotene and then to prolycopene. These two reactions are catalyzed by the enzyme Z-ISO and ZSO, respectively. This prolycopene, which is orange in color, is converted to red colored compound lycopene. This conversion is catalyzed by the enzyme CrtISO [20, 76]. Wang et al. [52] have identified and characterized the gene of these enzymes in different groups of algae-like Chlorophyta, Phaeophyta, and Rhodophyta. Various studies reported that, out of these four genes; the gene for PDS, ZDS and CrtISO are present in all three groups of algae, while gene for Z-ISO is absent in red algae (Rhodophyta). Wang et al. [52] also suggested that the absence of this gene in red algae does not affect the carotenoid biosynthesis.

3.5 Biosynthesis of carotenoids

Lycopene is the first link of carotenogenesis, which allows the biosynthesis of both α-carotene and β-carotene in algae. It is a most important branching point, where the ratio of α-carotenoids (lutein) to β-carotenoids (β-carotene) is to be decided. In α-carotene, one ω-ring and one β-ring are present at the extremity of lycopene. While, in β-carotene, two β-rings are present at the extremity of lycopene. Carotene with two ω-ionone rings rarely occurs in nature. This branch point reaction is catalyzed by two different enzymes; lycopene ω-cyclase (LCYE) and lycopene β-cyclase (LCYB) [77]. Formation of α-carotene from lycopene occurs in two sequential steps: in the first step, enzyme LCYE catalyzes the cyclization at the one open end and form δ-carotene; in the next step, LCYB catalyzes the β-ionone ring formation at another end and ultimately α-carotene is synthesized. In further sequential steps this α-carotene is converted to lutein. Similarly, β-carotene is also synthesized in two sequential steps, but here, both steps are catalyzed by the same enzyme LCY. In the first step, lycopene converted to γ-carotene and in the next step this γ-carotene is converted to β-carotene [58].

Based on chemical, GSMN and proteomic studies, it has been elucidated that algae are different in their lycopene cyclase enzyme compositions. Some algae contain both LCYE and LCYB, while other algae have only one class of LCY. Cui et al. reported that in green algae (except for C. reinhardtii (LCYE) and Chlorella sp. NC64A (LCYE), H. pluvialis (LCYB), D. salina (LCYB),) two distinct LCY (beta- and epsilon-type) enzymes are present, on the other hand, heterokontophyta have only lycopene beta-cyclase (LCYB) [77]. Recently, Inoue et al. [78] also reported the LCYB activity in brown alga Undaria pinnatifida. Macrophytic red algae have both LCYB and LCYE enzymes while microscopic algae have only the enzyme LCYB [52]. Some other studies also suggested that, synthesis of δ-carotene, α-carotene and lutein are absent in some groups of algae-like Bacillariophyceae, Chrysophyceae, Phaeophyceae, Xanthophyceae, and some red algae, while the β-carotene occurs in majority groups of algae and other photosynthetic organisms [18, 58]. Liang et al. [79] reported that the amino acid sequences of LCYB and LCYE are significantly similar. This report indicates that the gene duplication events in a common ancestor may be the possible reason behind the presence of two classes of LCY enzymes in algae. Deng et al. [58] suggested that LCYE, in green and red algae, evolved separately. Both enzyme LCYB and LCYE plays a major role in the metabolic flux of carotenoids. Sathasivam and Ki [80] reported that
treatment of redox-active heavy metals enhances the expression level of enzymes PSY, PDS, and LCYB and ultimately enhance the accumulation of lutein and \( \beta \)-carotene in brown alga *Tetraselmis suecica*.

### 3.6 Biosynthesis of xanthophylls

In algae, different types of xanthophylls like: lutein, cryptoxanthin, zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, fucoxanthin, diadinoxanthin, diatoxanthin, canthaxanthin, astaxanthin etc., are synthesized. Types and nature of xanthophyll molecules differ in various algal groups. Both \( \alpha \)- and \( \beta \)-carotene serves as precursor molecules for the biosynthesis of these xanthophyll compounds.

Lutein is a derivative of \( \alpha \)-carotene. It is synthesized in two successive steps: firstly \( \alpha \)-carotene is converted to zeinoxanthin or \( \alpha \)-cryptoxanthin and then in second step zeinoxanthin is converted to lutein. Both of these steps are catalyzed by P450-type enzymes \( \epsilon \)-hydroxylase and \( \beta \)-hydroxylase. Yang *et al.* [81] reported that red algal enzyme CYP97B29 has both \( \epsilon \)- and \( \beta \)-ring hydroxylase activity. Liang *et al.* [82] functionally identify the genes of carotene hydroxylases from alga *Dunaliella bardawil*. Various studies reported that, biosynthesis of lutein mainly occurs in green algae and some of the red algae. While other group of algae-like Bacillariophyceae, Chrysophyceae, Phaeophyceae and Xanthophyceae cannot synthesize lutein and other derivatives of \( \alpha \)-carotene.

Zeaxanthin is a double hydroxylation derivative of \( \beta \)-carotene. \( \beta \)-carotene is converted to \( \beta \)-cryptoxanthin and then \( \beta \)-cryptoxanthin is converted to zeaxanthin. Both of these hydroxylation reactions are catalyzed by the enzyme \( \beta \)-carotene hydroxylase (BCH). Biosynthesis of zeaxanthin occurs in all groups of algae as well as higher plants. Further, zeaxanthin is converted to violaxanthin in a two-step process. In the first step, zeaxanthin is converted to antheraxanthin and in the next step, antheraxanthin is converted to violaxanthin. Both of these steps are catalyzed by the enzyme ZEP. Enzyme ZEP catalyzes the epoxidation reaction of \( \beta \)-rings of zeaxanthin molecule [83]. Another enzyme VDE has also been reported which led to the formation of \( \beta \)-rings and ultimately reverses these reactions. This whole process of violaxanthin biosynthesis is also known as xanthophyll cycle-I. This cycle occurs in all members of green and brown algae while in the case of red algae it occurs partially [84].

In the case of brown algae some commercially important carotenoids like fucoxanthin and diadinoxanthin are also synthesized. Scientists have proposed two different pathways for the biosynthesis of these carotenoid compounds. According to the first pathway, violaxanthin is converted to neoxanthin and then neoxanthin is used as a precursor molecule for the biosynthesis of both fucoxanthin. An enzyme neoxanthin synthase (NXS) catalyzes the conversion of violaxanthin to neoxanthin in higher plants.

In the case of algae, recently, Dautermann *et al.* [85] reported a new enzyme violaxanthin de-epoxide-like (VDL), which is responsible for the conversion of violaxanthin to neoxanthin. They also reported that VDL is also involved in the synthesis of peridinin and vaucherixanthin. According to the second pathway, violaxanthin is used as a precursor for the biosynthesis of diadinoxanthin and then, the diadinoxanthin is converted into the fucoxanthin. The enzymes involved in this process are also still unknown. In some alga, diadinoxanthin can also be converted to diatoxanthin. This reaction is catalyzed by an enzyme de-epoxidase (DDE). This conversion of diadinoxanthin to diatoxanthin can be reversed by an enzyme diatoxanthin epoxide (DEP) under low light intensity. This pathway is called xanthophyll cycle-II. This cycle mainly occurs in members of Chrysophyceae, Bacillariophyceae, Phaeophyceae, and Xanthophyceae [54].

Astaxanthin, is another important carotenoid compound, which has strong antioxidant activity as compared to vitamin C and E, is synthesized by several bacteria, fungi, algae and higher plants. In algae several species have been reported which are involved in *de novo* synthesis of astaxanthin. The scientist has identified the two biochemical pathways for the biosynthesis of astaxanthin. According to the first pathway, \( \beta \)-carotene is converted to canthaxanthin and then, canthaxanthin is converted to astaxanthin. These two reactions are catalyzes by enzyme BKT and BCH, respectively. According to another pathway, zeaxanthin is converted to adonixanthin and then adonixanthin is converted to astaxanthin. In this second pathway, both of the reactions are catalyzed by the same enzyme BKT. Mao *et al.* [75] reported that, under sulfur stress conditions, genes LCYE and ZEP are down-regulated while gene LCYB, BCH and BKT are up-regulated. This regulation of gene expression enhances the accumulation of astaxanthin in green alga *Chromochloris zoﬁngiensis*. Among the algae, *Haematococcus pluvialis* considered as the richest source of natural astaxanthin. Astaxanthin in algae is present in esterified form in contrast to non-esterified form in yeast and synthetic form.

### 4. Chemistry of different carotenoid

Carotenoids are naturally occurring chemically diverse pigments covering yellow, orange, red, or dark green color, which are biosynthesized by diverse plants, fungi, algae, and microorganisms [86]. Carotenoids possess various biological functions, including light-catching, antioxidant activity, photoprotection from harmful ionizing radiation and medicinal properties and they are used as preventives against diseases such as cancer, diabetes, and cataract. Carotenoids are also used in food supplements, cosmetics, and pharmaceuticals. These compounds are largely isoprenoid chromophore-bearing polyene pigments having 3–13 conjugate double bonds containing two terminal rings. The presence of \( \pi \)-electron conjugation in the structure of
carotenoids (Fig. 2) is responsible for the unique spectroscopic properties of carotenoids. The polyene conjugation pattern is repeated in all carotenoids. The color of carotenoids can be determined by the presence of a number of conjugated double bonds in their skeleton. Carotenoids with a higher number of conjugated double bonds (such as lycopene and astaxanthin) generally show red color and are good antioxidants [87].

The unique spectroscopic property is mainly due to a strong symmetry allowed the electronic transition from the electronic ground state, $S_0$ to the lowest photoactive singlet excited state, $S_2$ which is relaxed by internal conversion (IC) [88]. The transition from the ground state, $S_0$ to the lowest-lying excited state, $S_1$ is optically forbidden due to a lack of change in symmetry [89]. The $S_0$–$S_2$ transition generally shows characteristic three-peak absorption spectra attributed to the transition to the three vibrational levels (0, 1, 2) of the $S_2$ state as depicted in Fig. 3.

Many studies have demonstrated that the energy of the $S_0$–$S_2$ transition decreases with the extended conjugation [90]. A few recent studies on analogues of the same carotenoid having different conjugation lengths rationalized the dependence of energy of the $S_2$ state on conjugation length [91].

Although conjugation length governs most of the spectroscopic properties of carotenoids, but it was reported that the presence of specific functional groups, such as conjugated aryl ring, carbonyl group, can significantly affect the energy of excited states. Aryl ring exhibiting carotenoids such as chlorobactene, $\beta$-isorenieratene, isorenieratene and okenone shown in Fig. 4, are pigments responsible for light-harvesting and photoprotective agents in green sulfur bacteria [92]. It was also reported that certain cyanobacteria are also capable of synthesizing aryl carotenoids such as synechoxanthin, which has been identified in Synechococcus sp. PCC 7002 [93].

Moreover, carotenoids with a conjugated carbonyl group are widely available pigments in plants and microorganisms and these carotenoids are most abundant in nature. Astaxanthin peridinin, fucoxanthin, and siphonaxanthin (Fig. 5) are carotenoids contain conjugated carbonyl groups found in algae and bacteria. These carotenoids are most studied as light-harvesting agents and excitation energy transfer agents to chlorophylls.
Carotenoids play a vital role in food industries as they offer natural color and flavors to various foods. Carotenoid-derived aroma compounds have been detected in leaf (tobacco, tea, and mate), essential oils, fruits (grapes, passionfruit, starfruit, quince, apple, nectarine), vegetables (tomato, melon), spices (saffron, red pepper), as well as coffee, oak wood, honey, seaweeds, etc. [94]. Degradation of carotenoids leads to different volatile flavor compounds [95]. Carotenoids produce a broad spectrum of aroma compounds (called apocarotenoids) in plants by oxidative cleavage, giving rise to volatile compounds responsible for the aroma of flowers, fruits, and leaves, as well as the well-known phytohormones such as abscisic acid and strigolactones [96]. The important volatile fragments of carotenoids with a 9–13 carbon skeleton frequently detected in nature. The important carotenoids-derived aroma compounds are: \( \beta \)-ionone, \( \beta \)-damascone, megastigmanes comprising C-13 skeleton; \( \beta \)-homocyclocitral and dihydroactinidiolide containing C-11 skeleton; \( \beta \)-cyclocitrinal, \( \alpha \)-cyclocitrinal, and safranal containing C-10 skeleton; 2,2,6-trimethylcyclohexanone, 2,2,6-trimethylcyclohexane1,4-dione comprising C-9 skeleton as depicted in Fig. 6.

5. Extraction of high value carotenoids

5.1 Conventional extraction methods

Carotenoids are extracted from microalgae utilizing conventional solvent extraction methods using organic solvents. Conventional extraction using organic or aqueous solvents depends on the polarity, solubility, and chemical stability of carotenoids to be extracted. Therefore, the selection of a suitable solvent system is necessary which can selectively and efficiently extract carotenoids with high purity. Non-polar solvents (n-hexane, dichloromethane, dimethyl ether, diethyl ether) and polar solvents (acetone, methanol, ethanol, biphasic mixtures of several organic solvents) can be used based on the polarity of the target carotenoid. The use of green solvents (environmentally safe and non-toxic solvents) such as ethanol, limonene, and biphasic mixtures of water and organic solvents has been investigated for the recovery of carotenoids from microalgae. However, the commercial reality of carotenoid extraction from microalgal species is still challenging due to the high cost of production, and usage of enormous amounts of solvents. The uses of non-conventional extraction methods are therefore gaining interest in recent years. These non-conventional extraction methods have several advantages including rapid extraction, low solvent consumption, better recovery, and higher selectivity. These different extraction approaches for various carotenoids along with their relative yield are mentioned in Table 2 (Ref. [97–112]).

5.2 Microwave-assisted extraction (MAE)

Microalgal cells are difficult to disrupt due to algaenan and sporopollenin within their cell wall [113]. Further, conventional techniques used for cell disruption and extraction methods have low efficiencies. MAE is an efficient method that takes advantage of microwave irradiation to accelerate the extraction of a diversity of compounds from natural matrices. MAE generates high-frequency waves (ranging from 300 MHz to 300 GHz) with wave-
lengths of 1 mm to 1 m. Microwave radiation when applied at a frequency near 2.45 GHz causes vibration of polar molecules resulting in inter and intra-molecular friction. The friction, together with the movement and collision of a large number of charged ions, results in the rapid heating (within few seconds) of the matrix. Intracellular heating leads to the breakdown of cell walls and membranes and therefore there is a faster transfer of the compounds from the cells into the extracting solvent. There are two major types of microwaves; closed and open vessels. In open vessels microwave application is performed at atmospheric pressure while in closed vessels, samples are irradiated by microwave under controlled pressure and temperature. The extraction temperature depends on the polarity of the solvent. Solvents with higher dielectric constant (ε′) absorb greater energy and thus achieve faster extraction and therefore polar solvents are better extractants than non-polar solvents. Pasqueta et al. [110] investigated the performance of microwave irradiation compared to conventional processes to extract pigments from two marine microalgae Dunaliella tertiolecta (Chlorophyta) and Cylindrotheca closterium (Bacillariophyta). All processes performed on D. tertiolecta led to rapid pigment extraction. Though the presence of frustule in the diatom C. closterium acted as a mechanical barrier to pigment extraction, MAE was found to be the best extraction process for C. closterium pigments with advantages like rapidity, reproducibility, homogeneous heating and high extraction yields. Fabrowska et al. [114] examined the efficiency of microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE) and conventional soxhlet extraction in three freshwater green algae species: Cladophora glomerata, Cladophora rivularis, and Ulva flexuosa. MAE and UAE were proved to be cost-effective techniques with higher yield compared to traditional solvent extraction techniques. Microwave-assisted extraction (MAE) has been applied for the extraction of astaxanthin from Haematococcus pluvialis which has the highest astaxanthin content [115]. For optimal extraction of astaxanthin, parameters like microwave power (W), extraction time (s), solvent volume (mL), and the number of extractions, were optimized using response surface methodology. The study suggested that optimized conditions of MAE viz. microwave power 141 W, extraction time 83 sec, solvent volume 9.8 mL, the number of extraction four times led to the extraction of about 594 ± 3.02 μg astaxanthin per 100 mg of dried powder.

5.3 Ultrasound-assisted extraction (UAE)

Ultrasound assisted extraction is based on ultrasonic cavitation. Ultrasonic extraction has been used to extract bioactive compounds like vitamins, polyphenols, pigments and other phytochemicals. UAE is cost-effective and significantly reduces the extraction time, whilst resulting in increased extraction yields. The ultrasound can be divided into two distinct categories: low intensity-high frequency (100 kHz–1 MHz) and high intensity-low frequency (20–100 kHz). Ultrasonic extraction is achieved with high intensity and low-frequency ultrasound waves. Ultrasound waves when traveling through liquid creates alternating high-pressure and low-pressure cycles resulting in the production of cavitation bubbles in the solvent. Cavitation bubbles form in the liquid during the expansion phase. The ability to cause cavitation depends on the frequency of the ultrasound wave, the solvent properties, and the extraction conditions. During the compression cycle cavitation bubble implodes on the surface of the matrix (cell, tissue or any particle) and a high-speed micro-jet is created leading to the generation of effects like surface peeling, particle breakdown, sonoporation and cell disruption. Sonoporation (perforation in cell walls and membranes) exerts a mechanical effect, allowing greater penetration of solvent into the sample matrix. This leads to increased extraction efficiency in less time. Using ultrasound-assisted extraction, 4.66 mg β-carotene per g of dry weight has been obtained from microalgae Spirulina platensis [116]. Various parameters (amplitude, duty cycle, sonication time, and depth of horn immersed into the solution) were optimized for intensified extraction. The optimized condition for the maximum extraction of β-carotene from this alga was 80% amplitude, 33% duty cycle, 0.5 cm depth of horn immersed in the solution, and 10 min ultrasoundication time. UAE has also been applied for the extraction of lutein, β-carotene, and α-carotene from Chlorella vulgaris [117]. The maximum extraction achieved were 4.844 ± 0.780, 0.258 ± 0.020, and 0.275 ± 0.040 mg/g of dry weight biomass, respectively.

5.4 Electrotechnologies-assisted extraction

Electrotechnologies, such as pulsed electric field (PEP), moderate electric field (MEF), high-voltage electric discharges (HVED) are emerging, non-thermal, and green extraction techniques for targeting intracellular compounds from bio-suspensions. In the pulsed electric field (PEP), the sample matrix is exposed to repetitive electric frequencies (Hz–kHz) with an intense (0.1–80 kV/cm) electric field for very short periods (from several nanoseconds to several milliseconds). In the moderate electric field (MEF), the sample matrix is exposed to low electric fields (between 1 and 1000 V/cm) with electric frequencies in the range of Hz up to tens of kHz. While high-voltage electric discharges (HVED) typically have 40–60 kV/cm for 2–5 μs electrical property. All of these electrotechnologies have their mechanism of delivering electrical current through the processed biomaterial, they all promote electro-permeabilization allowing the extraction of analytes. The selective extraction of intracellular compounds can be achieved by controlling the pore formation, which is dependent on various factors such as the intensity of the applied electric field, pulse duration, treatment time, and the cell characteristics (i.e., size, shape, orientation in
<table>
<thead>
<tr>
<th>Microalga</th>
<th>Pigment</th>
<th>Technical approach</th>
<th>Carotenoid yield</th>
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<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin</td>
<td>Integrated ultrasound-assisted liquid biphasic flotation</td>
<td>Maximum recovery yield, extraction efficiency, and partition coefficient of astaxanthin were 95.08 ± 0.02%, 99.74 ± 0.05%, and 185.09 ± 4.78, respectively</td>
<td>[97]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin</td>
<td>Biocompatible protic ionic liquids-based microwave-assisted liquid-solid extraction</td>
<td>high purity (97.2%) of free astaxanthin was achieved</td>
<td>[98]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin</td>
<td>cell permeabilizing ionic liquids</td>
<td>More than 70%</td>
<td>[99]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin, lutein, β-carotene and canthaxanthin</td>
<td>Supercritical carbon dioxide extraction</td>
<td>92% recovery of carotenoids was obtained at the pressure of 300 bar and the temperature of 60 °C, using ethanol as a co-solvent</td>
<td>[100]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin and lutein</td>
<td>Supercritical carbon dioxide extraction</td>
<td>98.6% and 52.3% recovery of astaxanthin and lutein respectively, was achieved at 50 °C and 550 bars</td>
<td>[101]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin and lutein</td>
<td>Supercritical carbon dioxide extraction</td>
<td>highest astaxanthin and lutein recoveries were found at 65 °C and 550 bar, with 18.5 mg/g dry weight (92%) astaxanthin and 7.15 mg/g dry weight (93%) lutein</td>
<td>[102]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin</td>
<td>Pressurized extraction solvent</td>
<td>extraction yield of 20.7 mg/g dry weight</td>
<td>[103]</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>Lutein</td>
<td>Pulse electric field</td>
<td>the concentration of lutein was around 4.5-fold higher when the fresh biomass was previously electroporated at 40 °C by a PEF of 25 kV/cm for 75 μs</td>
<td>[104]</td>
</tr>
<tr>
<td><em>Chlorella sorokiniana</em> MB-1</td>
<td>Lutein</td>
<td>Pre-treated by bead-beating and high-pressure cell disruption methods, followed by harvesting with reduced pressure extraction method</td>
<td>Extraction with tetrahydrofuran as solvent resulted in high lutein recovery efficiencies of 99.5% (40 min) at 850 mbar and 25 °C. In contrast, using ethanol as the solvent, 86.2% lutein recovery was achieved under 450 mbar, 35 °C and 40 min extraction</td>
<td>[105]</td>
</tr>
<tr>
<td><em>Tetradesmus obliquus</em></td>
<td>α-tocopherol, canthaxanthin, γ-tocopherol, lutein, phytofluene, retinol, and menaquinone-7</td>
<td>Supercritical fluid extraction (SFE)</td>
<td>The highest extraction of alpha-tocopherol, gamma-tocopherol, and retinol was achieved at a pressure of 30 MPa and a temperature of 40 °C</td>
<td>[106]</td>
</tr>
<tr>
<td><em>Chlorella zoofingiensis</em></td>
<td>Cantaxanthin</td>
<td>High-speed counter-current chromatography (HSCCC)</td>
<td>The recovery of canthaxanthin was 92.3%. Canthaxanthin at 98.7% purity from 150 mg of the crude extract</td>
<td>[107]</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>Fucoxanthin</td>
<td>maceration, ultrasound-assisted extraction, soxhlet extraction, and pressurized liquid extraction</td>
<td>ethanol provided the best fucoxanthin extraction yield (15.71 mg/g freeze-dried sample weight). Fucoxanthin content in the extracts produced by the different methods was somewhat constant (15.42–16.51 mg/g freeze-dried sample weight)</td>
<td>[108]</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum</em></td>
<td>Fucoxanthin</td>
<td>microwave-assisted treatment</td>
<td>ethanol was preferable for the extraction of fucoxanthin than other solvents in terms of the fucoxanthin yield (ethanol/methanol, 48.01 ± 0.35%; ethanol/acetic acid, 53 ± 0.46%) under the continuous microwave-assisted treatment time of 1 min</td>
<td>[109]</td>
</tr>
<tr>
<td><em>Cylindrotheca closterium</em></td>
<td>Fucoxanthin</td>
<td>Microwave assisted extraction, vacuum microwave assisted extraction and ultrasonic assisted extraction</td>
<td>Extraction yield UAE: 4.95 ± 0.27 mg/g; Rt soaking in acetone for 60 min: 7.48 ± 0.21 mg/g; hot soaking in acetone for 30 min: 9.31 ± 0.44 mg/g; MAE: 8.65 ± 0.29 mg/g; VMAE (vacuum-microwave assisted extraction): 5.25 ± 0.04 mg/g</td>
<td>[110]</td>
</tr>
<tr>
<td><em>Dunaliella salina</em></td>
<td>Carotenoids (not specified)</td>
<td>Supercritical carbon dioxide</td>
<td>highest carotenoids extraction yield (115.43 mg/g dry algae) was obtained at pressure of 400 bar and temperature of 55 °C</td>
<td>[111]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin</td>
<td>Solvent extraction hydrochloric acid pretreatment followed by acetone extraction (HCl-ACE), hexane/isopropanol (6:4, v/v) mixture (HEX-IPA), methanol extraction followed by acetone extraction (MET-ACE), and soy-oil extraction</td>
<td>HCl-ACE method yielded the highest astaxanthin content (19.8 ± 1.1%)</td>
<td>[112]</td>
</tr>
</tbody>
</table>
the electric field) [118]. Besides these factors, the temperature is another critical parameter affecting the efficacy of the electrotechnology assisted extraction [104]. Pulses of milliseconds (5 kV/cm-40 ms) or microseconds (20 kV/cm-75 μs) have improved the efficiency of carotenoids extraction from Chlorella vulgaris by 80%. In the microalgae Heterochlorella luteoviridis the application of MEF combined with ethanol as solvent (180 V, 75 mL/100 mL of ethanol solution) resulted in up to 73% of carotenoid extraction [119]. Moderate electric field (MEF) (0–180 V) has been evaluated as a pre-treatment for carotenoid extraction at different temperatures followed by extraction step using ethanol/water as solvent (75% of ethanol, v/v). The highest extraction yield, 86% of the total carotenoid content was achieved at both 40 and 50 °C with the MEF pre-treatment [120]. HVED treatment has been applied and favored the selective recovery of intracellular compounds from algae Parachlorella kessleri and Nannochloropsis oculture [121, 122].

5.5 Pressurized liquid extraction (PLE)

The main purpose of using PLE is that it allows rapid extraction and reduces solvent consumption; therefore, it is sometimes referred to as accelerated solvent extraction. PLE involves the extraction using solvents at elevated temperature and pressure but always below their critical points. This normally falls in the ranges of 50–200 °C and 35–200 bars. During PLE, the interaction between the solvent and the biological sample is increased compared to common solvent extraction methods. Therefore, less solvent is required for extraction. PLE has been applied for the extraction of carotenoids from freeze-dried microalgae and macroalgal biomass. In Phaeodactylum tricornutum pressurized liquid extractions resulted in exceptional amounts of fucoxanthin up to 26.1 mg/g dw [123]. Pressurized liquid extraction has been successfully applied in the case of Neochloris oleoabundans for the recovery of bioactive carotenoids lutein, caroteneido monooesters and violaxanthin [124]. Pressurized liquid extraction (PLE) has been optimized for the extraction of carotenoids and chlorophylls from the green microalgae Chlorella vulgaris and showed higher extraction efficiencies than maceration (MAC), soxhlet extraction (SOX), and ultrasound-assisted extraction (UAE) [49]. PLE has been optimized for Nannochloropsis oceanica with ethanol as extraction solvent. A total carotenoids content of 115.1 ± 0.6 mg/g extract was obtained at the optimum extraction conditions of 57 °C and 3 extraction cycles [125].

5.6 Supercritical fluid extraction (SFE)

SFE involves extraction using supercritical fluids i.e., fluids at a temperature and pressure above its critical limit. Supercritical fluids provide better solvating and transport properties than liquids due to their low viscosity and high diffusivity. For the selective extraction of a broad range of compounds, the solvating power of supercritical fluid can be adjusted by manipulating the temperature and pressure of the fluid. Carbon dioxide is the preferable solvent which can easily achieve supercritical conditions and has benefits like high purity, low toxicity and low flammability compared to other fluids. Supercritical carbon dioxide is non-polar and its polarity can be modified by using co-solvents. Besides CO₂, ethane and ethylene are other SFE solvents that have been used for the extraction of carotenoids in some studies. In Scenedesmus obliquus the highest carotenoid yield was achieved at 250 bar and 60 °C using SFE [126]. Supercritical CO₂ extraction has been applied in Spirulina to obtain carotenoids, chlorophylls, and phycocyanins. The SFE method resulted in 3.5 ± 0.2 mg/g total carotenoid contents in Spirulina [127].

5.7 Subcritical fluid extraction

Subcritical fluid extraction is similar to SFE, where subcritical (liquefied) fluids are used as extraction solvent. Subcritical fluid extraction works at relatively low temperature and pressure than supercritical fluid extraction. In various studies, subcritical CO₂, 1,1,1,2-tetrafluoroethane and dimethyl ether (DME) have shown the potential to extract carotenoids from algae. Subcritical fluid extraction has been performed to extract fucoxanthin from Phaeodactylum tricornutum. The highest fucoxanthin content (0.69 mg/g) was achieved with a solvent-to-solid ratio of 200 : 1, 20 MPa, 35 °C at 120 rpm 60 min by subcritical extraction [128]. The carotenoids and chlorophyll-a from Laminaria japonica have been isolated using ethanol modified subcritical 1,1,1,2-tetrafluoroethane [129].

5.8 High pressure homogenization (HPH) treatment and enzyme-assisted extraction

Microalgal cells are difficult to disrupt, therefore, a physical or enzymatic pre-treatment before extraction can be opted to promote the recovery of carotenoids. High-pressure homogenization (HPH) is one such method, where, cell disruption is achieved by applying high intensity fluid stress (50–400 MPa). In comparison with other physical milling processes, it offers significant advantages such as ease of operation, commercial applicability, reproducibility and high throughput. High-pressure homogenization (HPH) found to be very effective in microalgae with a recalcitrant cell wall such as Nannochloropsis [130]. Cell disruption by high-pressure homogenization has been shown to increase carotenoid and ω3-LC-PUFA bio accessibility [131]. Enzyme-assisted extraction (EAE) methods use hydrolytic enzymes like cellulase and pectinase for improved extraction. Cellulase hydrolyzes the 1,4-β-d-glycosidic links of the cellulose, whereas, pectinase breaks down the pectic substances and pectin found in cell wall components. In a study, enzyme type (cellulase and pectinase), pH values, hydrolysis temperature, and time on the release of astaxanthin from Haematococcus pluvialis were evaluated. The results showed that enzymatic pre-treatment improve
the separation yield of astaxanthin. Pectinase release rate of astaxanthin from *H. pluvialis* was found to be significantly higher than cellulase [132].

6. Application of carotenoids

Non-photosynthetic organisms (humans and animals) are unable to synthesize carotenoid. However, they intake them through their food metabolize them for normal physiological functions [133]. Carotenoid possess boundless and expansive applications and therefore have commercial significance. Further, health is paramount to the consumer, which triggers the large-scale production of these pigments. In this section application of different carotenoid is discussed along with their commercial implications.

6.1 Astaxanthin

Astaxanthin is generally known as ‘Super Vit-E’ due to its compelling antioxidant properties [134]. Kishimoto et al. [135] bestowed it with ‘King of antioxidants’ because of its better antioxidant properties as it emulates Vit C, resveratrol, CoQ10, green tea catechins, and Vit E. Lipid peroxidation, LDL oxidation, and peroxide-induced cytotoxicity, etc. are inhibited by large-scale production of these pigments. Their roles as anti-inflammatory, hepatoprotector and protective influence on retinal, brain, and spinal cord neurons and in the prevention of cardiovascular disease have been reported [137]. Aquaculture feed; poultry and food industries use this tremendously as red colorant [138]. Astaxanthin favorably lowers the level of plasma reactive protein C, down regulate inflammatory cytokines, modulate mitogen-induced lymphoproliferation, and boost the immune response [139]. It protects the skin from the detrimental effects of UV-A photo ageing and is therefore widely used in cosmetics [140]. The inability of astaxanthin to convert into Vit A in the human body safeguards from hypervitaminosis A toxicity even after its overconsumption [141].

6.2 β-carotene

The role of β-carotene as anti-inflammatory, antioxidant, immunoprotector, dermoprotector, hepatoprotector, retinoprotective are well known [142]. The food colorant and cosmetic industries use this pigment on a large scale [143]. It has been reported that the beneficial role of this pigment in breast, colon, lung, liver, and skin cancers. β-carotene can trigger better functioning of gap junction intercellular communications and can diminish the harmful effect of H$_2$O$_2$ on the same and maintain the processes like cell differentiation, growth, and apoptosis [144]. Its regular consumption in the daily diet protects from night blindness and liver fibrosis.

6.3 Fucoxanthin

Fucoxanthin has well-marked use as antioxidant, anti-angiogenic, anti-inflammatory, photo- and neuroprotective, which makes it commercially valuable. Peng et al. [145] reported its role in hindering DNA damage and H$_2$O$_2$-induced apoptosis. In HL-60, PC-3, HT-29, DLD-1, and Caco-2 human cell lines it acts as an antiproliferative [146]. It reduces obesity by up-regulating uncoupling protein-1 (UCP 1) to speed up metabolism and energy expenditure [147]. Heo et al. [148] reported its role in reducing nitric oxide, prostaglandin, TNF-α, histamine levels in *in-vivo* models.

6.4 Zeaxanthin

The role of zeaxanthin in ophthalmological fields is significant and crucial. It is naturally present in the central macula of the eye, and protects the visionary organ from blue, near UV radiations, age-related macular degeneration [149]. Its promising property as natural color mark it use in pigmentation of poultry and fishes, in food, and as an antioxidant in cosmetics [44]. Regular consumption of zeaxanthin might prove useful in avoiding lung and pancreatic cancers in diabetic patients and found to be effective in cardiovascular problems [150]. This xanthophyll has the ability to trigger apoptosis in melanoma cells and therefore useful in adjuvant therapy.

6.5 Lycopene

Lycopene has antioxidant, photo-protective properties. It proves to be anti-cancerous against cell lines from the human colon, breast, prostate, liver, and lymphocytes [151]. It prevents from early arthrosclerosis and high blood pressure problems by boosting endothelial functioning and lowering oxidative stress. Lycopene, also act as hypolipidemic in a way similar to statins and maintain blood cholesterol levels [152]. The sufficient daily doses of lycopene strengthen the skeleton system, enhance gap junction intercellular communications, and maintain glucose homeostasis, which in turn prevent type-2 diabetes. Viuda-Martos et al. [151] reported lycopene-induced inhibition of tumor metastasis because of modulated cell cycle progressions. The poultry farms use this as feed to improve the health of poultry birds and it also relieves heat stress in commercial poultry. Lycopene is commercially available in form of capsules, tablets, and in gel forms. It has profound role in cosmetics and coloring agent in food sectors.

6.6 Lutein

Marigold is the chief source of lutein production at the industrial level. Like zeaxanthin, it protects eye from pernicious UV, blue light and therefore, referred to as eye vitamin. It lowers the level of plasma factor D and in turn plays a defensive role against age-related macular degeneration and cataract. It is effectual as a chemotherapeutic agent [153]. It is an active anti-tumor substance against prostate, breast, and colon cancer. Lutein consumption can also lessen the chance of early atherosclerosis and lung cancer as well. It alleviates the effects of neurodegenerative disorders arise due to inflammation. It has prodigious use in poultry as yolk color enhancer, feather coloration through
feed and as an additive in infant food, drug and cosmetics [154].

6.7 Other pigments

Canthaxanthin is a β-carotene derived product, is remarkably used in food and as an additive in animal feed to poultry and fish (salmon, rainbow trout). It is an FDA approved compound to be used as a fish color intensifier [44]. Its doses along with β-carotene prevent idiopathic photodermatosis. Capsanthin is another pigment that shows antioxidant activity and is used as a natural colorant in food and cosmetic. Nowadays, it has been widely used in chicken feed to intensify color of egg yolk. The food sector uses one more yellow-orange pigment i.e., Annatto, to provide color to meat products, beverages, and smoked seafood. Echineone is a by-product of astaxanthin, possess pro-vitamin A and antioxidant property. Another pigment, peridinin presents anti-carcinogenic activities. The neoxanthin might lessen the lung cancer risk through regular consumption of 9-(Z) neoxanthin [143].

7. Global carotenoid market

The global monetary value of carotenoids depends upon its demand by the consumer and its extraction cost. Major branches of the carotenoid market include feed, food, nutraceutical, pharmaceutical, cosmetic, and aquaculture sectors [154]. An amount of USD 1.5 billion market was expected at the global level but with compelling demand it is expected to surge to USD 2 billion by 2022 with a compound annual growth rate (CAGR) of 5.7%. Astaxanthin, β-carotene, lutein, fucoxanthin, zeaxanthin dominate the market due to their wider properties. A report by BCC research mentions that capsanthin occupied a mammoth share of the total carotenoid market i.e., 285 million USD (19.7%), followed by astaxanthin (18.5%) of the total share, accounting 267.5 million USD, β-carotene—246.2 million USD (17% of total), lutein totaled 225 million USD (15.6%), annatto (Bixin)- 170 million USD (11.8%), and lycopene at 107 million USD with (7.4%) of total market [155]. The market value of astaxanthin is estimated to reach USD 426.9 million by 2022 from USD 288.7 million in 2017 at CAGR of 8.1%. As per the estimations of Astaxanthin-Global Market Outlook (2017–2023), the global astaxanthin value is estimated to elevate from $615.19 million in 2016 to $1226.14 million by 2023 with a CAGR of 10.3%. The utility of lutein will take the market value of pigment to 357.7 million USD by 2022 from 263.8 million USD in 2017 at CAGR of 6.3% from 2017. The average market price of β-carotene is near to USD 300–1500/kg [156]. Capsanthin witnessed a monetary value of USD 300 million in 2017 (20% of the total market) which will be projected to reach about 385 million USD by 2022 with CAGR of 5.1%. Europe (Germany, France, Italy, Netherlands, Spain) wrest the carotenoid market in terms of consumption followed by Asia and Pacific region (China and Japan) with the second largest consumption zone. Australia and New Zealand own the smallest market share in terms of consumption, while the Canadian market is unflagging with a declining CAGR (0.9% in 2017 to 1.4% in 2019–2022). Brazil and Mexico govern the market in Latin America, Russia, Poland, Slovakia, and the Czech Republic are the growing markets of Eastern Europe [157].

There are several desperate elements and factors, which govern the carotenoid market. From production point of view, the revamping of the selected entity depends upon their cultural conditions i.e., temperature, irradiance, pH, salinity, nutrients, and presence of oxidizing substances. Secondly, as consumers gaining interest and awareness to have an immune diet rather than muscle full supplement and balanced consumption is imperative in governing the carotenoid market and foster the overall demand and industrial production. The global carotenoid market estimates the major market value for the feed segment by 2023 because of less restricted regulations. Additionally, an increase in meat, poultry, and dairy products will accelerate the rise of the feed market. Feed segment is going to witness colossal demand because of a pandemic outbreak, animal diseases, and the requirement of quick-service restaurants both at the domestic and international level. The investment and participation of well-known cosmetic industries like Hindustan Unilever, L’Oreal, Henkel, and Beiersdorf may be lucrative for the growth of the European carotenoid market [9]. The high commercial demand and potential values inflate the market as per the demand and supply curve. Deficiency of raw material, efficient tools, and methods both for up streaming (extraction) and for the down streaming process (purification) attribute to high monetary value [158]. The presence of another compound along with required under amount substance make the large-scale production technically difficult and costly. Besides, legal and strict regulatory and approval checklist related to the health care issues, effect on environment and animals, constraint the market of carotenoids. The build-out of the carotenoid market and its constituent products depends on several ethical, social, commercial, and biotechnical factors. Biotechnologists are endeavouring for the burgeoning of production scale by developing green, renewable resources, efficacious technologies and companies are trying to propitiate consumers by sophisticated presentation and sale of their products. With the growing exigency for natural products and declining traditional resources, key commercial entities are investing in carotenoid production at a large scale and augmenting the carotenoid market competitiveness. The pre-eminent producers of carotenoid are listed in Table 3 along with their web address.
Table 3. Major companies involved in carotenoid production at commercial level.

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9. Author contributions

AK, VV and H conceptualize this review article. AKG, KS, KM and MM wrote the original draft. PKB wrote and interpreted about chemistry of different carotenoids. AK, VV and H edited the manuscript and prepared the tables. All authors read and approved the final manuscript.

10. Ethics approval and consent to participate

Not applicable.

11. Acknowledgment

The authors are grateful Head, Department of Botany, Mohanlal Sukhadia University, Udaipur for providing necessary laboratory facility.

12. Funding

Part of this work is supported by a grant from the Department of Science and Technology, New Delhi (SERB File Number: EEQ/2020/000011). VV is thankful to DST-Nanomission (Govt. of India) project number (SR/NM/NT-1090/2014(G) for financial support.

13. Conflict of interest

The authors declare no conflicts of interest.

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Abbreviations: AACT, acetoacetyl-CoA thiolase; ABA, abscisic acid; BCH, β-carotene hydroxylase; BKT, β-carotene ketolase; CAGR, Compound annual growth rate; CMK, 4- (cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase; Crt-ISO, carotenoid isomerase; DMAPP, dimethylallyl diphosphate; DXR, 1-deoxy-D-xylulose 5-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; EHY, ε-carotene hydroxylase; ESTs, expressed sequence tags; FPPS, farnesyl diphosphate synthase; GGPP, geranyl geranyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase; GPPS, geranyl diphosphate synthase; GSMN, genome scale metabolic network; HDR, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; HDS, 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HMG-CoA, β-Hydroxy β-methylglutaryl-CoA; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; IDI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; MCT, 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase; MDS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MEP, methylerythritol phosphate; MVA, mevalonate; MVD, mevalonate diphosphate decarboxylase; MVK, mevalonate kinase; NXS, neoxanthin synthase; PDS, phytoene desaturase; PMK, phosphomevalonate kinase; PSY, phytoene synthase; SL, strigolactone; VDE, violaxanthin de-epoxidase; ZDS, ζ-carotene desaturase; ZEP, zeaxanthin epoxidase; Z-ISO, ζ-carotene isomerase.

Keywords: Astaxanthin; Biosynthesis; Carotene; Fucoxanthin; High-Value compounds

Send correspondence to: Harish, Department of Botany, Mohanlal Sukhadia University, 313 001 Udaipur, Rajasthan, India, E-mail: harish.botany1979@gmail.com

Ashwani Kumar, Metagenomics and Secretomics Research Laboratory, Department of Botany, Dr. Harisingh Gour Central University, 470003 Sagar, MP, India, E-mail: ashwaniiitd@hotmail.com