REVIEW





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Abstract

Microalgae are promising sources of valuable carotenoids like β -carotene and astaxanthin with numerous health benefits. This review summarizes recent studies on producing these carotenoids in microalgae under different salinity and light-intensity conditions, which are key factors influencing their biosynthesis. The carotenoid biosynthesis pathways in microalgae, involving the methylerythritol phosphate pathway in chloroplasts, are described in detail. The effects of high salinity and light stress on stimulating astaxanthin accumulation in species like *Haematococcus pluvialis* and *Chromochloris zofingiensis* and their synergistic impact are discussed. Similarly, the review covers how high light and salinity induce β -carotene production in *Dunaliella salina* and other microalgae. The diverse health-promoting properties of astaxanthin and β -carotene, such as their antioxidant, antiinflammatory, and anticancer activities, are highlighted. Strategies to improve carotenoid yields in microalgae through environmental stresses, two-stage cultivation, genetic engineering, and metabolic engineering approaches are evaluated. Overall, this review highlights advancements in β -carotene and astaxanthin production reporting the different microalgal capability to produce carotenoids under different stress level like 31.5% increase in β -carotene accumulation in *Dunaliella salina* and astaxanthin productivity reaching 18.1 mg/L/day in *Haematococcus lacustris*. It also explores novel biotechnological strategies, including CRISPR–Cas9, for enhancing carotenoid yield.

Keywords Salinity stress, Light stress, Astaxanthin, β -Carotene, Antioxidants, Microalgae

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Introduction

Microalgae are vital components of ecosystems, functioning as photosynthetic microorganisms that harness light energy along with inorganic nutrients such as nitrogen, phosphorus, and carbon dioxide (CO₂) to produce biomass and a range of bioactive compounds [1, 2]. Additionally, they are capable of synthesizing highvalue metabolites, including carotenoids, which are of significant ecological and industrial importance [3]. Recent research has focused on microalgae as a promising source of bioactive compounds with the potential to enhance nutritional value and provide significant health benefits for both humans and animals [4, 5]. A varied class of terpenoid pigments found in many photosynthetic organisms are called carotenoids [6]. Apart from their vital roles in photosynthesis and photoprotection for phototrophs, carotenoids are also key parts of human diets and are vital for human health [7]. Carotenoids are produced in the chloroplasts around the nucleus of plants and algae, and their buildup only occurs in response to specific stressors [8]. Two prominent carotenoids produced by microalgae are β -carotene and astaxanthin. Astaxanthin and β -carotene are considered more important than many other carotenoids due to their unique and potent properties, making them highly valuable in health, nutrition, and commercial applications. β -Carotene is an orange-coloured carotenoid known as a provitamin A compound [3]. β -Carotene is a highly valuable biochemical compound functions as a dietary pigment, a precursor to vitamin A [9], and a potent antioxidant [10]. Microalgae are the primary source for the biosynthesis of β -carotene [11, 12]. Salt is a major environmental stressor for freshwater microalgae. Salt stress can impede photosynthesis and development which causes oxidative damage, and degrade chlorophyll [13]. Microalgae have developed a survival strategy to ensure the equilibrium of growth and stress responses in response to salinity stress by accumulating secondary metabolites like astaxanthin [14]. Under various stress situations, astaxanthin accumulation in lipid bodies outside of the chloroplast rises [15]. In Haematococcus sp., it accumulates in the cytoplasm while xanthophylls are localized and synthesized within the plastids of certain green microalgae [16]. A scant number of organisms possess the ability to synthesize astaxanthin like Xanthophyllomyces dendrorhous, Haematococcus pluvialis, Chromochloris zofingiensis, Chlamydomonas nivalis, and certain species of Phaffia and Paracoccus [6]. Haematococcus pluvialis, which is one of the main natural astaxanthin source available on the market at the moment [8] and Chromochloris

zofingiensis, which may develop vigorously to reach high cell densities in a variety of trophic circumstances [17–19] and is thought to be a possible substitute astaxanthin producer [20].

One of the best approaches for promoting astaxanthin and β -carotene synthesis in microalgae is high light intensity and saline conditions [21]. Microalgal carotenoid production is significantly influenced by stress conditions such as high light intensity, salt stress, nutrient deficiency, and excessive irradiation, which enhance secondary carotenoid synthesis by increasing reactive oxygen species (ROS) and mitigating photo-oxidative damage. While primary carotenoids like lutein degrade under stress, secondary carotenoids such as β -carotene and astaxanthin accumulate, with β -carotene reaching high concentrations in lipid bodies. Cultivation techniques, including two-stage and continuous processes, further enhance astaxanthin production, while certain species maintain stable lutein production under stress, making them viable for large-scale cultivation. For lipid production, autotrophic and heterotrophic strategies are employed, with key stress factors such as light intensity, temperature, and nitrate availability playing crucial roles. Effective stress management is critical for optimizing metabolite and lipid yields in laboratory and pilotscale systems as shown in Fig. 1 [22]. When exposed to intense light, astaxanthin and β -carotene productivity positively increases in a variety of microalgal species [23]. High salinity and light intensity induce the accumulation of β -carotene and astaxanthin in the green microalgae as a protective response to osmotic and oxidative stress [24]. Studies on microalgae have examined the routes and related enzymes involved in carotenogenesis. Algae and cyanobacteria produce carotenoid synthesis using distinct enzymes and genes [25]. Well-researched contenders for the synthesis of economically significant carotenoids include H. pluvialis, D. salina, and Chlorella sp. [26]. Carotenoids have several health benefits because of their antioxidative qualities, carotenoids are utilized as food supplements, colourants in aquaculture feed, and colouring agents in human food. They serve as antiinflammatory, anticarcinogenic, and antibacterial agents in medicinal preparations and cosmetics [27, 28]. While other carotenoids like lutein, zeaxanthin, and canthaxanthin also have health benefits, astaxanthin and β-carotene offer a broader range of applications and more potent biological effects. In the reports suggested by BCC Research, the global market value of carotenoids in 2022 was estimated to be \$2.0 billion USD, whereas according to the forecasts the global carotenoid market value will increase to \$2.7 billion USD by 2027 at a compound annual growth rate of 5.7% [29].



Fig. 1 Schematic representation showing the impact of stresses on carotenoid and biomass production (modified by Minhas et al. [22])

This review highlights recent advances in β -carotene and astaxanthin production under salinity and light stress, their health benefits, carotenoid biosynthesis pathways, stress-induced carotenogenesis mechanisms, and metabolic engineering strategies for enhanced yields. This review provides a comprehensive synthesis of recent advancements in β -carotene and astaxanthin production under stress conditions also it emphasizes the molecular mechanisms driving carotenogenesis and explores cutting-edge biotechnological strategies, including CRISPR–Cas9 and metabolic engineering, for optimizing carotenoid yields in microalgae.

Carotenoid biosynthesis pathways in microalgae

Carotenoids are synthesized in microalgae through the methylerythritol phosphate (MEP) pathway [30, 31] located in the chloroplast [14]. The chloroplast is where primary carotenoids, which are vital for cellular viability, are made. When under stress, secondary carotenoids generate cytoplasmic lipid vesicles that are bigger [32]. Isopentenyl pyrophosphate (IPP) is a precursor for quinines, sterols, phytol of chlorophylls, and carotenoids. The IPP can be synthesized from two independent pathways. One is the mevalonate pathway, whereas the other is the non-mevalonate pathway (1-deoxy-D-xylulose phosphate pathway) [25]. The pathway starts with the synthesis of the five carbon precursors isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) [33]. These precursors are combined by geranyl diphosphate synthase (GPPS) and geranylgeranyl pyrophosphate synthase (GGPPS) to produce the 20-carbon geranylgeranyl pyrophosphate (GGPP) [34]. In some microalgae like H. pluvialis, GGPPS alone can catalyse the full conversion from the 5-carbon precursors to GGPP [35]. IPP isomerase has been found in two copies of *H. pluvialis*. Due to the high light intensity, one of the two isomerase enzymes that accumulate carotenoids in the cytoplasm had enhanced expression and upregulation [36].

GGPP molecules are then condensed by phytoene synthase (PSY) to form the first carotenoid phytoene. Phytoene is subsequently desaturated by phytoene desaturase (PDS) and converted to lycopene via enzymes like ζ -carotene isomerase (Z-ISO), ζ -carotene desaturase (ZDS) and carotene isomerase [37]. At this point, lycopene can enter either the α -branch or the β -branch of the pathway. In the α -branch, lycopene is cyclized by lycopene ε -cyclase (LCYE) to α -carotene, which is then converted to lutein by cytochrome P450 enzymes like β -hydroxylase (CYP97A) and epsilon hydroxylase (CYP97C) [38]. In the β -branch, lycopene is cyclized by lycopene β -cyclase (LCYB) to β -carotene [39]. β -Carotene can be converted to zeaxanthin by β -carotene hydroxylase (CHYB) or to astaxanthin via the ketolase

Production dynamics of β-carotene

and astaxanthin in microalgae under light and salt stress

Impact of light stress on microalgal physiology, biomass, and carotenoid accumulation

Microalgal cells are susceptible to significant effects from light stress. Microalgae can initiate lipid synthesis when exposed to high light levels, with different species and strains needing differing levels of light to produce the most lipids [41]. Microalgae have evolved defence mechanisms against intense light, such as quickly lowering their chlorophyll-a concentration to prevent photodamage and preserve a high cell density. Light intensity also affects the spatial arrangement of cells within biofilms, which has an impact on the microalgae's general physiology and structure [42]. Microalgal cells' physiology is impacted by light exposure because it causes oxidative stress, which can harm cellular constituents. Microalgae enhance the production of β -carotene and astaxanthin, as potent antioxidants to mitigate oxidative stress and protect themselves under adverse conditions. By neutralizing reactive oxygen species (ROS) and converting surplus light energy to heat, these carotenoids can shield the photosynthetic system from harm [43, 44]. ROS can be produced when there is an excessive reduction in the electron transport chain in chloroplasts due to high light intensity. The xanthophyll cycle supported by carotenoids such as astaxanthin plays a crucial role in non-photochemical quenching [45].

Furthermore, light stress can change how genes involved in carotenoid biosynthesis are expressed, which increases the amount of these pigments produced. It has been found that light causes certain microalgae, including *H. pluvialis*, *D. salina*, *C. zofingiensis*, and *Euglena gracilis*, to accumulate carotenoids [46, 47]. High light intensity significantly enhances the biomass productivity of various microalgae, with species-specific responses observed. For instance, *D. salina* thrives at 1000 µmol photons/m²/s [48], while *Oscillatoria* sp. achieves 3 g DCW/L under 3000 lux [49]. A detailed comparison of different microalgae and their responses to high light conditions is summarized in Table 1.

The ability of microalgae to accumulate carotenoids in large quantities makes them valuable objects for biotechnological production [60]. Microalgae such as *D. salina*, *H. lacustris*, and some *Chlorella* spp. are rich sources of carotenoids [48, 61]. In *H. lacustris* [21, 62], *D. salina* [63], *C. zofingiensis* [17], and *T. obliquus* [41] exposure to high-intensity lighting stimulates the production of



Fig. 2 Biosynthesis pathway of carotenoids. The following enzymes are displayed: β -LCY, β -cyclase; β -OHase, β -carotene hydroxylase; CRTISO, carotenoid isomerase; DMAPP, dimethylallyl diphosphate; DXP, deoxy-D-xylulose 5-phosphate; DXS, DXP synthase; ϵ -LCY, ϵ -cyclase; ϵ -OHase, ϵ -carotene hydroxylase; G3P, glyceraldehyde-3-phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, GGPP synthase; MEP, methylerythritol E 4-phosphate; PDS, phytoene desaturase; PSY, phytoene synthase; ZDS, ζ -carotene desaturase; Z-ISO, ζ carotene isomerase [40]

carotenoid pigments. The two most intriguing carotenoids available in the global market are astaxanthin and β -carotene [60]. *D. salina* is the most commercially significant microalga for natural β-carotene synthesis, with the ability to accumulate up to 14% of its dry weight [53]. An increase in light intensity from 100 to 200 μ mol photons/m²/s resulted in a 31.5% increase in β -carotene accumulation in *D. salina* Y6 [63] and other strains of Dunaliella also showed an increase in productivity of β -carotene when exposed to intensity above 200 μ mol photons/m²/s. A separate study showed, when light intensity was adjusted from 100 to 1000 µmol photons/m²/s, D. salina's β -carotene content increased as well, reaching 3.1% of dry cell weight as shown in Table 2 [64]. D. bardawil contained 0.16% β-carotene on a dry weight basis. When exposed to high light intensity (250 μ mol photons/m²/s), β -carotene content increased to 8-14% dry weight [66]. A secondary carotenoid in the xanthophyll group is astaxanthin and its forerunner is β -carotene. According to reports, many microalgae produce and accumulate a considerable amount of astaxanthin when subjected to stressful conditions [75]. H. lacustris [76] and C. zofingiensis [20, 77] are recognized producers of astaxanthin. Chlamydomonas nivalis [78], Scenedesmus vacuolatus [79], and Neochloris wimmeri [79] were also reported to contain lower concentrations of astaxanthin. One of the best conditions for promoting astaxanthin production in microalgae is high light intensity [21]. Under high light conditions (>400 μ mol/m²/s), astaxanthin accumulates massively in the cytoplasm and lipid vesicles of H. pluvialis cells to act as a photoprotective agent against excessive energy absorption and singlet oxygen formation [80]. According to a separate study, 9.5 µg m/L of astaxanthin productivity occurs under high light in *H. pluvialis* [68]. There are positive results of an increase in the productivity of astaxanthin when exposed to high-intensity light (100 and 400 μ mol photons/m²/s) on heterotrophically grown cultures of H. lacustris NIES-14 [23]. In additional research, H. lacustris JNU35 used 150 μ mol photons/m²/s to produce 18.1 mg/L/day [67]. According to a study light source and photoperiod significantly impacted H. pluvialis growth. Under low light intensity (40.6 \pm 3.05 μ mol/m²/s), white plasma light with 24:0 light/dark cycle produced the best results like highest specific growth, maximum biomass and cell density. One study showed that 90 μ mol/m²/s light intensity led

S. No	Microalgae species	Optimal light intensity	Biomass/lipid	Biochemical response	References
1	Chlorella kessleri	120 µmol photons/m²/s	Highest biodiesel content of 24.19% of TVSS	Optimal for biomass accumula- tion and biodiesel productivity	[50, 51]
2	Chlorella protothecoides	30 µmol photons/m²/s	Biodiesel content of 19.48% of TVSS	Suitable for COD removal	[50]
3	Desmodesmus sp.	300 μE /m²/s	Biomass yield of 1.4 g/L	High lipid accumulation, poten- tial biofuel source	[52]
4	Scenedesmus obliquus	300 μE m ² /s	Biomass yield of 1.2 g/L	Biofuel potential identified	[52]
5	Chlorella vulgaris	420–520 µmol photons/m²/s	Lipid content increased signifi- cantly (7.9% to 22.2%)	Lipid enhancement observed under white and red LEDs	[53]
6	<i>Chlorella vulgaris</i> (biofilm)	100–300 µmol photons/m²/s	Enhanced cell density in bio- films	Chlorophyll content adjusted under excess light	[42]
7	Desmodesmus sp. CHX1	180 μmol photons/m²/s	High nutrient removal efficiency (NH4-N: 86.53%, TP: 99.15%)	Strong potential in wastewater treatment	[54]
8	Chlamydomonas reinhardtii	Up to 500 µmol photons/m²/s	9.7-fold increase in cell number	Cell size enlargement observed	[55]
9	Dunaliella salina	1000 µmol photons/m²/s	Maximum biomass growth observed	High carotenoid production	[48]
10	Chlorella sp. 800	200–500 µmol photons/m²/s	Biomass productivity increased from 0.423 to 0.707 g/L/day	Biomass enhancement with increasing light intensity	[56]
11	Chlorella sorokiniana	162 μmol photons/m²/s	High ammonium nitrogen removal and lipid accumulation observed	Biomass composition varied with light conditions	[57]
12	<i>Oscillatoria</i> sp.	3000 lx	Biomass of 3 g DCW/L achieved	Higher productivity under light stress	[49]
13	Dunaliella salina (two-stage)	600 W red light	Enhanced carotenoid content and high-quality protein	Red light improved protein and amino acid composition	[58]
14	Microalgae (general)	Heterotrophic vs. autotrophic conditions	Significant increase in biomass productivity under hetero- trophic growth	Indicates adaptability of micro- algae for biomass enhance- ment	[59]

Table 1 Microalgal diverse strategies and responses to varying light intensities and growth conditions

 Table 2
 Microalgal carotenoid production under different stress conditions

Microalgal species	Stress condition	Carotenoid produced	Amount of productivity	References
Dunaliella salina	Light intensity: 100–200 µmol photons/ m²/s	β-Carotene	31.5% increase	[63]
	Salinity: 1–3 M NaCl	β-Carotene	2-4 times increase	[65]
Dunaliella bardawil	Light intensity: 250 μ E/m ² /s, salinity	β-Carotene	8–14% of dry weight	[66]
Haematococcus lacustris	Light intensity: 150 µmol photons/m²/s	Astaxanthin	18.1 mg/L/day	[67]
Haematococcus pluvialis	High light intensity	Astaxanthin	9.5 μg/mL	[68]
	Salinity: 0.17 M NaCl for 10 days	Astaxanthin	17.7 mg/g	[69]
	Salinity: 1 M NaCl, Light: 800 µmol/m²/s	Astaxanthin	> 5 times higher than control	[65]
Scendesmus obliquus	Salinity: 0–50 mM NaCl	Astaxanthin	Up to 18% of dry weight	[70]
Chlorococcum	Light intensity: 90 µmol/m²/s	Astaxanthin	Higher content than no light	[71]
	Salinity: 0.5–1.0 M NaCl	Astaxanthin	0.9–1.8% of dry weight	[71]
Chromochloris zofingiensis	Salinity, high light	Astaxanthin	7.53-Fold increase compared to control	[72]
Chlorella zofingiensis	Strong light, salt stress	β-Carotene, zeaxanthin	7.18, 7.00 mg/g DW	[14]
Chlorella sorokiniana	Salinity: 20% NaCl (w/v), linoleic acid	Astaxanthin	1.25-Fold increase	[73]
Scenedesmus sp.	High light intensity and NaCl	Astaxanthin	0.59 mg/g	[74]

to higher astaxanthin content compared to no light [71]. According to one study, *H. pluvialis* also produces 9.5 μ g/mL of astaxanthin when exposed to high light intensity

[68]. In a different study the quality of light was also used to check its effect on the biomass and carotenoid production in photoperiod of 12 h:12 h light/dark cycle and light intensity of 100 μ mol photon/m²/s, the mixed red and blue light showed more balanced growth and high photosynthetic activity. The individual blue light stimulated carotenoid biosynthesis and individual red light primarily supports biomass accumulation. The mixed ratio of blue and red light with high proportion of blue and red light enhanced carotenoid and biomass accumulation, respectively. Thus, different light intensity and light quality can be used to stimulate the synthesis of carotenoids in microalgae mainly β -carotene and astaxanthin.

Impact of salt stress on microalgal physiology, biomass, and carotenoid accumulation

Microalgae are susceptible to many effects of salt stress, including decreased cell development, decreased chlorophyll concentration, inhibition of photosynthesis, and morphological alterations [13]. When *H. pluvialis* is exposed to salt stress, its cell wall hardens, its volume increases, and eventually it turns into immovable cysts. These morphological modifications are linked to several intricate signalling events [13].

Early signalling

Calcium signalling Calcium (Ca²⁺) acts as a universal secondary messenger in response to salt stress, regulating downstream effector proteins, such as calmodulin (CaM), Ca²⁺ dependent protein kinases, and CaM-dependent protein kinases [81, 82]. Ca^{2+} has several properties that make it an ideal ubiquitous signalling molecule. Its cytosolic concentration is easily mutable because it is low compared to other internal or exterior areas. Numerous proteins are capable of identifying and halting (Ca^{2+}) alterations. Numerous Ca²⁺-permeable channels or transporters are available to accurately control these variations in concentration [81]. The decarboxylation of glutamate to γ -aminobutyric acid (GABA) is regulated by the Ca²⁺/ CaM protein [83]. y-Amino butyric acid (GABA) is a water-soluble, non-proteinaceous, four-carbon molecule that has been shown to accumulate in response to various abiotic stressors [84].

GABA and nitric oxide signalling GABA is a signalling molecule involved in cell growth and enhancing abiotic stress tolerance in plants [85]. Under salt stress, it also helps to maintain the C/N balance and acts as a scavenger of reactive oxygen species (ROS) [86]. It has been proposed that GABA improves abiotic stress tolerance in plants by increasing photosynthetic efficiency and development, as well as by activating some defence mechanisms, such as enzymatic and non-enzymatic antioxidants [87, 88]. Using GABA during salt stress alters the nitrate-uptake system's signalling and the antioxidant enzyme functions of the N metabolic pathways [89].

It is also related to nitric oxide (NO) accumulation under stress conditions [85]. Nitric oxide is an important molecule involved in plant growth, development, and tolerance to abiotic stress [90]. It can activate antioxidant enzyme defence against oxidative stress induced by salt stress and aid in the recovery of the damaged photosynthetic system [90]. When subjected to different abiotic stressors have demonstrated decreased chlorophyll loss followed by repair due to exogenous NO application using NO donors [91].

Reactive oxygen species signalling ROS are second messengers induced by salinity stress, also associated with Ca²⁺ signalling [83]. It can regulate cell growth and metabolite synthesis but can also cause oxidative damage [92]. Salinity raises the ROS concentration, which results in anomalies in the organelle ultrastructure and the loss of beneficial electrolytes [93]. Antioxidant enzymes and antioxidants, such as carotenoids, are essential mechanisms for eliminating excessive ROS in microalgae (Fig. 3) [24].

Downstream signalling

Gene expression Salinity-induced signals can affect the gene expression of microalgae cells, and the effects are related to salt concentrations [94]. Low salt concentrations can promote the growth of some microalgae by upregulating growth-related genes, such as *rbcL*, *rbcS*, and *NR* [94, 95].

Osmotic homeostasis Under high-dose salt stress, microalgae need to maintain ionic and osmotic homeostasis [96]. Strategies include regulating intracellular Na⁺ and K⁺ concentrations through ion transporters and accumulating compatible solutes like glycerol to maintain water potential [97]. Through the exclusion of Na⁺, selective transporters of Na⁺ preserve the balance of Na⁺ and K⁺ in cells. Furthermore, several studies have shown that maintaining the K⁺/Na⁺ ratio contributes to salinity tolerance. Thus, the ability of a large concentration of K⁺ to persist aids in the exclusion of Na⁺ from the cell. [98].

Metabolic adjustments Salt stress can upregulate genes related to starch catabolism and downregulated genes for gluconeogenesis, providing building blocks for the storage of lipids and carotenoids [33]. Expression of acetyl-coenzyme A carboxylase (ACCase) for fatty acid synthesis is induced under salt stress in various microalgae species [99, 100].

Omics approaches Omics approaches, such as genomics, transcriptomics, and metabolomics, are employed to study the changes occurring under salt stress. Integrating



Fig. 3 Possible mechanisms explaining microalgae responses to salt stress

diverse "omics" methodologies such as transcriptomics, proteomics, metabolomics, and genomes is novel and extensively used instrument for managing adaptation and tolerance to salinity. Studies using "omics" to examine control and salinity-stressed plants have allowed for the clear demonstration of modifying characteristics, genes, proteins, and metabolomes that are essential for giving salt stress tolerance [101]. Transcription factors like MYB, WRKY, and bHLH play important roles in regulating gene expressions under salinity [92].

Effect of salt stress on biomass and carotenoid accumulation in microalgae

Strict salt stress can be toxic and inhibit growth in microalgae. Therefore, an optimal salt concentration should be chosen carefully based on the microalgal species and the cultivation goals (biomass or carotenoid production) [72]. Research suggests that β -carotene levels can be increased by 2–4 times at 1–3 M NaCl concentrations as opposed to low salinities [65]. Astaxanthin production is induced in *H. pluvialis* as a response to various stress factors. High salinity stress promotes astaxanthin accumulation by inducing oxidative stress via osmotic and ionic imbalances [102]. Studies have shown that exposing *H. pluvialis* to high NaCl concentrations (>0.5 M) can elevate astaxanthin levels by more than 2.0-fold [103]. According to the study 1% (0.17 M) NaCl treatment for 10 days increased astaxanthin content from 3.53 to 17.7 mg/g in H. pluvialis [69]. In a separate study of H. pluvialis, melatonin (MT) addition enhanced carotenogenic gene expression and induced a 1.20-fold increase in astaxanthin accumulation under N-starvation and 1 g/L salt stress [104]. Also, under 0.8% of NaCl, astaxanthin productivity of 80 pg/ml is seen in H. pluvialis [105]. In S. obliquus, when concentration is increased from 0 to 50 Mm NaCl, then astaxanthin accumulation occurs up to 18% of its dry weight [70]. The highest fucoxanthin yield of 79.40 ± 0.95 mg/g dry weight (DW) was obtained with 36.27 g/L salt addition in *Tisochrysis lutea* [106]. In Coccomyxa onubensis 100 mM NaCl significantly boosted growth rate and biomass productivity. 500 mM NaCl enhanced lutein content by 47% to 7.80 mg/g DW, although cell growth was inhibited [107]. In another study, Coccomyxa onubensis under 100 mM NaCl significantly boosted growth rate and biomass productivity [108].

NaCl treatment amplified the effect of linoleic acid (LA) on boosting astaxanthin accumulation, with LA increasing astaxanthin content by 1.25-fold in the presence of 20% NaCl (w/v) in *Chlorella sorokiniana* [73]. One study found that increasing NaCl concentration from 0.5 M to 1.0 M significantly increased astaxanthin

content from around 0.9–1.8% of dry weight in *Chlorococcum* [71]. Research suggests that β -carotene levels can be increased by 2–4 times at 1–3 M NaCl concentrations as opposed to low salinities [65]. Increasing salinity from 1.5 M NaCl to 3.5 M NaCl also increased β -carotene accumulation [66]. Since *D. salina* can grow in high salinity, it is economical to cultivate it in seawater with a high β -carotene output, perhaps avoiding microbial contamination according to research prominent productivity of the order of 3,267 µg/mL carotenoid was obtained by the marine strain *D. salina* (DUN5), particularly at mean salt concentrations of 2.5 M [109].

Effect of combined stress (light and salinity) on biomass and carotenoid accumulation in microalgae

The combined effect of salinity and highlight shows a synergistic impact. For example, H. pluvialis cultivated under 1 M NaCl and 800 µmol/m²/s light exhibited astaxanthin levels over 5 times higher than cultures grown under regular conditions [65]. In C. zofingiensis, salinity treatment combined with high light increased astaxanthin yield by 7.53-fold compared to the control [72]. In another study, H. pluvialis at 0.25 mM y-aminobutyric acid (GABA) facilitated a 3.24-fold increase in astaxanthin productivity under high light with 2 g/L salinity treatment [110]. In Scenedesmus sp., 0.59 mg/g of astaxanthin was produced under the combined effect of high light intensity and salt stress. For the accumulation of β -carotene, the combined effects of salt and light stress are also found to work synergistically. When compared to non-stressed control cultures, D. salina cells were grown in 2 M NaCl, and 1000 μ mol/m²/s light had a β -carotene concentration that was nearly 6 times higher. In D. salina under salt stress, combined with high light at high temperatures and nutrient deficiency, β-carotene content achieved 13% of DW [65]. D. bardawil accumulates large amounts of β -carotene under conditions of high light intensity and salinity stress [111]. At 3.5 M NaCl and 250 μ mol/m²/s light, β-carotene content reached 14–16% dry weight. β -Carotene was found to be localized in globules within the inter-thylakoid spaces of the chloroplast. The globules were composed of β -carotene, lipids, and protein. β-Carotene likely serves a photoprotective role in D. bardawil under high light and salinity stress [66]. Cells, dietary β -carotene is connected to enhanced immunological function [112]. Under conditions of strong light and salt stress, a mutant C. zofingiensis bkt1 may accumulate large levels of three carotenoids: lutein (13.81 mg/g DW), β -carotene (7.18 mg/g DW), and zeaxanthin (7.00 mg/g DW) [14]. All the results of different stresses are summarized in Table 2.

Health benefits of microalgal-based astaxanthin and $\boldsymbol{\beta}\text{-carotene}$

In the food sector, microalgae are becoming crucial each year, more and more foods incorporating microalgae are being introduced to the market [75]. Because of their potential for commercialization, microalgae have garnered significant attention in studies recently [76]. Microalgae are a potent source of carotenoids. Provitamin A compounds such as α -carotene, β -carotene, astaxanthin, violaxanthin, neoxanthin, and violaxanthin can be produced in significant amounts by Spirulina, Chlorella, Dunaliella, and Haematococcus [77]. There are several bioactive compounds that have been approved as the source of human food and are derived from some microalgae. For instance, to a certain extent, oil high in astaxanthin from H. pluvialis and docosahexaenoic acid (DHA) from Schizochytrium sp. can be ingested [78]. It is anticipated that thousands of bioactive chemicals can be isolated from eukaryotic microalgae and more than 200 from cyanobacteria [79]. Numerous bioactivities have been demonstrated by β -carotene, a pigment that is widely present and involved in the photosynthetic process in microalgae [113]. The most prevalent dietary provitamin A can be transformed into vitamin A (VA, retinol), a micronutrient essential to human health [100].

Astaxanthin

The natural pigment astaxanthin is found in many aquaculture species [114]. With a potency of 110 times more than that of vitamin E and ten times greater than other carotenoids, it is recognized as the most potent naturally occurring antioxidant [115]. Due to astaxanthin's exceptional ability to scavenge free radicals as well as its antiaging, antiinflammatory, antihypertensive, and anticancer qualities (Fig. 4) [116]. It is frequently used in functional foods, medications, and cosmetics [35]. As of right now, astaxanthin produced from microalgae accounts for just 1% of the global astaxanthin market. Furthermore, the cost of producing natural astaxanthin (USD 1800/kg) is significantly higher than that of producing it chemically (USD 1000/kg) [117]. Therefore, increasing microalgae's astaxanthin output is crucial for commercialization. The Food and Drug Administration (FDA) has effectively approved astaxanthin generated from microalgae for direct human consumption [118]. Regarding the current primary natural astaxanthin source, H. pluvialis has been utilized in the biotechnology industry to produce astaxanthin with an annual yield of 300 tons of biomass [119]. With attached cultivation, this species can accumulate up to 5% DW of astaxanthin [120]. H. pluvialis changes from green, motile cells to red cyst cells with a high astaxanthin concentration when growth conditions



Fig. 4 Diagram depicting the powerful health benefits of astaxanthin and β -carotene and their sources

are unfavourable [102]. Numerous studies have taken advantage of tactics to increase astaxanthin accumulation, including nutrient deficiency, intense light, and chemical addition. The synthetic production of astaxanthin involves the use of petrochemical sources, raising concerns about food safety, possible toxicity in the finished product, pollution, and sustainability [121]. Figure 4 illustrates astaxanthin's primary impacts.

As a defence mechanism or in response to physical harm, inflammation is a complicated series of immunological responses that can be prevented or treated with astaxanthin [122]. As it can prevent inflammation from starting, astaxanthin is crucial in preventing illnesses related to the central nervous system [123, 124]. This beneficial carotenoid also reduces bacterial infectionsinduced stomach irritation. It has been claimed that astaxanthin generated from microalgae can decrease the generation or expression of inflammatory mediators and cytokines, as well as the expression of cyclooxygenase-2 and inducible nitric oxide synthase that can causes diseases like, atherosclerosis, or inflammatory bowel disease and brain inflammatory disease [125].

β-Carotene

 β -Carotene is a hydrocarbon carotenoid that is pure and is found in algae, fungi (Phaffia rhodozyma), and plants (carrots, mangos, and pumpkins, among others) [126, 127]. β -Carotene is a precursor to vitamin A and has antioxidant and antiinflammatory properties [128, 129]. It can be converted to vitamin A as needed by the body [130]. Vitamin A is essential for vision, immune function, growth, and development [126]. D. salina has high quantities of β -carotene [131]. Microalgae have a high concentration of carotenoids, which typically make up 0.1–0.2% of their dry mass [132]. Up to 13% of D. salina's biomass is produced as β -carotene. Carotenoids that have been chemically synthesized currently account for 97-98% of the market [133]. The Food and Drug Administration (FDA), the European Community, Japan, the World Health Organization (WHO), and other international organizations and experts have acknowledged β-carotene as a food additive, a nutritional supplement, and a precursor to VA [53]. The significant biological action of organically generated β -carotene makes it useful for treating eye illnesses, cancer prevention, antioxidation,

antiaging, animal colouring, and boosting immunological and reproduction in animals [128, 134]. Epidemiological studies suggest an inverse correlation between β -carotene intake and risk of certain cancers like lung, prostate, and stomach cancer [135]. The antioxidant properties of β-carotene may help protect cells from oxidative damage that can lead to cancer. As an antioxidant that scavenges radicals, β -carotene lowers lipid peroxidation when present [136]. β -Carotene may help reduce the risk of cardiovascular disease. It inhibits the oxidation of LDL cholesterol, preventing plaque buildup in arteries [137]. Some studies found higher β-carotene levels associated with lower risk of coronary artery disease. By stimulating spleen cells, dietary β -carotene is connected to enhanced immunological function [112]. Currently, β -carotene products on the market are predominantly synthetic, while products extracted from natural sources occupy only a small fraction. Synthetic all trans-\beta-carotene dominates due to factors such as lower production costs and scalability [138]. However, natural cis-\beta-carotene isoforms, extracted from sources like microalgae, have been shown to be more bioactive and potent than their synthetic counterparts. This superiority highlights the untapped potential of natural β -carotene and suggests a shift towards large-scale extraction and purification from natural sources in the future.

Therapeutic benefits of astaxanthin and β -carotene

The health advantages have been the subject of several investigations and published research publications. Here is a summary of a few therapeutic advantages based on published study data. As a result, a large number of synthetic carotenoids made by isomerization, condensation, and dehydration of carbonyl compounds are being marketed. To produce chemical carotenoids (Witting processes), two phosphonium salts and one dialdehyde molecule are usually mixed [139]. These compounds are subsequently isomerized to produce carotenoids such as astaxanthin, and β -carotene [28].

Anticancer activity

Carotenoid's anticancer properties may offer protection against a range of human cancers, including intestinal, lung, oral, prostate, breast, and leukemic cancers [140]. However, among other ways that carotenoids fight cancer, they also cause restricted cell proliferation. β -Carotene, fucoxanthin, astaxanthin, lutein, and zeaxanthin have all been linked to a decreased risk of cancer in earlier research [103]. Initial evidence of vitamin A's potential as an anticancer agent first came to light in the 1960s. These discoveries subsequently motivated scientists and researchers to look into β -carotene's potential as an anticancer agent because it was the precursor [141]. According to the study's authors, arrest of the cell cycle, apoptosis, and metastasis are the most frequent processes that carotenoids follow [140]. Astaxanthin helps to reduce tumour size and burden [142].

Antioxidant activity

Reactive oxygen species (ROS), free radicals that are produced as a byproduct of regular metabolic processes [143]. At lower concentrations, though, these free radicals function as a signalling molecule and are essential for preserving cellular homeostasis and function [144]. Among the most varied classes of pigments, carotenoids have strong antioxidant properties and function as a brake on free radicals. Furthermore, it inhibits the production of free radicals by stopping oxidation processes [145, 146].

The most prevalent carotenoids in microalgae are fucoxanthin, violaxanthin, β -carotene, zeaxanthin, and astaxanthin [147]. These compounds have antioxidant qualities. Examples of good sources of β -carotenes are D. salina, Scenedesmus almeriensis (lutein and β-carotene), and Chlorella vulgaris (a strong source of astaxanthin) [148, 149]. Astaxanthin decreases the production of ROS within cells by modifying the enzymes that react to oxidative stress and by inhibiting the Sp1/NR1 signalling pathway, a symptom of oxidative stress [150, 151]. Astaxanthin boosted the amount of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase as well as the synthesis of Nrf2 and Nrf2-targeted proteins [151]. Additionally, it was discovered that when carotenoids were coupled with several other antioxidants, such as vitamin E, their ability to scavenge free radicals was increased.

Antiinflammatory activity

 β -Carotene has been shown in various preclinical in vitro experiments to prevent and decrease diabetes, a chronic low-grade inflammatory illness with common consequences [152]. Numerous studies indicate that consuming a diet high in carotenoid-rich foods may be beneficial in preventing the cardiovascular problems associated with diabetes [153]. According to reports, oxidative stress generated in adipose tissue causes dysregulated adipocyte production of proinflammatory adipokines, which is linked to the aetiology of obesity and diabetes [154]. The impact of carotenoids on stroke has been the subject of numerous investigations. Nevertheless, no additional research or trials have been conducted to demonstrate that carotenoids shield the body from stroke. Many studies have shown that some carotenoids and vitamins can lower homocysteine levels, which has led to the belief that carotenoids may protect blood vessels from stroke.

A larger-scale clinical experiment is currently being conducted to investigate the impact of carotenoids on stroke and brain health [155, 156]. Astaxanthin has been shown in several studies to have anticancer properties. The activation of many tumour metastasis suppressors, including maspin, Kai1, breast cancer metastasis suppressor 1, and mitogen-activated protein kinase kinase 4, has been revealed to be the mechanism by which astaxanthin functions as a new metastasis inhibitor on the human breast cell line T47D [157]. Furthermore, astaxanthin has been shown to have a cytotoxic effect on ovarian cancer cells by inactivating the NF-κB signalling system and promoting apoptosis [158]. By inhibiting PPAR-γ, astaxanthin has also demonstrated antiproliferative effects in leukae-

Antidiabetes activity

mia K562 cells [159].

Carotenoids have been shown to have promise in the treatment and management of diabetes, according to a recent study. It has been demonstrated that carotenoids reduce the risk of type 2 diabetes mellitus in humans [160]. It has also been discovered that there is a negative correlation between glucose levels and carotene intake [161]. Prior research has indicated an antagonistic correlation between β -carotene and fasting blood sugar levels [128]. Consuming foods high in antioxidants may reduce the risk of type 2 diabetes [161].

Advance strategies and genetic modifications employed in different microalgae species to increase astaxanthin and β-carotene accumulation

One effective strategy is the application of abiotic stress conditions, such as salt stress and light stress, which can induce these carotenoids accumulation in microalgae as a protective mechanism against oxidative stress (Table 3). There are several strains which has undergone random mutagenesis, chemical mutagens like N-methyl-N'nitro-N-nitrosoguanidine (MNNG) and ethyl methane sulfonate (EMS) are used to induce random mutations,

Table 3 Genetic modifications in different microalgae species to increase carotenoid accumulation

Microalgae	Genetic modification	Carotenoid produced	Result	References
Chlamydomonas reinhardtii	Overexpression of PSY from con- sensus regions	β-Carotene	31.8 mg/g DCW of β-carotene, 3 times more than wild type	[162]
Chlamydomonas reinhardtii	Overexpression of endogenous orange (OR) protein	β-Carotene	1.3-Fold increase in β -carotene	[163]
Chlamydomonas reinhardtii	Heterologous expression of CrtYB (phytoene-β-carotene synthase)	β-Carotene	Increase (38%) in β -carotene	[164]
Chlamydomonas reinhardtii (strain UVM4)	Synthetic redesign of BKT gene	Astaxanthin	Able to produce astaxanthin	[165]
Haematococcus pluvialis	Overexpression of endogenous PDS in chloroplasts	Astaxanthin	Up to 34.3 mg/L astaxan- thin, higher than wild type (18.0 mg/L)	[26]
Dunaliella salina	Heterologous expression of BKT	Keto-carotenoids (e.g., astax- anthin)	Able to produce keto-carote- noids not naturally present	[166]
Chlorella sp. AE10	Adaptive evolution for salt tolerance	-	Upregulated genes for CO ₂ fixa- tion under salt stress	[167]
Haematococcus pluvialis MT 2877	MNNG mutagenesis	Astaxanthin	4.0-fold increase in astaxanthin	[168]
Chromochloris zofingiensis bkt1	MNNG mutagenesis	β-Carotene	High levels of zeaxanthin and β -carotene	[169]
Chromochloris zofingiensis	Modified norflurazon-resistan- tendogenous <i>pds</i> gene	Astaxanthin	Up to 54.3% higher astaxanthin	[35]
Dunaliella salina	Transformation with <i>bkt</i> and <i>chyb</i> genes from <i>Haemato-</i> <i>coccus pluvialis</i>	Astaxanthin	Astaxanthin biosynthesis, better tolerance to high light	[170]
Haematococcus pluvialis	Overexpression of β-carotene ketolase (BKT)	Astaxanthin	2.0- to 3.0-fold increase in total carotenoids and astaxanthin	[171]
Chlamydomonas reinhardtii	Overexpression of phytoene synthase (PSY)	β-Carotene	3.0-Fold increase in β -carotene	[172]
Haematococcus pluvialis	Overexpression of endogenous PDS in chloroplasts	Astaxanthin	Up to 34.3 mg/L astaxanthin (higher than wild type)	[170]
Chlamydomonas reinhardtii	Heterologous expression of CrtYB (bifunctional phytoene synthase-lycopene cyclase)	β-Carotene	38% increase in β -carotene	[170]

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potentially leading to strains with enhanced carotenoid accumulation. In Coelastrum sp. C1-G1, carotenoid content increased about 2.0-fold over the mother strain using EMS mutagenesis [174]. The MNNG mutant strain H. pluvialis MT 2877, known as the astaxanthinoverproduction strain, produced four times as much astaxanthin as the WT strain [168]. Under various culture conditions, C. zofingiensis bkt1, a chemical mutagen via MNNG, can accumulate high concentrations of three important carotenoids, e.g., zeaxanthin, lutein, and β -carotene [169]. A method called adaptive evolution involves subjecting microalgae strains to gradual selective pressures, such as salt stress or specific compound exposure, to obtain strains with desired phenotypes. Chlorella sp. AE10 was subjected to adaptive evolution for 138 days (46 cycles) to improve its tolerance to 30 g/L salt, resulting in the strain *Chlorella* sp. S30 with upregulated genes related to CO_2 fixation under salt stress [167]. Another method that involves the introduction or overexpression of salt-tolerance genes like nhaP (Na⁺/H⁺ antiporter), codA (choline oxidase), and Dps (DNA-binding proteins) in various microalgae species called targeted genetic engineering [175]. Genetic engineering of *C. zofingiensis* with a modified norflurazon-resistant endogenous pds gene resulted in up to 54.3% higher astaxanthin production [35]. Transformation and expression of *dxs* and *psy* genes in Phaeodactylum tricornutum, increased fucoxanthin content by 2.4-fold and 1.8-fold, respectively [172]. Transgenic D. salina with bkt and chyb genes from H. pluvialis, enabling astaxanthin biosynthesis and better tolerance to high light [170]. Manipulating the key enzymes and genes involved in the carotenoid biosynthesis pathway can also help in enhancing carotenoid production like overexpression of endogenous phytoene desaturase (PDS) in the chloroplasts of *H. pluvialis* led to astaxanthin yields of up to 34.3 mg/L, surpassing the wild-type production of 18.0 mg/L [62]. In Chlamydomonas reinhardtii, deletion of the zeaxanthin epoxidase (ZEP) gene using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 resulted in a 56-fold increase in zeaxanthin concentration [11], while the double deletion of ZEP and CpFTSY enhanced growth and zeaxanthin content [176].

CRISPR/Cas9-mediated genome modification is one of the most intriguing new gene editing methods because of its ease of use and adaptability [173]. It has been demonstrated to work well in a range of microalgae [177]. Although, modifications were required to lessen the harmful impact of Cas9 on the algae strain, the first successful application of CRISPR/Cas9 in microalgae was shown in 2014 on *C. reinhardtii* [176, 178]. Since then, *Chlamydomonas* transformation using the CRISPR system has been carried out several times, for example, to increase the content of lipids and pigments [179], increase the productivity of triacylglycerol [180-182], increase lipid accumulation [183]. Various other research also successfully used CRISPR/Cas9 to increase the production of several carotenoids in D. salina [184] and C. reinhardtii [176]. Overexpression of phytoene synthase (PSY) from consensus regions of multiple species increased β -carotene production by 3-fold (31.8 mg/g DCW) [19]. Additionally, overexpression of endogenous orange protein (OR) and its mutant form (CrORHis) boosted β-carotene, and total carotenoid levels in C. reinhardtii [163, 185]. Heterologous expression of genes has also been explored. The expression of the bifunctional *CrtYB* (phytoene- β -carotene synthase) gene from *Xan*thophyllomyces dendrorhous in Chlamydomonas rein*hardtii* increased β -carotene by 38%, respectively [164]. Furthermore, the introduction of the *bkt1* gene from *H*. pluvialis into C. reinhardtii enabled the production of keto-carotenoids, which are not naturally synthesized by this microalga [165, 186]. To capitalize on this potential of astaxanthin and β-carotene, advancements in extraction technologies are crucial. One promising innovation is supercritical carbon dioxide (CO₂) extraction, a method that has gained traction for isolating carotenoids and other bioactive compounds. This technique leverages supercritical CO2's unique properties, such as low viscosity and high diffusivity, to achieve efficient, selective, and environmentally friendly extraction [138]. Supercritical CO₂ extraction is already widely applied and holds promise for improving the yield and purity of natural cis- β -carotene, paving the way for its broader adoption in industries like food, pharmaceuticals, and cosmetics.

Advances in processing technologies and sustainability

Recent innovations in extraction, encapsulation, and stabilization methods have enhanced the functionality and industrial applicability of microalgal-derived products, particularly carotenoids. Also, new extraction and encapsulation methodologies, optimized for large-scale production, have paved the way for microalgal-based bioactives to transition from niche markets to mainstream applications. The solvent extraction method is a widely utilized technique for isolating natural compounds, such as pigments, from microalgal sources. However, this approach is associated with several limitations, including high solvent and energy demands, potential toxicity of certain solvents, and suboptimal extraction efficiencies [187, 188]. To address these challenges, a range of alternative methods, collectively referred to as green extraction techniques, have been developed. These advanced methodologies include ultrasound-assisted extraction, microwave-assisted extraction, pulsed electric field-assisted

extraction, pressurized liquid extraction, aqueous twophase systems, and supercritical fluid extraction, all of which aim to enhance efficiency, reduce environmental impact, and ensure safety [189]. Encapsulation technologies enhance the dispersibility, stability, and bioavailability of carotenoids while reducing degradation caused by heat, light, and oxygen. Carotenoids derived from microalgae, such as astaxanthin and fucoxanthin, demonstrate significant antioxidant properties and are more potent compared to other pigments [190]. Advanced green extraction techniques, such as microwave-assisted extraction (MAE) and high-voltage electric discharge (HVED), improve the yield, purity, and sustainability of carotenoid extraction [191]. In a study by Georgiopoulou et al. [192], Chlorella vulgaris was subjected to MAE for recovery of high-added value bioactive compounds. Results showed, MAE yielded more quality yield than conventional solid-liquid extraction (SLE). Encapsulation reduces chemical degradation and preserves sensory attributes like colour and aroma, enhancing product shelf life and consumer acceptability. Encapsulation supports the incorporation of carotenoids into diverse food matrices without compromising their bioactivity [193]. Technologies like spray drying and nano-emulsion formation facilitate the design of stable, bioavailable food-grade carotenoid delivery systems [191]. Despite technological advances, commercial applications of encapsulated carotenoids are limited by regulatory ambiguities and high production costs, more research is needed to standardize encapsulation materials and methods for broader industrial adoption. Microalgae-based products, while promising for their economic potential and sustainability applications, face significant environmental challenges due to energy-intensive cultivation, dewatering, and drying processes. Autotrophic systems have higher energy and water demands compared to heterotrophic systems, with substantial impacts on global warming potential, ecotoxicity, and resource use [194]. Pigment and polyunsaturated fatty acid production are particularly energy-intensive [195-197]. Strategies like pressurized filtration with lyophilization and transitioning to renewable energy sources can reduce environmental footprints by up to 95% [198]. Integrating microalgae cultivation into biogas plants using the stacked modular open raceway pond (SMORP) system improves sustainability. While the traditional biogas setup showed better environmental performance, the microalgae-integrated system demonstrated economic feasibility with a net present value of €2.57 million and an internal rate of return of 14.1%, leveraging waste heat, CO_2 , and digestate from biogas plants. Challenges include high energy use, material costs, and optimizing nutrient and CO₂ efficiency [199]. Despite these hurdles, the system shows promise for reducing chemical fertilizer use, CO_2 emissions, and producing high-value bioproducts, aligning with circular economy goals and sustainable waste management strategies. Achieving sustainability and economic viability requires process optimization, renewable energy integration, and technological advancements, supported by policy initiatives and market demand for eco-friendly products.

Conclusion and future perspective

Microalgae are a promising natural source of high-value carotenoids like astaxanthin and β -carotene that offer numerous health benefits. Environmental factors, particularly high light intensity, and salinity, can induce the accumulation of these carotenoids in microalgae as a protective response against oxidative stress. Elucidating the carotenoid biosynthesis pathways and the molecular mechanisms underlying stress-induced carotenogenesis has provided valuable insights for metabolic engineering efforts. Strategic application of abiotic stresses like high light intensity and salinity can substantially elevate carotenoid accumulation in microalgae, though the optimal conditions are species-specific. H. pluvialis and D. salina are the current industrial workhorses, other oleaginous green microalgae like C. zofingiensis and Tetraselmis sp. are also promising alternate sources. Further improvements in carotenoid productivity are anticipated through advanced biotechnological interventions such as genetic engineering to overexpress rate-limiting enzymes, promoter modifications and the CRISPR-Cas9 system. CRISPR-Cas9 and metabolic engineering are powerful tools for optimizing β -carotene and astaxanthin production by precisely modifying metabolic pathways. CRISPR-Cas9 enables targeted gene knockouts, activation, or repression to enhance flux through carotenoid biosynthesis while reducing competing pathways. Metabolic engineering complements this by overexpressing key enzymes (e.g., PSY, PDS, LCYB, crtW, crtZ), increasing precursor availability (e.g., GGPP), and improving host tolerance to carotenoid accumulation. Synthetic biology further allows pathway redesign and dynamic regulation in microbial hosts, ensuring balanced flux and high yields. These strategies make carotenoid production more efficient and economically viable for industrial applications. Adaptive laboratory evolution and mutagenesis approaches could also generate strains with superior carotenogenic capabilities. Integrating upstream and downstream bioprocesses through biorefinery concepts will be crucial for commercial viability. As microalgal carotenoids transition from high-value niche products to commodity nutraceuticals and food ingredients, resolving challenges around scalability, cost-effectiveness, and regulatory compliance will facilitate their

widespread adoption across industries like food, feed, pharmaceutics, and cosmetics. Scaling microalgal production of β -carotene and astaxanthin to industrial levels faces challenges like low yields, cultivation inefficiencies, energy-intensive harvesting, and high costs. Solutions include genetic and metabolic engineering to enhance productivity, hybrid cultivation systems combining open ponds and photobioreactors, and low-energy harvesting methods like flocculation or supercritical CO₂ extraction. Standardizing processes and leveraging co-products can improve economic viability, while integrating wastewater recycling, renewable energy, and carbon capture reduces environmental impact. These innovations, combined with compliance with regulatory standards, are essential for sustainable and cost-effective industrial-scale production. Integrating microalgal cultivation with wastewater treatment or carbon capture and utilization processes can contribute to the sustainability and economic feasibility of the process. Overall, this multidisciplinary research arena holds immense potential for rewarding fundamental insights and transformative biotechnological applications. These advancements collectively provide a pathway for achieving sustainable, high-yield production of carotenoids at an industrial scale.

Abbreviation

Abbrevia	itions
ROS	Reactive oxygen species
IPP	Isopentyl pyrophosphate
MEP	Methylerythritol phosphate
DMAPP	Dimethylallyl diphosphate
GPPS	Geranyl diphosphate synthase
GGPPS	Geranylgeranyl pyrophosphate synthase
GGPP	Geranylgeranyl pyrophosphate
PSY	Phytoene synthase
PDS	Phytoene desaturase
ISO	Isomerase
ZDS	ζ-Carotene desaturase
LCYE	Lycopene ε-cyclase
LCYB	Lycopene β-cyclase
CHYB	β-Carotene hydroxylase
BKT	β-Ketolase
DCW/L	Dry cooling coil water/Leave
CaM	Calmodulin
C/N	Carbon/nitrogen
GABA	Gamma amino butyric acid
NO	Nitric oxide
rbcL	Ribulose bisphosphate carboxylase
rbcS	Ribulose bisphosphate carboxylase small subunit
ACCase	Acetyl-coenzyme A carboxylase
MYB	Myeloblastosis viral oncogene homolog
bHLH	Basic helix loop helix
MT	Melatonin
DW	Dry weight
LA	Linoleic acid
FDA	Food and Drug Administration
VA	Vitamin A
LDL	Low density lipoproteins
Nrf2	Nuclear factor erythroid-2-related factor
MNNG	N-Methyl-N'-nitrosoguanidine
CRISPR	Clustered regularly interspaced short palindromic repeats
HVED	High-voltage electrical discharges
MAE	Microwave-assisted extraction
SLE	Solid–liquid extraction

SMORP Stacked modular open raceway pond

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Author contributions

A: conceptualization, writing—original draft, writing draft—review and editing. R.B.: conceptualization, writing—original draft, writing draft—review and editing. A.Y.: conceptualization, writing—original draft, writing draft—review and editing. P.S.: investigation, conceptualization, resources, data curation, writing—original draft, writing draft—review and editing, supervision, project administration. M.M.: investigation, conceptualization, resources, data curation, writing—original draft, writing draft—review and editing, project administration. All the authors reviewed and edited the contents. All authors have read and agreed to the published version of the manuscript.

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The authors declare no competing interests.

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