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Bioaccessibility, Bioavailability and Bioactivities of Carotenoids in Microalgae: A Review

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ABSTRACT

In recent years, carotenoids, as photosynthetic pigments, have drawn increasing interest due to their health benefits. This is because carotenoids have possessed enormous potential in lowering the risk of cardiovascular, cataracts, macular degradation and malignant tumours. Although many carotenoids can be produced via chemical synthesis, the commonly used petroleum-derived extraction method can be problematic for the environment and potentially contaminate the isolated carotenoids. Therefore, in contrast to chemosynthesis, microalgae can be considered an excellent alternative natural source to synthetic ones. Varying in microalgal strains, a variety of different carotenoids can be produced, for instance, the β-carotene from *Dunaliella salina*, the fucoxanthin from *Undaria pinnatifida* and the astaxanthin from *Haematococcus pluvialis*. Besides, the pharmacological action of carotenoids is highly associated with their bioaccessibility and bioavailability, which is one of the research directions and affecting factors of the carotenoid application in the food, pharmaceutical, nutraceutical and cosmeceutical industries. Despite there being numerous publications about carotenoids, a comprehensive review on the microalgal carotenoids and their triggered bioactivities is still lacking. Considering the significance of bioavailability, this review presents extensive information on microalgal carotenoids, including their classifications, bioaccessibilities, absorption process mechanisms, bioactivities and relevant health benefits.

KEYWORDS

Microalgae; carotenoids; bioaccessibility; bioavailability; health benefits; application

Introduction

Carotenoids, also well-known as tetra-terpenoids, are commonly synthesised naturally or chemically for pigment purpose. Statistically, over 1,100 types of carotenoids can be sourced from the plants, algae, fungi and photosynthetic bacteria in nature.[1] Common carotenoids absorb in the violet and blue-green regions of the solar spectrum and can be used as colourants in food, beverage and feed industries.[2] Many systematic reviews and meta-analyses published recently have indicated health benefits of carotenoids.[3] It stands to reason since carotenoids have been affirmed to have outstanding antioxidant activities by possessing strong free radical scavenging and retina damage-repairing abilities.[4,5] The specific driven mechanisms have been reported previously and can be summarised as either A) working as a direct scavenging agent, or indirect affecting factors via transcription like the nuclear factor erythroid 2–related factor 2 (NRF-2) and nuclear factor kappa B (NF-κB); or B)

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activating the nuclear hormone receptor pathways including retinoic acid receptor (RAR), retinoid × receptor (RXR) or peroxisome proliferator-activated receptors (PPARs) via carotenoid metabolites.[6] Contributed by the extending applications among industries, the carotenoids market has been expanded, from around $1.24 billion in 2016 to over $1.5 billion in 2021.[7] However, 80–90% of the market is still dominated by the chemically synthesised carotenoids, with only 10–20% sourced from the nature.[8]

Indeed, the demand for natural carotenoids is driven by consumers' increasing food safety awareness. In contrast to terrestrial plants, microalgae have many favourable conditions for carotenoid production due to their shorter growing period, more efficient oxygen photosynthesis, and lower nutrient requirements.[9] Due to these advantages and developments, microalgae have already become commercial carotenoid sources. Specifically, β-carotene, astaxanthin, fucoxanthin, and lutein are commonly acquired from *Dunaliella salina*, *Haematococcus pluvialis*, *Undaria atifida* and *Chlorella* sp., respectively.[10–13] While from the biological perspective, carotenoids play essential and necessary roles within and beyond the photosystems.On the one hand, carotenoids existing in plant plastids and chloroplasts make constructive contributions to maintaining the cellular structure and protecting the functional integrity of the photosynthetic process.[14] On the other hand, they act as secondary electron donors in the photosystem, which make the formation of cellular reducing power [15] and the generation of non-photochemical quenching induced by the reactive oxygen species become possible.[16] In terms of the roles played beyond the photosystems, the benefits of carotenoids to human health have nowadays received more researchers' attention. Particularly, carotenoids have been identified for their ability of reducing the risk of cataracts, malignancies, coronary heart disease and age-related macular degeneration.[17] This is because they can inactivate the free radicals presenting in the human body through the two specific driven mechanisms summarised previously, thereby mitigating the dangers from oxidative stress, atherosclerosis and severe cancer-like diseases.[6,14] At the same time, the by-products from the oxidative cleavage of carotenoids, apocarotenoids and diapocarotenoids can also be used as additives, vitamin supplements and cosmetics.[18] Besides, some specific carotenoids, such as β-carotene,[19] can positively boost the immune systems by enhancing the cell function.

Even though there are many existing reviews on the extraction and purification methods of carotenoids or the cultivation of carotenoids-rich microalgal strains, the bioaccessibility and bioavailability of microalgal carotenoids still need to be further evaluated. Therefore, to better understand the potential health benefits of carotenoids and farther assist their application in food and pharmacological industries, this comprehensive review has systematically classified the carotenoids presenting in microalgae and elaborated the extensive knowledge of their bioavailabilities and absorption process mechanisms in the human body.

Literature research methodology

This review summarises and critiques over 150 published articles on microalgal carotenoids over the past two decades (2000 – present) in databases (including PubMed, Google Scholar, ScienceDirect, Scopus and Web of Science). The keywords used for the data search were microalgae carotenoids, carotenoids classification, carotenoids content, microalgae carotenoids absorption, microalgae carotenoids bioaccessibility, microalgae carotenoids bioavailability, microalgae carotenoids health benefits, microalgae carotenoids application. During the search process, various Boolean operators (OR, AND, NOT) were used to combine the search keywords to find relevant studies in the academic database. Candidate articles were excluded if the experimental design cannot meet the requirements. Preferences were set to screen the papers published within the last ten years.

Table 1. Contents of different types of carotenoids from microalgae.

Microalgae	Carotenoids	Contenat	References
C. zofingiensisis	Astaxanthin	4.89–11.70 mg/g	[25–27]
H. pluvialis		~5.01 mg/g	[28]
Desmodesmus subspicatus		0.37 mg/g	[29]
Scenedesmus sp.	β-carotene	31.80 mg/g	[30]
Eustigmatos cf. *polyphem*		60.76 mg/g	[31]
Parachlorella sp.	Lutein	11.8 mg/g	[32]
Chlorella sorokiniana		5.86–9.57 mg/g	
Desmodesmus sp.		5.56 mg/g	
Scenedesmus obliquus		4.52 mg/g	
Chlamydomonas planctogloea		7.40 mg/g	[33]
C. planctogloea	canthaxanthin	1.49 mg/g	[34]
Eutetramorus fottii		0.38 mg/g	
Nannochloropsis sp.	Violaxanthin	0.62 mg/g	[29]
Chlorella sp.		0.82 mg/g	
P. tricornutum	Fucoxanthin	26.1 mg/g	[34]
Tisochrysis lutea		9.81 mg/L/d	[35]
Odontella aurita		7.96 mg/L/d	[36]

Classification of microalgal carotenoids

Carotenoids primarily consist of tetra-terpenoids (C_{40} structure) containing the same isoprene structural unit but different lengths of carbon chain.[20] They are commonly classified into two subdivisions, which are:

(i) Carotenes (α-, β-carotene, lycopene),
(ii) Xanthophylls (lutein, zeaxanthin, violaxanthin and others).

Carotenes are strict hydrocarbon carotenoids without oxygen atoms, whereas xanthophylls are oxygen-containing derivatives. In terms of specific structure, they can be further divided into four categories:

(i) Acyclic or linear carotenoids (phytoene, lycopene).
(ii) Cyclic carotenoids (α-, β-carotene) contain one or two cyclic structures.
(iii) Hydroxycarotenoids/carotenoids (lutein, zeaxanthin) that have at least one hydroxyl group (xanthophylls).
(iv) Epoxycarotenoids (violaxanthin) contain at least an epoxy group (xanthophylls).

Besides, some other carotenoids have been reported recently. It was found apocarotenoid derived by oxidative cleavage of tetra-terpenoids with being catalysed by carotenoid oxygenase contained abscisic retinoic acid, retinol and plant hormone acid but less than 40 carbon atoms in its polymeric structure.[21] Meanwhile, a few C_{50} or C_{30} carotenoids had also been identified and isolated from bacteria,[22] such as bacterioruberin (C_{50} carotenoid) from Halobacteria and 5-hydroxy-5,6-dihydro-4,4'-diapolycopene (C_{30} carotenoid) from *Planococcus* sp.[23,24] In summary, Table 1 has demonstrated the principal subdivision of carotenoids found in the microalgal species.[25–36]

β-Carotene

β-Carotene is the most prominent group of the carotenoid family. Based on the traditional carotenoid core 40-carbon structure, β-carotene contains two β-ionone ring substitutions.[37] The peak absorbance of β-carotene is at ~440 nm, which is the reason for its orange-to-red colour.[38] Regarding several isomers of β-carotene, all-*trans*-β-carotene is the dominant form and shows relatively higher bioavailability in contrast with the other isomers.[39] In fact, the high bioavailability of all-*trans*-β-

carotene can be mainly explained by its symmetrical structure. With the help of this structural feature, it can be metabolised to all-*trans* retinoic acid, which can undergo preferential uptake, transport and tissue accumulation. Besides, β-carotenes have been found in almost all human tissues, even the blood,[40] and evidence suggests they can protect the human body from the heart disease and cancer.[41] Moreover, β-carotene has been considered an efficient quencher of reactive oxygen species (ROS), including singlet oxygen and the excited triplet.[42] A clinical trial conducted in the 1980s, with the results of 133 patients suffering from erythropoietic protoporphyria improved, attested to the potential radical defence function of β-carotene.[43] In a more recent study, the application of β-carotene showed an active ability to reduce the susceptibility of low-density lipoprotein (LDL) cholesterol by inhibiting lipoprotein oxidation.[2] Even though there is widespread application of β-carotene, only 2–3% of the β-carotene is produced from bio-resources (such as fruits and vegetables, algae, fungi, and bacteria), while the rest mainly remains in the synthetic form. However, the developed extraction techniques of β-carotene from microalgae have enabled the partial replacement of synthetic β-carotene.[44] Over 95% (nearly 1,200 tons p.a.) of the total need for β-carotene can be supplied by *D. salina*.[45] *Dunaliella salina* is a halophilic green single-cellular microalga commonly found in hypersaline environments. The high β-carotene content in *D. salina* biomass contributes to its excellent antioxidant activities.[46] This bioactive application has been commercialised. In detail, *D. salina* completes cell growth in industrial production, followed by β-carotene production and accumulation under stress environments.[47] Then the orange cells (rich in β-carotene) are disrupted by the organic solvents, thereby β-carotene can be extracted. In the end, the extracted pigment can then be purified and directly used as a concentrated product or for other purposes via drying and encapsulation to produce capsules or tablets.[48]

Astaxanthin

Astaxanthin is one type of ketocarotenoid that contains ketone (carbonyl) groups. The main skeleton is 3,3′-dihydroxy-β,β-carotene-4,4′-dione, with three existing configurational isomers (3 R,3′R), (3 R,3′ S) and (3S,3′S). Compared to the primary carotenoids (photosynthetic apparatus compounds), astaxanthin is the secondary carotenoid accumulating in cytosolic lipid bodies.[49] It is commonly synthesised by microalgae, embryo plants, bacteria or fungi. In addition to its colourant property, astaxanthin has also possessed an extensive array of biochemical activities, such as antioxidant capacity, radiation scavenging ability, and immunomodulation effect.[50] Its hydroxyl and keto groups can neutralise the reactive oxygen species thereby not only limiting the peroxyl and hydroxyl radicals but also inhibiting the autoxidation chain process by quenching damaging singlet oxygen. At the same time, astaxanthin can also function as a chelator by converting metal prooxidants into non-toxic compounds.[51]

Many microalgae species have been considered ideal raw materials for astaxanthin production. For instance, *H. pluvialis* is a hyper-accumulator that was reported to have up to 7% astaxanthin content by its dry weight, with 3S,3'S stereoisomer being the predominant isomer.[52] This ideal microalgae strain can even accumulate carotenoids under harsh living environments, such as nutrient depletion, photon steradians, hypersaline, and temperature abnormalities.[53] Stimulated by these extreme environmental conditions, the microalgae's green motile flagellated and non-motile palmella will convert into resting cells (haematocysts or aplanospores). Subsequently, a massive amount of astaxanthin will be accumulated and gathered in cytosolic lipid bodies, giving the haematocysts a characteristic bright red colour.[54]

Lutein and zeaxanthin

Lutein and zeaxanthin are often evaluated together due to their benefits to the eye retina and lens.[55] They are classified as macular pigments which protect the retinal membrane from blue light and improve visual acuity.[56,57] Different from the other carotenoids (β-carotene or

lycopene), the contained hydroxyl functional groups allow the lutein, zeaxanthin and their isomers to cross the blood-ocular barrier (BOB) and the blood-brain barrier (BBB). Specifically, BOB refers to the boundary created by the endothelium of capillaries of the retina and iris, ciliary epithelium and retinal pigment epithelium,[58] while BBB describes the border formed by endothelial cells of the capillary wall, astrocyte end-feet ensheathing the capillary, and pericytes embedded in the capillary basement membrane).[59] Therefore, the two carotenoids are commonly used to treat age-related macular degeneration, cataracts, retinal nerve disease, Alzheimer's disease and diabetic retinopathy. Specifically, based on the locations of C=C in the rings, lutein and zeaxanthin distribute in different body parts. The central area of the retina is dominated by zeaxanthin, while lutein exists in the peripheral region.[60] Besides, another isomer, meso-zeaxanthin, is seldom present in the diet and is believed to be generated at the macula through metabolic transformations of ingested carotenoids.[61] In the centre of the retina (macula), meso-zeaxanthin can assist in filtering out the retina-damaging blue light and protect against the oxidative stress.[62]

Various classes of microalgae have been identified as the new potential sources of lutein and zeaxanthin production, such as *Cyanobacteria*, *Rhodophytes*, *Chlorophytes*, and types of *Heterokonts*.[63] In a research study conducted earlier, *Porphyridium cruentum* was the dominant species in zeaxanthin production among seventy red marine algae species, which was reported up to 97.4% production yield.[64] *Chlorella pyrenoidosa* contains an identical quantity of free lutein; meanwhile, it also shows inherent advantages in the extraction and separation process.[65] From the economic perspective, microalgal species with high lutein content (0.5–1.2% of dry weight) have cultivation cost than traditional sources like the marigold petals, which have been regarded as high-benefit commercial bioresources.[66]

Canthaxanthin

As a keto-carotenoid, canthaxanthin has been widely applied in the nutraceutical and pharmaceutical industries.[67] Besides, due to light absorption in the red wavelength range, it has been commonly utilised as a colouring agent in the aquaculture and poultry industries.[68] For example, canthaxanthin is often used as a yolk colour-enhancer in hen diets [69] and a colour-improver for shrimp, crabs and prawns.[70] Regarding its potential medical applications, canthaxanthin possesses powerful antioxidative properties and has been found to help reduce the risk of substantial human diseases, including ulcers and coronary artery disease.[68]

On the market, most available canthaxanthin is synthesised chemically. A novel chemical synthesis pathway proposed recently began with the epoxidation of a-ionone with metachloroperbenzoic acid. After conversion, Darzens condensation and twice Wittig – Horner condensation, epoxide 3-hydroxyl-β-ionone converts to 4,4′-dihydroxyl-β-carotene, followed by oxidation to afford the target product canthaxanthin.[71] Although the canthaxanthin extraction efficiency by using natural sources cannot meet the requirement of commercialisation, some green microalgae have been verified to produce canthaxanthin, such as *Chlorococcum* sp. and *Coelastrella striolata*.[72–74] But limited information about the canthaxanthin content in the other microalgal strains has been reported so far. Thus, future studies can focus on exploring the new microalgal strains ideal for canthaxanthin extraction.

Fucoxanthin

Fucoxanthin is a pigment with brown or olive-green colour and mainly absorbs the light distributed in the visible blue-green to the yellow-green region with absorbance peaks at ~510–525 nm. It accounts for more than 10% of the total carotenoids produced in nature.[75] Fucoxanthin shows strong antioxidant activity due to its polyene chain. While the containing epoxide, hydroxyl groups, allenic link and conjugated carbonyl group synergistically contribute to the high bioactivities of the fucoxanthin.

Nevertheless, the chemical synthesis of fucoxanthin involves a complex process that is hard to meet the requirements of large-scale industry production. Therefore, nowadays, fucoxanthin is mainly acquired from macroalgae such as *Laminaria japonica, Eisenia bicyclis, Undaria pinnatifida* and *Hijikia fusiformis*.[76] However, considering the relatively lower fucoxanthin content in macroalgae, today's scientists are proposing that microalga is a more promising acquisition source, based on its higher cellular content of fucoxanthin.[77] They analysed several microalgae strains for fucoxanthin, finding 26.6 mg g^{-1} DW in *Mallomonas* spp. (Synurophyceae), 21.67 mg g^{-1} DW *in Phaeodactylum tricornutum* and *Odontella aurita* (Bacillariophyceae or diatoms), and 18.23 mg g^{-1} DW in *Isochrysis galbana* (Prymnesiophyceae).[78] Among these four microalgae strains, the *Isochrysis* aff. *galbana* was believed as a feasible acquisition source of fucoxanthin for industrial-scale production.

Peridinin

Peridinin ($C_{39}H_{50}O_7$) is an apocarotenoid derived from carotenoids by oxidative cleavage, catalysed by the carotenoid oxygenase.[79] It is essential for photosynthesis in many organisms, like the photosynthetic dinoflagellates.[80] The absorbance of peridinin is between 470 nm to 550 nm (blue-green light) which is beyond the chlorophyll molecules' visible range. Peridinin is typically a part of the peridinin-chlorophyll-protein (PerCP) complex, consisting of a boat-shaped protein molecule with a large central cavity that includes peridinin, chlorophyll and lipid molecules in a 4:1 peridinin-to-chlorophyll ratio.[81] The PerCP preserves cellular photosynthetic machinery by scavenging free radicals and quenching singlet oxygen. In medical research, PerCP coupled with antibodies, proteins, and peptides is utilised for fluorescent immunolabelling for fluorescent-activated cell sorting. Peridinin from the dinoflagellates has been demonstrated to induce apoptosis in cancer cells [82] and inhibit eosinophil cell-mediated allergic response in mice.[83] Besides, many other bioactivities of PerCP have been identified, such as antioxidant, anti-inflammatory, and anti-cancer.[84] A recent study reported the accumulation machinery of PerCP in some microalgae species under specific circumstances.[85] This significant finding can be further exploited to expand the industrial production scale of PerCP.

Violaxanthin

In contrast, violaxanthin belongs to the xanthophyll group and exists in orange-colour fruits, green vegetables and microalgae. Violaxanthin is generally biosynthesised from zeaxanthin through epoxidation, converting the carbon-carbon double bond into 5,6-epoxy groups.[86] Previous reports have shown that violaxanthin presented strong radical scavenging activity, contributing to its powerful lipid peroxidation inhabitation and red blood cell homolysis abilities.[87] Additionally, violaxanthin can be regarded as a promising potential anti-inflammatory agent for clinical use or functional adjuvant purposes.[88] However, the food application of violaxanthin has been banned in many Occident countries.[89] One of the reasons is that resources cannot satisfy the efficient commercial supply of violaxanthin, which needs to be produced through heterologous microorganism pathways.[90]

With the development of microalgal biotechnology, *Chlorella ellipsoidea* was introduced to violaxanthin industry production, the content accounting for 87% of the total carotenoids.[91] Pasquet et al.[92] also isolated violaxanthin from *Dunaliella tertiolecta*, while Wang et al.[86] first illustrated the amount of violaxanthin that the microalgae could produce. Recently, a new strain of *Nannochloropsis* has been cultivated as a violaxanthin-enriched source, with 44% higher content than the previously used strains.[93]

Absorption and movement of carotenoids

The absorption of dietary carotenoids can be briefly defined as carotenoid movement in the gastrointestinal tract and the process of their metabolites entering lymphatic or portal circulation.[94] The several following processes have been affirmed necessary for carotenoids absorption, which include: A) the sufficient release from the food matrix through oral mastication; B) the mineralisation with lipids in the small intestine; C) the movement through intestinal epithelium cells via the transport proteins and enzymes; and D) the distribution to lymphatic or portal circulation. More detailed information has been evaluated in the following subsections.

Absorption process

Generally, it is believed that the carotenoid absorption mechanism follows the principle of other dietary fat-soluble compounds.[95] Once they are released from the food matrix, the fraction dissolves in the fat phase, followed by emulsifying into lipid droplets in the stomach and duodenum.[96] The pancreatic lipase existing in the small intestinal facilitates carotenoids combining with bile salt micelles, which is created by the interactions between triglyceride (TAG) enzymatic cleavage products (free fatty acids and monoglycerides), phospholipids and cholesterol esters.[97] Formed mono- or di-acylglycerides, lysophospholipids, and free cholesterol further diffuse to the surface of the enterocyte through the mucin layer [98] and bind with the transport proteins to enter the cell interiors, such as the retinol-binding protein receptor 2 (RBPR2) for dietary retinol acceleration [99] and Astar protein engaged in nonvesicular cholesterol transportation.[100] In addition to the mentioned transport proteins, scavenger receptor class B type I (SR-BI), Niemann-Pick C1 Like1 (NPC1L1) and cluster determinant 36 (CD36) have also get involved in the delivery of provitamin A carotenoids.[101] Moreover, the absorption competition has been observed between carotenoids, other fat-soluble micronutrients and lipid-digested products due to the selectivity of the transport proteins. However, no evidence could suggest the mechanism of transport proteins, whether it goes through the extracellular environment to the cell interior, serves as a membrane structure, or forms a protein-conducting channel that allows carotenoids to be internalised.[102] Before carotenoids are taken by liver parenchymal cells and released into the blood circulation, they are incorporated with chylomicron and high-density lipoprotein (HDL) to form chylomicron remnants and carotenoid-HDL.[103] In the liver cells, carotenoids are partly absorbed and stored for exerting bioactivities, or further secreted into other specific human tissues, or excreted from the human body. The very low-density lipoproteins (VLDL) contribute to the delivery of carotenoids by loading to the peripheral tissues and adipose tissues, then transform themselves into the LDL particles. At the same time, HDL is also associated with the circulation of xanthophylls and carotenes.[95] The mentioned absorption process has been detailed in Fig. 1.

Enzyme regulation

The enzyme family (especially carotenoid-oxygenases) have been discovered as the key players in carotenoid cleavage.[105] After entering the enterocyte, various intercellular enzymes begin to remodel carotenoids. In particular, β-carotene-15,15′-oxygenase (BCO 1) and β-carotene-9′,10′-oxygenase (BCO 2) have been reported as the main oxygenases cleave carotenoids existing in human or animal bodies.[106] In terms of BOC 1, it shows a high capacity for symmetric cleaving, which participates in the central cleavage of provitamin A carotenoids. For example, it cleaves β-carotene at the central double bond to yield two retinal molecules. Then those products can be transformed into all-*trans*-retinol by the esterification effects of lecithin retinol acyltransferase (LRAT) and acyl-CoA-dependent transferases.[107] All-*trans*-retinol is encapsulated in chylomicrons and secreted to lymph before storing in stellate cells, the primary storage site of vitamin A and carotenoids. In contrast to BOC 1, BOC 2, the second enzyme involved in carotenoid cleavage, presents in the mitochondria and is regulated by entirely different active sites. This enzyme is expressed in most human tissues (cardiac

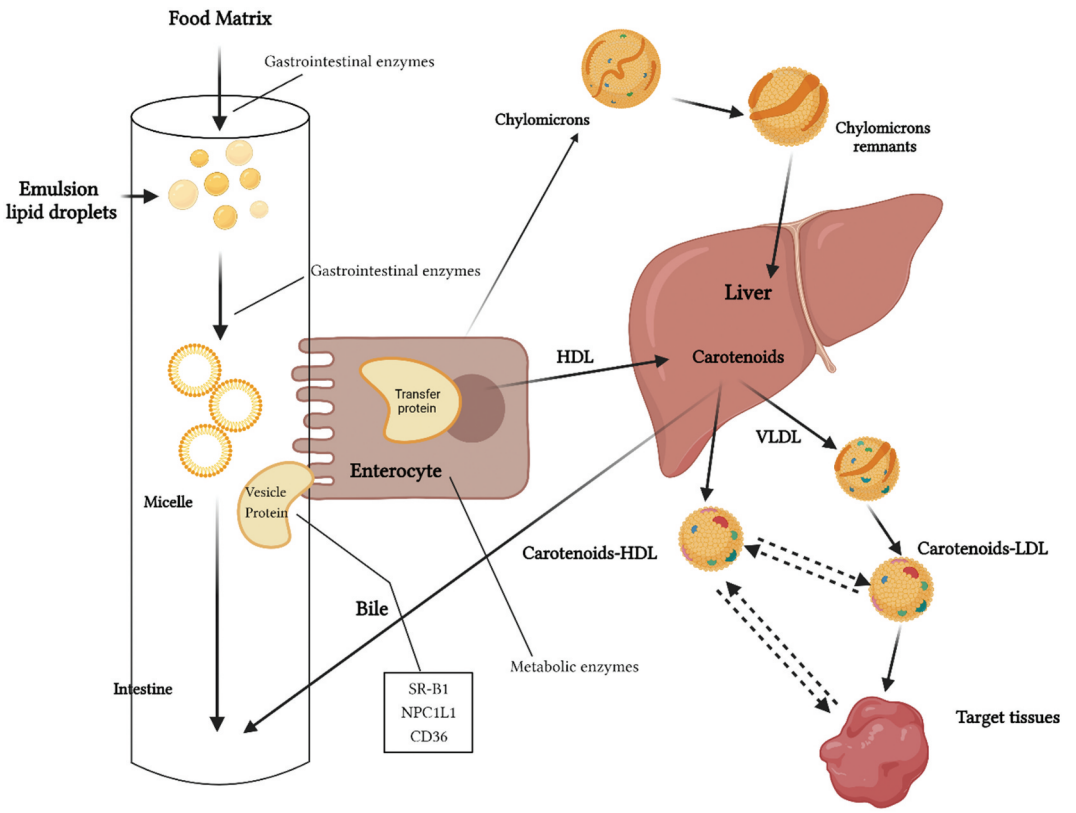

Figure 1. Absorption and Transportation of Carotenoids.[104] SR-B1, scavenger receptor class B type 1, NPC1L1, Niemann-pick C1-like 1, CD36, cluster of differentiation 36.

and skeletal muscle tissue, prostate, endometrial connective tissue and the pancreas), which cleaves eccentrically at the 9′, 10′ positions to produce an apo-10′-carotenoid and an ionone.[106]

From the substrate specificity perspective, BOC 1 prefers carotenes (provitamin A carotenoids), the β-ionone ring containing carotenoids and apocarotenoids, while BOC 2 tends to xanthophyll, hydroxylated apocarotenoids and lycopene isomers.[108] Non-provitamin A carotenoids (particularly lycopene) have been suggested as the substrates of BCO2 rather than BCO1.[109] Preferentially accumulation of lycopene was discovered in perigonadal adipose and liver tissues based on the BOC 2-lacking mice models. However, the level had a noticeable decrease in BOC 1-lacking mice, highly recommended as the cleavage activity acting by BOC 2. Interestingly, BOC 2-lacking mice were reported to accumulate dietary zeaxanthin and lutein, but fowls cannot,[110] thereby BOC 2 has also been regarded as the mammalian-existed carotenoid-oxygenase. Different catalytic activities on substrates also indicate the metabolic pathways of various carotenoids, which shows in more detail in Fig. 2.

Moreover, carotenoids are detected as free forms in plasma after absorption, which implies the occurrence of de-esterification at the process within the absorption.[111] In this case, pancreatic lipases help release xanthophyll and then transfer from emulsion particles to the micelles.[112] However, some researchers proposed that human pancreatic lipase showed lower efficiency in the hydrolysis of the xanthophyll.[113] It is probably due to the non-specific recognition triggered by the abovementioned competition between carotenoids and other lipid-soluble substances.

Except for the primary two carotenoid-oxygenases, some other enzymes have also been affirmed to contribute to carotenoid cleavage. For example, the isolated 9-*cis*-epoxycarotenoid dioxygenase can cleave 9-*cis* xanthophyll to xanthoxin and C25-apocarotenoids.[114] This pathway has been verified as

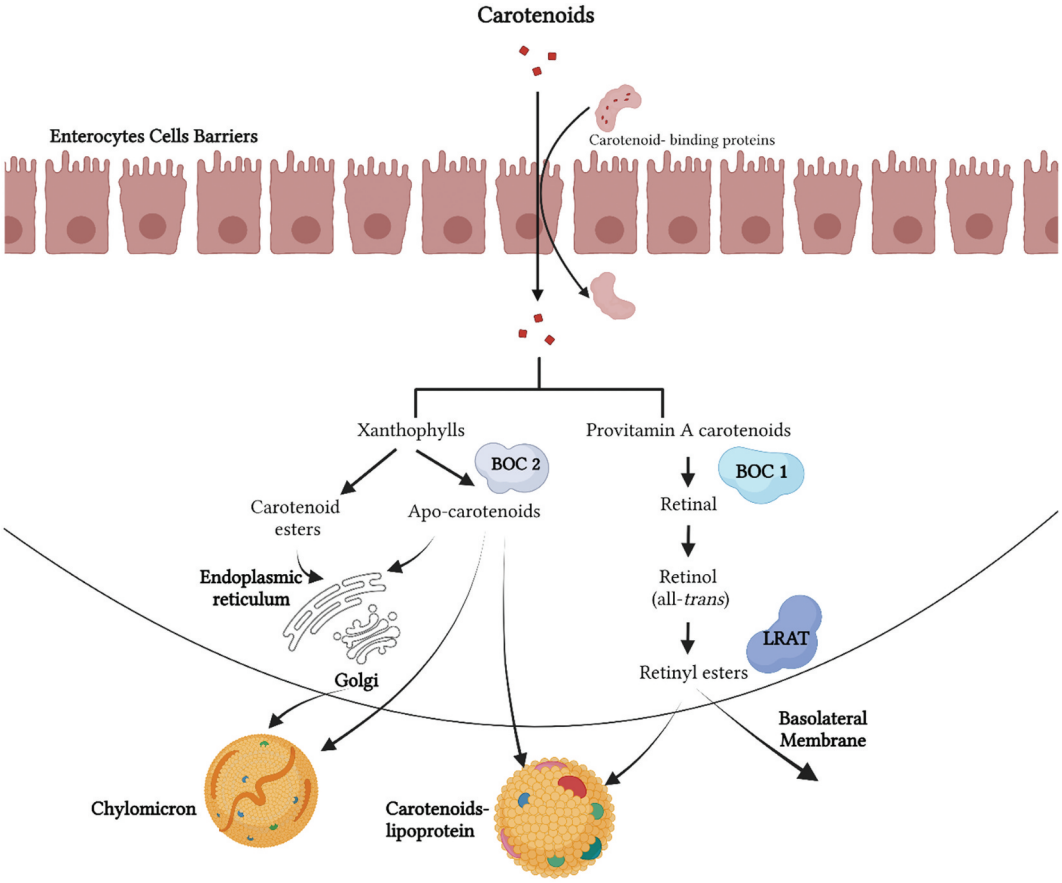

Figure 2. Enzyme-Mediated Carotenoids Cleavage and Conversion. BOC1, β-carotene-15,15′-oxygenase, BOC2, β-carotene-9′,10′-oxygenase, LART, lecithin retinol acyltransferase.

the rate-limiting step of abscisic acid biosynthesis in plants. Nevertheless, till today, the enzyme has only been observed in terrestrial plants. In addition, carboxyl ester lipase (CEL) was demonstrated to hydrolyse zeaxanthin, resulting in 12.3 times higher bioavailability from micelles.[115] Once the xanthophylls were cleaved by CEL in the gastrointestinal tract, astaxanthin diesters were first converted to the monoester, then hydrolysed to free astaxanthin.[116] Also, the CEL compensated for the substrate affinity on xanthophylls lacking by other pancreatic enzymes.

Canthaxanthin and astaxanthin have been first proposed as inducers of lung and kidney xenobiotic metabolising enzymes.[117] In the liver, after 15-day administration of canthaxanthin, the activities of nicotinamide adenine dinucleotide (NADH)- and nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome *C*-reductase have been significantly increased. Meantime, Cytochrome P450s (a group of heme-containing oxidases) content in rat liver increased, further with the activation of ethoxyresorufin *O*-deethylase (EROD) and methoxyresorufin *O*-demethylase (MROD).[118] Treatment with astaxanthin and canthaxanthin also enhanced the activity of the enzymes quinone reductase (QR) and *p*-nitrophenol UDP-glucuronosyl transferase (4NP-UGT) in phase II.[119] Previously, it has been suggested that lycopene mixture induced antioxidant-dependent transcription of phase II xenobiotic-metabolizing enzymes.[120] Thereby, the lycopene metabolites could act as enzyme inhibitors against the initiation, promotion and progression of carcinogenesis. As polar metabolites, lycopene and its isomers have been proven to interact with NF-κB and nuclear factor erythroid 2-related factor 2 (Nrf-2).[121] On the one hand, they are able to directly reduce the inflammatory gene expression by inhibiting NF-κB, thereby decreasing the levels of tumour necrosis factor. On the other hand, they can accelerate the

translocation of Nrf-2, which activate the activity of catalase (CAT) and superoxide dismutase (SOD). Furthermore, glutathione-*S*-transferase and reduced glutathione status were assessed, which assisted in determining the absorption of carotenoids by human tissues.[117]

Carotenoid bioaccessibilities and bioavailabilities

Except for the intake amount, same as the other bioactive compounds, carotenoids need to be released from the food matrix by the upper digestive system. The released proportion of the total carotenoid content is considered bioaccessible.[122] On the other hand, the degrees to that carotenoids reach the circulatory system has been regarded as their bioavailabilities.[123] Taking both affecting factors into account, the bioactivities of carotenoids can be investigated and evaluated further. These terms are highly dependent on the absorption and movement of carotenoids in the upper digestion system.

Determination of carotenoid bioaccessibilities

Generally, in *vitro* studies mimicking gastric and small intestinal digestion helps assess nutritional compounds' bioaccessibility.[124] Indeed, the bioaccessibilities of carotenoids are highly variable but generally at a relatively low accessibility level, which might be because of the numerous methodologies employed to analyse these characteristics (food matrix or molecule itself).[104] To avoid this difference, the INFOGEST network published a static method of *in vitro* digestion toward international consensus. This static model employs a constant ratio of food to enzymes and electrolytes, as well as a constant temperature and pH throughout each phase of digestion (oral, gastric, and intestine).[125] Later in 2019, INFOGEST 2.0 adaption was published, with the clarification of viscosity in the oral phase and the lipase in the gastric phase.[126] The adapted INFOGEST method was developed with the aim of reducing the effect of bile acids on carotenoid micellisation and stability, therefore assessing a more accurate and higher level of carotenoids bioaccessibility ($p < 0.05$), compared to those simplest protocols.[127]

Table 2 summarises some previous research on the bioaccessibility of carotenoids. For example, lutein and zeaxanthin commonly existed as the free form in *Scenedesmus almeriensis*, and both showed highly stable character during *in vitro* digestion.[132] The reported below 1% mineralisation also provided a reason for the poor bioaccessibility in the digestion process. However, with the addition

Table 2. Bioaccessibility of mentioned microalgae-based carotenoids.

Carotenoids	Microalgae	Bioaccessibility (%)	References
β-carotene	*Chlorella* sp.	0–2.6%	[128]
	C. reinhardtii	10%	[129]
	P. tricornutum	19–76%	[129]
	Nannochloropsis sp.	5–16%	[130]
Astaxanthin	*H. pluvialis*	Lipid-based formulation was 1.7–3.7 folder higher than commercial formulation	[131]
Lutein	*S. almeriensis*	Olive oil-based formulation was 25% higher in micellization	[132]
	C. reinhardtii	34.7% in intact microalgae 34.1% in disrupted microalgae	[133]
Zeaxanthin	*S. almeriensis*	Olive oil-based formulation was 45–60% higher in micellization	[132]
Fucoxanthin	*P. tricornutum*	20–62%	[129]
	Nitzschia laevis	11.4% in fucoxanthin 21.3% in fucoxanthinol	[134]
	Microalgae Species	17.6% in conventional emulsion 33.9% (NO.2 nanoemulsion) 44.3% (NO.3 nanoemulsion) 46.7% (NO.4 nanoemulsion)	[135]
Violaxanthin	*Scenedesmus bijuga*	17.02 ± 1.03% in 15-*cis*-isomer	[136]

Bioaccessibility: the amount fraction bioaccessible compared to total carotenoids ingested.

of olive oil, the micellisation of lutein and zeaxanthin rose considerably, reaching levels as high as 80–90% of the initial levels. It was verified that an oil auxiliary could increase the amounts of lutein and zeaxanthin extraction and bioaccessibility of lyophilised biomass.[137] In addition, a study showed that the bioaccessibility of lutein in different food matrices (intact and disrupted microalgae) was similar (34.7% and 34.1%, respectively).[133] With the inconstant results in β-carotene, they indicated that the food matrix could only affect the absorption of biologically active substances to a limited extent. The differences in β-carotene are probably due to the highly stable character within the natural cell environment. Still, once isolated, they become sensitive and cannot expose to light, heat or acids.[138] Moreover, although the isomerisation of lutein (*cis*-) showed a low content during *in vitro* digestion, it increased in the micellar phase. It showed greater bioaccessibility than the all-*trans* forms.[139] They are more soluble and better micellarised with bile salts pancreatin in the intestinal phase.[122]

For fucoxanthin, simulated gastrointestinal digestion was designed and explored its deacetylation kinetics in different formulations.[134] The final bioaccessibility of fucoxanthin (11.4%) and fucoxanthinol (21.3%) implied that almost 2/3 of fucoxanthin reached the small intestine and transformed into fucoxanthinol. Meanwhile, compared to the conventional emulsion, the bioaccessibility of fucoxanthin nanoemulsion with small particles increased to approximately 33.9–46.7%.[135] Due to the lower surface area of the emulsion containing big particles, the decreased access of lipase was highly related to the significant increase in bioaccessibility.[135]

Regarding the β-carotene, its bioaccessibility ranged from 0–2.6% by isolating from *Chlorella* sp., and up to 10% and 29% by extracting from *Chlamydomonas reinhardtii* and *P. tricornutum*, respectively.[128,129] The increase might be attributed to their less rigid cell walls. Cell disruption improved the *in vitro* bioaccessibility of carotenoids from microalgae. The cell wall and/or cell membrane served as a natural barrier for (lipophilic) nutrients during *in vitro* digestion.[130] The surface area available for digestive enzymes to target would increase since the mechanical disturbance would allow the carotenoids to be more releasable. Moreover, the lipid component and its structural properties, such as the degree of saturation and the fatty acid chain length, impacts the individual bioaccessibility of the β-carotene.[136]

Many research studies also considered the bioaccessibility of astaxanthin, violaxanthin, and canthaxanthin. Isomerisation was a determining factor in astaxanthin's bioavailability efficiency.[131 It exhibited a non-linear dose-response and selective absorption of cis isomers, which was also affected by the esterified or free-form.[140] However, a large proportion of released canthaxanthin was selectively distributed in the continuous phase rather than transferred into the micelle phase, further reducing their bioaccessibility.[141] Moreover, the xanthophylls violaxanthin was significantly more bioaccessible than all-*trans*-β-carotene.[136] Violaxanthin is a hydrophobic molecule commonly associated with membranes and/or involved in non-covalent binding to a specific light-harvesting protein, violaxanthin-chlorophyll (VCP),[142] which impacts their bioaccessibility negatively. Nevertheless, few articles focus on its bioaccessibility due to the protein-binding property of peridinin,[143] which can be considered a future research direction.

Affecting factors

Intrinsic factors are the prominent affecting contributors to the absorption and distribution of carotenoids. Matrix interferents have been regarded as the main inner affecting factors, such as constituents of the matrix cell walls (proteins, dietary fibres, minerals and lipids), physicochemical molecule properties and genetic aspects related to the host cell. Furthermore, the natural physical (cell wall) and chemical (interaction with other components) barriers of the food matrix, as well as the physicochemical features of the chemicals, have also been thought as one of the most aggravating reasons.

Lipids

Triglycerides are the most focused lipids that can increase the free type and ester form carotenoids by facilitating their transfer to the aqueous micellar fraction during digestion.[144] Compared to the control group, 5 g and 10 g of fat sufficiently increased the carotenoid uptake.[145] It agreed with another study, which suggested fat can effectively improve the concentration of β-carotene in the plasma.[146] They indicated lipids acted as accumulators in the micelle, which agreed with the conclusion of Failla et al.[147] Lipids, to some extent, would influence the esterification and solubilisation of lutein in the fat phase.[146] The raised solubilisation of lipid-carotenoid droplets also triggers higher bioavailability. Besides, triglycerides encourage chylomicron secretion to increase carotenoid absorption outside the enterocyte, preventing intracellular build-up.[148] However, some scientists were against this conclusion. Plasma carotenoid concentrations would drop significantly to a limited amount if the lipid addition were unabsorbable or slightly absorbable. Therefore, sucrose polyesters were suggested as a dietary fat replacer due to their unabsorbed property and physical trapping capacity on carotenoids. They have been reported to decrease the plasma carotenoid concentration (β-carotene 31%, lutein 18% and zeaxanthin 13%) without negative effects on oxidation markers, eye health, cardiovascular health or immune system status.[149] In contrast, the metabolic route of triglycerides affects the bioavailability of carotenoids. The portal vein is the primary absorption position of medium-chain triglycerides, which would limit the rate of chylomicron formation.[146]

Dietary fibres

Dietary fibre is another group that would reduce the bioaccessibilities and bioavailabilities of carotenoids. They limit the formation of micelles by physically entrapping carotenoids or intermingling with bile salts, thereby decreasing the passive absorption of carotenoids.[150,151] From the soluble fibre point of view, they have the ability to enhance the viscosity of gastric contents and slow gastric emptying, which interferes with the absorption of lipids and carotenoids.[152] A previous study has indicated that the medium- (80 mPa·s) and high- (300 and 500 mPa·s) viscosity alginates, which are the dominant fibres sourced from marine organisms, inhibited the β-carotene micellisation than the fibre-free control group over 92.7%, 71.6% and 73.5%, respectively.[153] Similar results were found in κ-carrageenan, they significantly reduce the bioaccessibility of β-carotene.[154] κ-Carrageenan have verified their acid self-structuring and ionotropic gelation performances, which lowered the β-carotene bioaccessibility, ranging from 34–38.9% under 0.4–1.0% fibre concentrations, compared to 55.4% in the control group.

Divalent minerals

Several divalent mineral cations (Mg, Ca, Zn and Na) have been reported to be involved in carotenoid digestion by hampering the micellisation process, specifically via bile salt complexation and fatty acid precipitation.[155] Based on an *in vitro* digestion model coupled to Caco-2 cells, both mineralisation and intestinal uptake of neoxanthin, β-carotene and violaxanthin were significantly suppressed with metal and zinc ion presence in a concentration-dependent manner.[156] Compared to the blank controls, fraction mineralisation and uptake were significantly reduced (to 22.5 and 5.0%, respectively; $p < 0.001$) by 12.5 mmol/L Fe^{2+}. The effects of Mg have also been assessed, weaker than other competitors, only uptake was decreased significantly to 69.2% of the control value at 25 mmol/L concentration ($p < 0.001$). Besides, a high concentration of divalent cations has been observed in their correlation with increasing surface tension, which suggested the depletion of surfactants (bile salts).[155] As an endogenous surfactant, bile salts contribute to the transportation of various carotenoids across biological barriers,[157] directly affecting their bioaccessibilities.

Table 3. Bioactivity of microalgae carotenoids and their brief mechanism.

Carotenoids	Bioactivity	Microalgae	Model	Mechanisms		Reference
				Main Results	Specific doses & Outcomes	
Fucoxanthin (Fucoxanthinol)	Anti-angiogenic	*U. pinnatifida*	Embryonic Stem Cell-Derived Embryoid Bodies (EBs)	Suppressed development of blood vessel-like structures	10 μM (19.9% inhibitory, $p < 0.05$) 20 μM (50% inhibitory, $p < 0.05$)	[159]
		U. pinnatifida *C. fragile*	HUVECs	Attenuation of intracellular proliferation and survival signals	1.0/5.0 μM (reduced 50% phosphorylation, $p < 0.05$)	[160]
		U. pinnatifida	SK-Hep-1 human hepatoma cells	• Enhance gap junctional intercellular communication • Increase protein and mRNA expressions of C×43 and C×32	1–20 μM (IC_{50} = ca. 9.4 μM, $p < 0.05$)	[161]
	Anti-inflammatory	*U. pinnatifida*	Murine macrophage RAW 264.7 cells	(a) Express TNF-α and IL-6 (b) Inhibit iNOS/NO pathway	Chloroform fraction (87.5% inhibitory, $p < 0.01$) Hexane fraction (66.3% inhibitory, $p < 0.01$)	[162]
			Human monocytic leukemia cell line	(a) Reduce TNF-α, IL-6, ROS (b) Down-regulate COX-2 and iNOS (c) Inhibit NF-κB and MAPK pathway	50 μM, $p < 0.001$	[163]
	Antioxidant (Anti-Alzheimer's disease)	*P. tricornutum*	*In vitro*	Inactivate butyrylcholinesterase	2% fucoxanthin *cis*-isomer enhanced scavenging capacity: DPPH (21.0%, $p < 0.05$) Hydrogen peroxide (10.3%, $p < 0.05$) Superoxide anion (16.0%, $p < 0.05$) Reducing power (19.7%, $p < 0.05$)	[164]
	Cardioprotective	*Sargassum hemiphyllum*	Mice	(a) Interfere TCA cycle, glycolysis and steroid hormone biosynthesis (b) Enhance ventricular rhythm	500 mg/kg oral administration for 28 days 36.43% lower ROS level, $p < 0.05$	[165]

(*Continued*)

Table 3. (Continued).

Carotenoids	Bioactivity	Microalgae	Model	Mechanisms		Reference
				Main Results	Specific doses & Outcomes	
Astaxanthin	Anti-inflammatory	*H. pluvialis*	Mouse RAW 264.7 macrophages cells	(a) Reduce IL-6 and IL-12 (b) Inhibit NO pathway	10 µg/mL (50.5% inhibitory, p <0.05) 20 µg/mL (47.2% inhibitory, p <0.05) 50 µg/mL (45.5% inhibitory, p < 0.05)	[166]
		H. pluvialis	Multiple cells model	(a) Reduce TNF-α, IL-6 and IL-1β (b) Inhibit NO pathway	5 µM, around 50% − 90% inhibitory, p < 0.0001	[167]
	Hepatoprotective	*H. pluvialis*	Rats	Activate antioxidant enzymes catalase, glutathione, superoxide dismutase and lipid peroxidase levels	250 µg/kg $_{b.w.}$ oral administration for 14 days 30% − 50% reduced of the lipid peroxidation	[168]
		S. platensis *D. salina*	Rats	Reduce activity of serum glutamate pyruvate transaminase	100 µg/kg $_{b.w.}$ Control (CCl4-treated): 128.68 units/mL SGPT *Dunaliella*-treated: 62.83 units/mL SGPT, p < 0.05 *Spirulina*-treated: 76.83 units/mL SGPT, p < 0.05	[169]
	Memory enhancement	*H. pluvialis*	Rats	Neuron modification	1.3 mg/kg, Day 6 (p < 0.01) Day 7 (p < 0.05)	[170]
	Cognitive function		*In vitro*	Cross blood brain barrier	Neurological severity score improved, p < 0.007 Object recognition test, p < 0.035	[171]
	Immunomodulatory effects		*In vitro* *Ex vivo*	Potentiate LPS-induced cell proliferation *ex vivo*	1.4 mg/kg, 100% enhanced cell viability, p < 0.05 7 mg/kg around 50% enhanced cell viability, p < 0.05	[172]
	Reduce blood pressure		*In vivo*	Xanthine/XOD related	50 mg/kg, 20% incidence of stroke (Control: 80%)	[173]

(*Continued*)

Table 3. (Continued).

Carotenoids	Bioactivity	Microalgae	Model	Mechanisms		Reference
				Main Results	Specific doses & Outcomes	
β-Carotene	Anti-aging	*D. salina*	Drosophila	Increase mitochondrial function	Optimised concentrate (10 μM) 6% enhanced lifespan in male ($p < 0.05$) 21% enhanced lifespan in female ($p < 0.01$)	[174]
	Antioxidant	*S. maxima*	Animal model	Reduce oxidized compounds production	5 mg for 2/7 weeks 71% of plasma antioxidant capacity than 54% in control group, $p < 0.05$	[175]
	Antagonistic	*D. salina*	Rats	(a) Suppress AST, ALT, MDA and some other hepatic content (b) Reduce fibrosis, centrilobular necrosis, and inflammatory cell infiltration	12.5 mg/kg, 25 mg/kg or 50 mg/kg for 6 weeks 12.5 mg/kg, 37% reduction of ALT 25 mg/kg, 41% reduction of ALT, 44% reduction of ALP 50 mg/kg, 38% reduction of ALT, 51% reduction of ALP $p < 0.05$	[176]
	Provitamin A effect	/	Human	Convert into vitamin A	30 μg for one week Mean absorption of D6 β-carotene in all subjects was 2.235 ± 0.925%	[177]
Lutein	Anti-cataracts	/	Human	No effect on AMD	Lutein (10 mg) + zeaxanthin (2 mg) for 5 years Treat 2.0%, control 0.9% $p = 0.04$	[178]
	Anti-arthritis	/	Rat	(a) Activate Nrf-2 (b) Reduce oxidative stress (c) Down-regulate inflammatory proteins and pro-inflammatory cytokines	1 μM, 95% cell viability, $p < 0.001$	[179]
		Other plants (*Tagetes erecta*)	Animal study	Suppress arthritis scores and paw swelling	1.5 mg/kg for 31 days suppressed the proliferation of synovial fibroblast (1.86 ± 0.53; $p = 0.003$), accumulation of plasma cells (1.29 ± 0.38; $p = 0.009$), formation of pannus (1.14 ± 0.39; $p = 0.003$), new bone (1.21 ± 0.37; $p = 0.003$)	[180]

Bioactivity and health benefits of carotenoids from microalgae

Remarkably, microalgal carotenoids contribute to health-promoting effects owing to bioaccessibility and bioavailability. Numerous studies have proved that a carotenoids-rich diet can reduce the incidence of human illness during the last few decades. Carotenoids are regarded as oxidation "defensors" by scavenging free oxygen radicals and preventing lipid peroxidation.[158] Detailed information about carotenoid bioactivities is listed in Table 3.

Fucoxanthin

Based on previous studies, fucoxanthin and fucoxanthinol have been reported for their potential anti-angiogenic effects.[181] In the first place, fucoxanthin is able to stop the differentiation of endothelial cells and limit the development of blood vessel-like structures to great extents. Concrete research on *Undaria pinnatifida* has reported fucoxanthin dose-dependently suppressed the growth of blood vessel-like structures in embryonic stem cell-derived embryoid bodies.[159] In detail, applied 10 μM and 20 μM fucoxanthin showed 73.9% and 43.8% inhibitory rates, which were more efficient in contrast to the untreated control group (93.8%). In addition, fucoxanthin can also regulate various angiogenic biomarkers.[160] For example, fibroblast growth factor 2 (FGF-2) was significantly ($p < 0.05$) suppressed by 1 μM and 5 μM fucoxanthin after 6-hour treatment, which were around 60% and 50%, respectively. A cellular experiment has demonstrated the positive effect of fucoxanthin on angiopoietin-2 (Ang2), a growth factor belonging to the angiopoietin signalling.[182] The one-day fucoxanthin treatment (0–20 μM) triggered an increasing expression of Ang 2, ranging from 100–125 relative densities (normalised by glyceraldehyde 3-phosphate dehydrogenase, GAPDH). Furthermore, the factor further weakened the cell-to-cell junction by regulating the expression of vascular endothelial cadherin (VE-cadherin), which plays a key role in maintaining vascular integrity.[183] At the same time, fucoxanthin has also been found to enhance the gap junction intercellular communication by regulating the expression of connexin 32 (Cx32) and 43 (Cx43) protein based on human cancer cell models.[161] It presumably caused the enhancement of intracellular signalling that promoted cell cycle arrest and apoptosis.

In terms of the anti-inflammatory property of fucoxanthin, three primary mechanisms have been confirmed, including effects on cytokines, transcription factors and enzymes. First, fucoxanthin is capable of regulating pro-inflammatory cytokines. For instance, fucoxanthin treatment diminished the increasing expression of interleukin 1β (IL-1β), IL-6 and tumour necrosis factor α (TNF-α) in inflamed RAW 264.7 cells induced by palmitate.[184] All three markers showed a significant decrease (~60%, $p < 0.001$) compared to the bovine serum albumin (BSA) control group after being treated with 50 μM fucoxanthin. Extracts of microalgae *Phaeodactylum tricornutum* have been evaluated for their regulation effects. The expression of the IL-1β was significantly reduced ($p < 0.001$) in cells with microalgal extracts due to the presence of 40 μM fucoxanthin 4 h prior to lipopolysaccharides (LPS) and adenosine triphosphate (ATP) stimulation.[185] Besides, fucoxanthin also showed inhibitory effects on some transcription factor-mediated signalling pathways like NF-kB [185] and mitogen-activated protein kinases (MAPK).[186] Isolated from *Conticribra weissflogii*, fucoxanthin was found to inhibit phosphorylation of the NF-kB signalling pathway, thereby reducing the nuclear translocation of NF-kB. In particular, fucoxanthin (0.3–30 μM) produced a significant dose-dependent reduction of NF-kB activity, with the half maximal inhibitory concentration (IC 50) at 11.08 ± 0.78 μM on the THP1-Lucia™ NF-κB Cells.[187] The last but not least mechanism is the regulation of inflammatory-related enzymes. Fucoxanthin has been found inhibiting effects on inducible nitric oxide synthase (iNOS) expression. The relative iNOS expression level was effectively reduced to ~45% under the pre-treatment with 50 μM fucoxanthin based on $A\beta_{42}$ (a 42 amino acid proteolytic product from the amyloid precursor protein)-induced BV2 cell models.[188]

In relation to Alzheimer's disease, fucoxanthin exhibited activities on butyrylcholinesterase (BuChE) and acetylcholinesterase (AchE),[189] two enzymes participating in regulating brain acetylcholine (ACh)

levels. Fucoxanthin has been proven to potent inhibition against BuChE levels of Alzheimer's patients, with an IC 50 value at 1.97 mM.[190] It also directly prevented the AChE enhancement induced by scopolamine (inhibitor constant at 42 μM) under the *in vitro* assays with three concentrations of fucoxanthin (25, 50 and 75 μM).[189] Moreover, the varying isomer compositions of fucoxanthin showed different free radical scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide, superoxide anion, relying on its *cis*-isomer ratios. With the amount of *cis*-isomer increased by 2%, the scavenging decreased by 21.0%, 10.3% and 16.0%, respectively.[190] The cardioprotective effects of fucoxanthin have been identified as another pharmaceutical function. High-stability fucoxanthin significantly reduced the cardiac fibrosis formation in the ventricle areas compared to the control group after 28-day oral administration in aging mice.[191] The potential mechanism was explained as the interference of metabolism, including the pathway of the tricarboxylic acid (TCA) cycle, glycolysis and steroid hormone biosynthesis. Moreover, in recent decades, Khaw et al.[192] conducted a bibliometric analysis of fucoxanthin research. Anti-diabetic, anti-obesity,[193] neuroprotective, skin protection [194] and anti-cancer [195–197] were the most commonly cited topic of fucoxanthin and its metabolites fucoxanthinol.

Astaxanthin

It has been suggested that astaxanthin makes generous contributions due to its superior antioxidant capacities.[198] Astaxanthin at concentrations 10, 20 and 50 μg/mL has been conducted significantly inhibited nitric oxide (NO) production, a signalling molecule playing key roles in the pathogenesis of inflammation, at 50.5%, 47.2% and 45.5%.[166] The antioxidant activities of macrophages have eventually been raised by down-regulating the level of IL-6 from 198.24 pg/mL to 132.55 pg/mL and 136.10 pg/mL at concentrations of 10 μg/mL and 20 μg/mL astaxanthin treatment. Further, compared among three types of astaxanthin ester, monoesterified (Ast-mE), diesterified (Ast-dE) and nonesterified (Ast-N), Ast-mE has been confirmed as the most efficient inhibitor of various immunologically active mediators based on murine macrophage RAW 264.7 cells.[167] Different astaxanthin samples (with different ester form compositions) substantially decreased the mRNA levels of IL-1β, IL-6, and TNF-α, with 68.1% Ast-mE contained sample inhibiting two-fold than the other two counterparts and three-fold than the control group ($p < 0.001$).

In addition, the hepatotoxicity effects of astaxanthin and astaxanthin esters have also been observed.[168] A 30–50% increase was found in the level of antioxidant enzymes catalase, glutathione, superoxide dismutase (SOD) and lipid peroxidase after feeding 10 mg/kg astaxanthin every day for 2 weeks to carbon tetrachloride (CCl4)-treated rats.[199] The results suggested astaxanthin achieved hepatoprotection activity by activating related enzymes, in agreement with mechanisms concluded by Chiu et al.[200] A comprehensive evaluation of microalgal carotenoids confirmed the high antioxidant activity of mixed carotenes and xanthophylls.[169] The activity of serum glutamate pyruvate transaminase (SGPT) reduced from 128.68 units/mL to 62.83 units/mL for *Dunaliella* sp. and 76.83 units/mL for *Spirulina* sp., which indicated the mixtures exerted their action against lipid peroxidation induced by liver injury. In particular, astaxanthin suppresses the generation of free radicals and protects the inner oxidation.[201]

In terms of the memory enhancement function of astaxanthin, an animal experiment has been previously designed in a Morris water maze. The rats were pre-supplied with different doses of *H. pluvialis* powder, from 0, 0.26, 1.3 to 6.4 mg/kg, and then were monitored by automatic video recording.[170] The middle-dosage group (1.3 mg/kg) significantly shortened the latency and distance required for mice to find a hidden platform. At the same time, astaxanthin supplied group could easily find the original hidden place while the control group quickly forgot the location. Furthermore, because of the BBB penetration ability of astaxanthin, the rats' cognitive behaviours were also attributed to the neuroprotective effects.[171] Therefore, it could be an efficient supplement for prophylactic or remediation against neuronal disorders.

β-Carotene

Data from β-carotene, especially 9-*cis*-isomer, indicated a positive consequence in alleviating ageing and age-related diseases. One drosophila study suggested that 9-*cis*-β-carotene, produced from *D. salina*, increased their mean lifespan and mobility, probably due to the increased mitochondrial function.[174] After the treatment of 10 μM total β-carotene solutions, the median life span of male drosophila significantly increased by 32.6% ($p < 0.01$), whereas a slightly lower magnitude was found in female flies. However, studies of microalgal carotenoids for improving mitochondrial function have not been continuous, differing in concentrations, sources, and models. Given the synergy of phenolic acids, tocopherols and β-carotene, the *in vivo* and *in vitro* animal studies were conducted with *Spirulina maxima* strains.[175] After two- and seven-week administration, the treatment group had a two-fold higher plasma antioxidant capacity, which simultaneously reduced the production of oxidised compounds in the liver.

Activation of hepatic stellate cells (HSC) has been linked to several clinical hepatic disorders. Researchers have investigated the hepatoprotective and antagonistic effects of β-carotene on HSC of adult male albino Wistar rats. Two dose levels of *D. salina* powder extracts (25 & 50 mg/kg) triggered a significant decrease in serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), while 12.5 mg/kg dose reduced the value of ALT and AST.[176] According to histological analysis of the liver, β-carotene also reduced the probability of fibrosis, centrilobular necrosis and inflammatory cell infiltration induced by the thioacetamide.[176] The results agreed with the previous study by Murthy et al.[169] But they indicated mixed carotenoids from *D. salina* had a better hepatoprotection than β-carotene alone extracted from *S. platensis*.

In addition, β-carotene is a provitamin A carotenoid, preventing vitamin A deficiency. In this case, eleven volunteers participated in a daily vitamin determination. After being dosed with D6 β-carotene, 6 participants had sufficient plasma concentrations of retinol that could be detected.[177] However, the organiser also pointed out the limitation. The mean conversion ratio of β-carotene-to-retinol was only 0.0296%, with an equally low β-carotene absorption at 2.235%. Microalgal-based β-carotene also showed various bioaccessibility levels, except for *P. tricornutum*, ranging from 19 to 76%.[129] It was assumed that *P. tricornutum* would be a good source of supplemental provitamin A.

Lutein

Lutein protects eye tissues from light damage by acting as an 'eye vitamin'. Lutein reduces oxidative damage triggered by light and reactive oxygen species (ROS).[202] Related enzymes, such as catalase, glutathione peroxidase and transferase, would be significantly activated by adding lutein, which further down-regulated cytokines (C-reactive protein, CRP, tumour necrosis factor-alpha, TNF-α, interleukin-1, IL-1).[203] However, the age-related macular degeneration (AMD) study supported by the National Institutes of Health disagreed with this. They thought lutein (with zeaxanthin) treatment had neither positive nor negative effects on advanced AMD.[178] The competitive absorption between lutein and inherent nutritional status might be an underlying issue [148] that needs further assessment.

Moreover, activation of Nrf-2 was the primary mechanism of lutein in osteoarthritis protection.[204] The addition of lutein enhanced the antioxidant defence capacity to reduce oxidative stress, consequently showing anti-arthritis effects. Inflammatory proteins and pro-inflammatory cytokines were down-regulated, supporting the powerful cytoprotection ability.[205] The point was approved by conducting an animal model study and proved by lutein administration. Repeat injections of lutein at a dose of 1.5 mg/kg for 31 days significantly suppressed the arthritis scores and hind paw swelling in collagen-induced arthritis model rats.[180]

Table 4. Applications of Microalgae Carotenoids.

Category of carotenoids	Microalgae species	Applications/Functions	References
β-Carotene	*D. salina* *C. zofingiensis* *Arthrospira* *Chlorella fusca* *Chlorella vurgaris* *Botryococcus sudeticus* *Pandorinamorum* *Chlorococcus*	(i) Pigmentation (food and feed), (ii) Pro-vitamin A (retinol), (iii) Antioxidants (additive to cosmetics and health food), (iv) Anti-inflammatory, immunomodulatory, hepatoprotective agent, and multivitamin formulations.	[]206–208
Astaxanthin	*H. pluvialis* *Chloromonas nivalis* *B. braunii* *Chlamydocapas* spp. *Scenedesmus* sp. *C. zofingiensis* *Chlorococcum* sp. *Scotiellopsis oocystiformis* *P. botryoides* *N. wimmeri*	(i) Aquaculture and poultry industries colourant, (ii) Nutraceutical, pharmaceutical, cosmetics, and food industry applications (Dietary supplements), (iii) Antioxidants, (iv) Vitamin E-rich biomass.	
Lutein	*S. almeriensis* *Muriellopsis* sp. *Chlorella protothecoides* *Coccomyxa acidophila* *Chlamydomonas acidophila*	(i) Colourants and functional additives in baby food, cosmetics, and pharmaceuticals, (ii) Prevent macular degeneration and some cancers effectively.	
Canthaxanthin	*C. zofingiensis* *Nannochloropsis oculate* *H. pluvialis* *C. vurgaris* *Coelastrella striolata* var. *multistriata*	(i) The pigment used in aquaculture, poultry, cosmetics (tanning agent), (ii) Medicine, nutraceuticals, and pharmaceuticals	
Fucoxanthin	*Chaetoceros gracilis* *Cylindrotheca closerium* *Isochrysis* aff. *galbana* *Isochrysis galbana* *P. tricornutum* *Nitzschia* sp. *O. aurita*	(i) In nutraceuticals and more recently in nutricosmetics (ii) As a bioactive component in traditional foods (e.g., milk, rice, bread, and pasta) to improve nutritional value and sensory attributes, (iii) Anti-obesity medicines, sometimes known as "oral cosmetics," are another option.	
Zeaxanthin	*P. cruentum* *Chromochloris zofingiensis* *D. salina* *Chlorella ellipsoidea*	(i) A powerful antioxidant and high-value bioproduct used as a natural colourant and addition in cosmetics and food, (ii) Preventing age-related macular degeneration, (iii) Cardiovascular disease and some forms of cancer prevention.	
Peridinin	*I. galbana* *Amphidinium carterae*	(i) Medical uses as a medicinal agent and technological applications (ii) Anticancer (e.g., colon cancer cells) (iii) Tumour prevention	
Violaxanthin	*C. ellipsoidea* *Nannochloropsis oceanica* *Nannochloropsis salina* *Nannochloropsis gaditana*	(i) Protection against UVB-induced skin damage (e.g., wrinkles) (ii) Cosmetics	

Practical Applications and Functions

Triggered by the benefits mentioned above, the research interest in microalgal carotenoids and their extraction techniques has increased.[206] Therefore, these micro-produce molecules have been broadly applied in many areas, such as pharmaceutical, cosmeceutical, (functional) food and animal feed industries.[207] Also, they have their prospective advantages in the healthcare sector, biofertiliser aspects, wastewater treatment and biofuel production.[208] Table 4 briefly summarises the portions of several application sectors of the carotenoid industry.

Generally, carotenoids from microalgae account for substantial market shares with functional properties. In the carotenoid sector, the research focuses on developing efficient microalgal growing techniques. Scientists target intelligent microalgal screening and strain optimisation to make carotenoid extraction more effective and environment-friendly. From this point of view, the knowledge of carotenoid structure and bioactivities is essential and draws increasing interest from researchers and industries. Emerging carotenoids, innovative carotenoid extraction methods and downstream procedures were also covered in a greater depth.[209]

Despite the positive outlook of natural sources of carotenoids, production techniques can be labour-intensive and time-consuming. In addition, low target carotenoid productivities and disobedience make carotenoid extraction more difficult.[209] Due to the chirality of isomers, it is hard to see the difference between natural and synthetic products. Only specialised procedures (such as isotope ratio mass spectrometry) can help tell genuineness properly.[210] Hence, it is straightforward for the adulterated version (synthetic pigments) to enter the market while falsely claiming to be natural to make a profit. Ultimately, governments try to issue strict restrictions and legislation to ensure the quality and order of the industry. In other words, these measures will oppositely hamper the development of microalgal-based carotenoid products and their commercialisation. Only specialised procedures (such as isotope ratio mass spectrometry) can help tell genuineness properly.[210] Hence, it is straightforward for the adulterated version (synthetic pigments) to enter the market while falsely claiming to be natural to make a profit. Ultimately, governments try to issue strict restrictions and legislation to ensure the quality and order of the industry. In other words, these measures will oppositely hamper the development of microalgal-based carotenoid products and their commercialisation.

Conclusion

Carotenoids are a large group of terpenoid pigments with structural diversity. Benefiting from their structural properties, carotenoids have played an essential and necessary role in the pharmaceutical, cosmeceutical, nutraceutical, (functional) food, and feed industries. In recent years, microalgae have attracted increasing research interest due to their relatively higher carotenoid content than many terrestrial plants. Microalgae are particularly abundant in several types of carotenoids, such as β-carotene, fucoxanthin, lutein, zeaxanthin and violaxanthin. Same as lipid compounds, carotenoids must be released from the food matrix and incorporated into mixed micelles to be absorbed. Although the bioaccessibility of carotenoids is limited, they show a variety of bioactive and body-protective properties which help people away from degenerative illnesses or other health problems. Neurological diseases, cardiovascular, radiation sickness, inflammatory and mutagenic concerns are the most discussed, while memories and cognitive effects have also been evaluated. However, there are still many research directions in shadow. Further studies can focus on the screening of non-toxic extraction solvents which are vastly able to retain carotenoid bioactivity, the improvement of carotenoid bioaccessibilities and bioavailabilities by nano/microencapsulation delivery, or the integration in the simultaneous production of multiple produces derived from microalgae to develop a more economically attractive marine source industry.

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Disclosure statement

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References

[1] Yabuzaki, J. Carotenoids Database: Structures, Chemical Fingerprints and Distribution Among Organisms. *Database*. 2017, *2017*. DOI: 10.1093/database/bax004.
[2] Mezzomo, N. and S. Ferreira, R. S. Carotenoids Functionality, Sources, and Processing by Supercritical Technology: A Review. *J. Chem*. 2016, *2016*, 1. DOI: 10.1155/2016/3164312.
[3] Cooperstone, J.; Schwartz, S. Recent Insights into Health Benefits of Carotenoids. In *Handbook on Natural Pigments in Food and Beverages*, eds. ; Carle, R., Schweiggert, R. W, Ed(s). ; Woodhead Publishing: United Kingdom, 2016; pp 473–497. DOI: 10.1016/B978-0-08-100371-8.00020-8.
[4] Qiang, S.; Su, A. P.; Li, Y.; Chen, Z.; Hu, C. Y.; Meng, Y. H. Elevated β-Carotene Synthesis by the Engineered Rhodobacter Sphaeroides with Enhanced CrtY Expression. *J. Agric. Food Chem*. 2019, *67*(34), 9560. DOI: 10.1021/acs.jafc.9b02597.
[5] Ren, Y.; Sun, H.; Deng, J.; Huang, J.; Chen, F. Carotenoid Production from Microalgae: Biosynthesis, Salinity Responses and Novel Biotechnologies. *Mar. Drugs*. 2021, *19*(12), 713. DOI: 10.3390/md19120713.
[6] Bohn, T.; Bonet, M. L.; Borel, P.; Keijer, J.; Landrier, J. -F.; Milisav, I.; Ribot, J.; Riso, P.; Winklhofer-Roob, B.; Sharoni, Y. Mechanistic Aspects of Carotenoid Health Benefits–Where are We Now? *Nutr. res. rev*. 2021, *34*, 1. DOI: 10.1017/S0954422421000147.
[7] Sathasivam, R.; Ki, J. S. A Review of the Biological Activities of Microalgal Carotenoids and Their Potential Use in Healthcare and Cosmetic Industries. *Mar. Drugs*. 2018, *16*, 1. DOI: 10.3390/md16010026.
[8] Saini, R. K.; Keum, Y. -S. Carotenoid Extraction Methods: A Review of Recent Developments. *Food Chem*. 2018, *240*, 90. DOI: 10.1016/j.foodchem.2017.07.099.
[9] Guedes, A. C.; Amaro, H. M.; Malcata, F. X. Microalgae as Sources of Carotenoids. *Mar. Drugs*. 2011, *9*(4), 625. DOI: 10.3390/md9040625.
[10] Masojídek, J.; Torzillo, G. Mass Cultivation of Freshwater Microalgae. In *Reference Module in Earth Systems and Environmental Sciences*, eds. ; Jørgensen, S. E., Fath, B. D. Ed(s).; Oxford: Elsevier, 2014; pp 2226–2235. DOI: 10.1016/B978-008045405-4.00830-2.
[11] Shah, M. M. R.; Liang, Y.; Cheng, J. J.; Daroch, M. Astaxanthin-Producing Green Microalga *Haematococcus Pluvialis*: From Single Cell to High Value Commercial Products. *Front. Plant Sci*. 2016, *7*. DOI: 10.3389/fpls.2016.00531.

[12] Sun, H.; Yang, S.; Zhao, W.; Kong, Q.; Zhu, C.; Fu, X.; Zhang, F.; Liu, Z.; Zhan, Y.; Mou, H., et al. Fucoxanthin from Marine Microalgae: A Promising Bioactive Compound for Industrial Production and Food Application. *Crit. Rev. Food Sci. Nutr*. 2022, 1. DOI: 10.1080/10408398.2022.2054932.
[13] Miyazawa, T.; Nakagawa, K.; Kimura, F.; Nakashima, Y.; Maruyama, I.; Higuchi, O.; Miyazawa, T. *Chlorella* is an Effective Dietary Source of Lutein for Human Erythrocytes. *J. Oleo Sci*. 2013, *62*(10), 773. DOI: 10.5650/jos.62.773.
[14] Zulfiqar, S.; Sharif, S.; Zia-Ul-Haq, M.; Rasool, N. Carotenoids Synthesis and Isomerism. In *Carotenoids: Structure and Function in the Human Body*; Zia-Ul-Haq, M., Dewanjee, S. and Riaz, M., Eds.; Springer International Publishing: Cham, 2021; p. 77.
[15] Hashimoto, H.; Uragami, C.; Cogdell, R. J. Carotenoids and Photosynthesis. In *Carotenoids in Nature: Biosynthesis, Regulation and Function*; Stange, C., Ed.; Springer International Publishing: Cham, 2016; p. 111.
[16] Swapnil, P.; Meena, M.; Singh, S. K.; Dhuldhaj, U. P.; Marwal, A. Vital Roles of Carotenoids in Plants and Humans to Deteriorate Stress with Its Structure, Biosynthesis, Metabolic Engineering and Functional Aspects. *Current Plant Biology*. 2021, *26*, 100203. DOI: 10.1016/j.cpb.2021.100203.
[17] Eggersdorfer, M.; Wyss, A. Carotenoids in Human Nutrition and Health. *Arch. Biochem. Biophys*. 2018, *652*, 18. DOI: 10.1016/j.abb.2018.06.001.
[18] Dutta, D.; Chaudhuri, U. R.; Chakraborty, R. Structure, Health Benefits, Antioxidant Property and Processing and Storage of Carotenoids. *Afr. J. Biotechnol*. 2005, *4*, 13. DOI: 10.4314/ajfand.v4i13.71773.
[19] Hughes, D. A. Effects of Carotenoids on Human Immune Function. *Proc. Nutr. Soc*. 1999, *58*(3), 713. DOI: 10.1017/S0029665199000932.
[20] Lohr, M. Chapter 21 - Carotenoids. In *The Chlamydomonas Sourcebook*, Second Edition), E ed.; Harris, H., Stern, D.B. and Witman, G.B., Eds.; Academic Press: London, 2009; p. 799.
[21] Felemban, A.; Braguy, J.; Zurbriggen, M. D.; Al-Babili, S. Apocarotenoids Involved in Plant Development and Stress Response. *Front. Plant Sci*. 2019, *10*. DOI: 10.3389/fpls.2019.01168.
[22] Vila, E.; Hornero-Méndez, D.; Azziz, G.; Lareo, C.; Saravia, V. Carotenoids from Heterotrophic Bacteria Isolated from Fildes Peninsula, King George Island, Antarctica. *Biotechnol. Rep*. 2019, *21*, e00306. DOI: 10.1016/j.btre.2019.e00306.
[23] Takemura, M.; Takagi, C.; Aikawa, M.; Araki, K.; Choi, S. -K.; Itaya, M.; Shindo, K.; Misawa, N. Heterologous Production of Novel and Rare C30-Carotenoids Using Planococcus Carotenoid Biosynthesis Genes. *Microbial cell factories*. 2021, *20*(1), 1. DOI: 10.1186/s12934-021-01683-3.
[24] Brocks, J. J.; Summons, R. E. 10.3 - Sedimentary Hydrocarbons, Biomarkers for Early Life. In *Treatise on Geochemistry*, 2nd ed.; Holland, H.D. and Turekian, K.K., Eds.; Elsevier: Oxford, 2014; p. 61.
[25] Pelah, D.; Sintov, A.; Cohen, E. The Effect of Salt Stress on the Production of Canthaxanthin and Astaxanthin by Chlorella Zofingiensis Grown Under Limited Light Intensity. *World J. Microbiol. Biotechnol*. 2004, *20*(5), 483. DOI: 10.1023/B:WIBI.0000040398.93103.21.
[26] Ip, P. -F.; Chen, F. Production of Astaxanthin by the Green Microalga Chlorella Zofingiensis in the Dark. *Process Biochem*. 2005, *40*(2), 733. DOI: 10.1016/j.procbio.2004.01.039.
[27] Liu, J.; Mao, X.; Zhou, W.; Guarnieri, M. T. Simultaneous Production of Triacylglycerol and High-Value Carotenoids by the Astaxanthin-Producing Oleaginous Green Microalga Chlorella Zofingiensis. *Bioresour. Technol*. 2016, *214*, 319. DOI: 10.1016/j.biortech.2016.04.112.
[28] Ding, W.; Cui, J.; Zhao, Y.; Han, B.; Li, T.; Zhao, P.; Xu, J. -W.; Yu, X. Enhancing Haematococcus Pluvialis Biomass and γ-Aminobutyric Acid Accumulation by Two-Step Cultivation and Salt Supplementation. *Bioresour. Technol*. 2019, *285*, 121334. DOI: 10.1016/j.biortech.2019.121334.
[29] Paliwal, C.; Ghosh, T.; George, B.; Pancha, I.; Maurya, R.; Chokshi, K.; Ghosh, A.; Mishra, S. Microalgal Carotenoids: Potential Nutraceutical Compounds with Chemotaxonomic Importance. *Algal Res*. 2016, *15*, 24. DOI: 10.1016/j.algal.2016.01.017.
[30] Chen, C. Y.; Kao, A. L.; Tsai, Z. C.; Shen, Y. M.; Kao, P. H.; Ng, I. S.; Chang, J. S. Expression of Synthetic Phytoene Synthase Gene to Enhance β□carotene Production in Scenedesmus Sp. CPC2. *Biotechnol. J*. 2017, *12*(11), 1700204. DOI: 10.1002/biot.201700204.
[31] Li, Z.; Ma, X.; Li, A.; Zhang, C. A Novel Potential Source of β-Carotene: Eustigmatos Cf. Polyphem (Eustigmatophyceae) and Pilot β-Carotene Production in Bubble Column and Flat Panel Photobioreactors. *Bioresour. Technol*. 2012, *117*, 257. DOI: 10.1016/j.biortech.2012.04.069.
[32] Xie, Y.; Xiong, X.; Chen, S. Challenges and Potential in Increasing Lutein Content in Microalgae. *Microorganisms*. 2021, *9*(5), 1068. DOI: 10.3390/microorganisms9051068.
[33] Soares, A. T.; da Costa, D. C.; Vieira, A. A. H.; Antoniosi Filho, N. R. Analysis of Major Carotenoids and Fatty Acid Composition of Freshwater Microalgae. *Heliyon*. 2019, *5*(4), e01529. DOI: 10.1016/j.heliyon.2019.e01529.
[34] Neumann, U.; Derwenskus, F.; Flaiz Flister, V.; Schmid-Staiger, U.; Hirth, T.; Bischoff, S. C. Fucoxanthin, a Carotenoid Derived from Phaeodactylum Tricornutum Exerts Antiproliferative and Antioxidant Activities in vitro. *Antioxidants*. 2019, *8*(6), 183. DOI: 10.3390/antiox8060183.
[35] Gao, F.; Teles, I.; Wijffels, R. H.; Barbosa, M. J. Process Optimization of Fucoxanthin Production with Tisochrysis Lutea. *Bioresour. Technol*. 2020, *315*, 123894. DOI: 10.1016/j.biortech.2020.123894.

[36] Xia, S.; Wang, K.; Wan, L.; Li, A.; Hu, Q.; Zhang, C. Production, Characterization, and Antioxidant Activity of Fucoxanthin from the Marine Diatom Odontella Aurita. *Mar. Drugs*. 2013, *11*(7), 2667. DOI: 10.3390/md11072667.
[37] Grune, T.; Lietz, G.; Palou, A.; Ross, A. C.; Stahl, W.; Tang, G.; Thurnham, D.; Yin, S. A.; Biesalski, H. K. Beta-Carotene is an Important Vitamin a Source for Humans. *J. Nutr*. 2010, *140*(12), 2268S. DOI: 10.3945/jn.109.119024.
[38] Sen Gupta, S.; Ghosh, M. *In Vitro* Antioxidative Evaluation of Alpha- and Beta-Carotene, Isolated from Crude Palm Oil. *J. Anal. Methods Chem*. 2013, *2013*, 351671. DOI: 10.1155/2013/351671.
[39] Deming, D. M.; Teixeira, S. R.; Erdman, J. W., Jr. All-Trans β-Carotene Appears to Be More Bioavailable Than 9-Cis or 13-Cis β-Carotene in Gerbils Given Single Oral Doses of Each Isomer. *J. Nutr*. 2002, *132*(9), 2700. DOI: 10.1093/jn/132.9.2700.
[40] Bogacz-Radomska, L.; Harasym, J. β-Carotene—properties and Production Methods. *Food Qual. Saf*. 2018, *2*(2), 69. DOI: 10.1093/fqsafe/fyy004.
[41] Osganian, S. K.; Stampfer, M. J.; Rimm, E.; Spiegelman, D.; Manson, J. E.; Willett, W. C. Dietary Carotenoids and Risk of Coronary Artery Disease in Women. *Am. J. Clin. Nutr*. 2003, *77*(6), 1390. DOI: 10.1093/ajcn/77.6.1390.
[42] Fiedor, J.; Burda, K. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. *Nutrients*. 2014, *6*(2), 466. DOI: 10.3390/nu6020466.
[43] Mathews-Roth, M. Treatment of Erythropoietic Protoporphyria with Beta-Carotene. *Photo-Dermatology*. 1984, *1*(6), 318.
[44] Marino, T.; Casella, P.; Sangiorgio, P.; Verardi, A.; Ferraro, A.; Hristoforou, E.; Molino, A. J.; Musmarra, D. Natural Beta-Carotene: A Microalgae Derivate for Nutraceutical Applications. *Chem. Eng. Trans*. 2020, *79*, 103.
[45] Pourkarimi, S.; Hallajisani, A.; Alizadehdakhel, A.; Nouralishahi, A.; Golzary, A. Factors Affecting Production of Beta-Carotene from *Dunaliella Salina* Microalgae. *Biocatal. Agric. Biotechnol*. 2020, *29*, 101771. DOI: 10.1016/j.bcab.2020.101771.
[46] Xu, Y.; Harvey, P. J. Carotenoid Production by Dunaliella Salina Under Red Light. *Antioxidants (Basel)*. 2019, *8*(5), 123. DOI: 10.3390/antiox8050123.
[47] Lamers, P. P.; Janssen, M.; De Vos, R. C.; Bino, R. J.; Wijffels, R. H. Carotenoid and Fatty Acid Metabolism in Nitrogen-Starved Dunaliella Salina, a Unicellular Green Microalga. *J. Biotechnol*. 2012, *162*(1), 21. DOI: 10.1016/j.jbiotec.2012.04.018.
[48] Hejazi, M. A.; Holwerda, E.; Wijffels, R. H. Milking Microalga *Dunaliella Salina* for Beta-Carotene Production in Two-Phase Bioreactors. *Biotechnol. Bioeng*. 2004, *85*(5), 475. DOI: 10.1002/bit.10914.
[49] Han, D.; Li, Y.; Hu, Q. Astaxanthin in Microalgae: Pathways, Functions and Biotechnological Implications. *Algae*. 2013, *28*(2), 131. DOI: 10.4490/algae.2013.28.2.131.
[50] Olaizola, M.; Huntley, M. *Recent Advances in Commercial Production of Astaxanthin from Microalgae*; Ed., Nagabhushaman, M.F.A.R. Science Publishers: New Hampshire, 2003 p. 143.
[51] Brotosudarmo, T. H. P.; Limantara, L.; Setiyono, E.; Heriyanto. Structures of Astaxanthin and Their Consequences for Therapeutic Application. *Int. J. Food Sci*. 2020, *2020*, 2156582. DOI: 10.1155/2020/2156582.
[52] Al-Bulishi, M. S. M. Health Aspects of Astaxanthin: A Review. *Can. J. Clin. Nutr*. 2015, *3*(2), 71. DOI: 10.14206/canad.j.clin.nutr.2015.02.08.
[53] Wayama, M.; Ota, S.; Matsuura, H.; Nango, N.; Hirata, A.; Kawano, S. Three-Dimensional Ultrastructural Study of Oil and Astaxanthin Accumulation During Encystment in the Green Alga *Haematococcus Pluvialis*. *PLoS One*. 2013, *8*(1), e53618. DOI: 10.1371/journal.pone.0053618.
[54] Gwak, Y.; Hwang, Y. -S.; Wang, B.; Kim, M.; Jeong, J.; Lee, C. -G.; Hu, Q.; Han, D.; Jin, E. Comparative Analyses of Lipidomes and Transcriptomes Reveal a Concerted Action of Multiple Defensive Systems Against Photooxidative Stress in *Haematococcus Pluvialis*. *J. Exp. Bot*. 2014, *65*(15), 4317. DOI: 10.1093/jxb/eru206.
[55] Roberts, J. E.; Dennison, J. The Photobiology of Lutein and Zeaxanthin in the Eye. *J. Ophthalmol*. 2015, *2015*, 687173. DOI: 10.1155/2015/687173.
[56] Krinsky, N. I.; Landrum, J. T.; Bone, R. A. Biologic Mechanisms of the Protective Role of Lutein and Zeaxanthin in the Eye. *Annu. Rev. Nutr*. 2003, *23*(1), 171. DOI: 10.1146/annurev.nutr.23.011702.073307.
[57] Fernández-Sevilla, J. M.; Acién Fernández, F. G.; Molina Grima, E. Biotechnological Production of Lutein and Its Applications. *Appl. Microbiol. Biotechnol*. 2010, *86*(1), 27. DOI: 10.1007/s00253-009-2420-y.
[58] Peiffer, R.; Petersen Jones, S. *Small Animal Ophthalmology: A Problem-Oriented Approach*; W.B. Saunders: Edinburgh, 2008.
[59] Widomska, J.; Subczynski, W. K. Why Has Nature Chosen Lutein and Zeaxanthin to Protect the Retina? *J. Clin. Exp. Ophthalmol*. 2014, *5*(1), 326. DOI: 10.4172/2155-9570.1000326.
[60] Quinn, N.; Csincsik, L.; Flynn, E.; Curcio, C. A.; Kiss, S.; Sadda, S. R.; Hogg, R.; Peto, T.; Lengyel, I. The Clinical Relevance of Visualising the Peripheral Retina. *Pro. Retinal Eye Res*. 2019, *68*, 83. DOI: 10.1016/j.preteyeres.2018.10.001.
[61] Bernstein, P. S.; Li, B.; Vachali, P. P.; Gorusupudi, A.; Shyam, R.; Henriksen, B. S.; Nolan, J. M. Lutein, Zeaxanthin, and Meso-Zeaxanthin: The Basic and Clinical Science Underlying Carotenoid-Based Nutritional Interventions Against Ocular Disease. *Pro. Retinal Eye Res*. 2016, *50*, 34. DOI: 10.1016/j.preteyeres.2015.10.003.

[62] Ma, L.; Liu, R.; Du, J. H.; Liu, T.; Wu, S. S.; Liu, X. H. Lutein, Zeaxanthin and Meso-Zeaxanthin Supplementation Associated with Macular Pigment Optical Density. *Nutrients*. 2016, *8*(7), 426. DOI: 10.3390/nu8070426.
[63] Bourdon, L.; Jensen, A. A.; Kavanagh, J. M.; McClure, D. D. Microalgal Production of Zeaxanthin. *Algal Res*. 2021, *55*, 102266. DOI: 10.1016/j.algal.2021.102266.
[64] Schubert, N.; García□mendoza, E.; Pacheco□ruiz, I. Carotenoid Composition of Marine Red Algae. *Journal of Phycology*. 2006, *42*(6), 1208. DOI: 10.1111/j.1529-8817.2006.00274.x.
[65] Fan, X. D.; Hou, Y.; Huang, X. X.; Qiu, T. Q.; Jiang, J. G. Ultrasound-Enhanced Subcritical CO2 Extraction of Lutein from *Chlorella Pyrenoidosa*. *J. Agric. Food Chem*. 2015, *63*(18), 4597. DOI: 10.1021/acs.jafc.5b00461.
[66] Wu, Z.; Wu, S.; Shi, X. Supercritical Fluid Extraction and Determination of Lutein in Heterotrophically Cultivated *Chlorella Pyrenoidosa*. *J. Food Process Eng*. 2007, *30*, 174. DOI: 10.1111/j.1745-4530.2007.00102.x.
[67] Honda, M. Nutraceutical and Pharmaceutical Applications of Carotenoids. In *Pigments from Microalgae Handbook*; Jacob-Lopes, E., Queiroz, M.I. and Zepka, L.Q., Eds.; Springer International Publishing: Cham, 2020; p. 449.
[68] Hua-Bin, L.; Fan, K. W.; Chen, F. Isolation and Purification of Canthaxanthin from the Microalga *Chlorella Zofingiensis* by High-Speed Counter-Current Chromatography. *J. Sep. Sci*. 2006, *29*(5), 699. DOI: 10.1002/jssc.200500365.
[69] Zaheer, K. Hen Egg Carotenoids (Lutein and Zeaxanthin) and Nutritional Impacts on Human Health: A Review. *CyTa - J. Food*. 2017, *15*(3), 474. DOI: 10.1080/19476337.2016.1266033.
[70] Niu, J.; Li, C. H.; Liu, Y. J.; Tian, L. X.; Chen, X.; Huang, Z.; Lin, H. Z. Dietary Values of Astaxanthin and Canthaxanthin in *Penaeus Monodon* in the Presence and Absence of Cholesterol Supplementation: Effect on Growth, Nutrient Digestibility and Tissue Carotenoid Composition. *Br. J. Nutr*. 2012, *108*(1), 80. DOI: 10.1017/S0007114511005423.
[71] Pi, S.; Xi, M.; Deng, L.; Xu, H.; Feng, C.; Shen, R.; Wu, C. Practical Synthesis of Canthaxanthin. *J. Iran. Chem. Soc*. 2020, *17*(2), 493. DOI: 10.1007/s13738-019-01784-2.
[72] Liu, B. -H.; Lee, Y. -K. Secondary Carotenoids Formation by the Green Alga *Chlorococcum* Sp. *J. Appl. Phycol*. 2000, *12*(3), 301. DOI: 10.1023/A:1008185212724.
[73] Abe, K.; Hattori, H.; Hirano, M. Accumulation and Antioxidant Activity of Secondary Carotenoids in the Aerial Microalga *Coelastrella Striolata* Var. *Multistriata*. *Food Chem*. 2007, *100*(2), 656. DOI: 10.1016/j.foodchem.2005.10.026.
[74] Zhang, Y.; Ye, Y.; Bai, F.; Liu, J. The Oleaginous Astaxanthin-Producing Alga *Chromochloris Zofingiensis*: Potential from Production to an Emerging Model for Studying Lipid Metabolism and Carotenogenesis. *Biotechnol Biofuels*. 2021, *14*(1), 119. DOI: 10.1186/s13068-021-01969-z.
[75] Dembitsky, V. M.; Maoka, T. Allenic and Cumulenic Lipids. *Prog. lipid res*. 2007, *46*(6), 328. DOI: 10.1016/j.plipres.2007.07.001.
[76] Kanazawa, K.; Ozaki, Y.; Hashimoto, T.; Das, S. K.; Matsushita, S.; Hirano, M.; Okada, T.; Komoto, A.; Mori, N.; Nakatsuka, M. Commercial-Scale Preparation of Biofunctional Fucoxanthin from Waste Parts of Brown Sea Algae *Laminalia Japonica*. *Food Sci. Technol. Res*. 2008, *14*(6), 573. DOI: 10.3136/fstr.14.573.
[77] Petrushkina, M.; Gusev, E.; Sorokin, B.; Zotko, N.; Mamaeva, A.; Filimonova, A.; Kulikovskiy, M.; Maltsev, Y.; Yampolsky, I.; Guglya, E., et al. Fucoxanthin Production by Heterokont Microalgae. *Algal Res*. 2017, *24*, 387. DOI: 10.1016/j.algal.2017.03.016.
[78] Kim, S. M.; Kang, S. -W.; Kwon, O. N.; Chung, D.; Pan, C. -H. Fucoxanthin as a Major Carotenoid in *Isochrysis* Aff. *Galbana*: Characterization of Extraction for Commercial Application. *J. Korean Soc. Appl. Biol. Chem*. 2012, *55*(4), 477. DOI: 10.1007/s13765-012-2108-3.
[79] Marasco, E. K.; Vay, K.; Schmidt-Dannert, C. Identification of Carotenoid Cleavage Dioxygenases from *Nostoc* Sp. PCC 7120 with Different Cleavage Activities. *J. Biol. Chem*. 2006, *281*(42), 31583. DOI: 10.1016/S0021-9258(19)84072-3.
[80] Yoon, H. S.; Hackett, J. D.; Bhattacharya, D. A Single Origin of the Peridinin- and Fucoxanthin-Containing Plastids in Dinoflagellates Through Tertiary Endosymbiosis. *Proc. National Academy Sci*. 2002, *99*(18), 11724. DOI: 10.1073/pnas.172234799.
[81] Carbonera, D.; Di Valentin, M.; Spezia, R.; Mezzetti, A. The Unique Photophysical Properties of the Peridinin-Chlorophyll-*α*-Protein. *Curr. Protein Pept. Sci*. 2014, *15*(4), 332. DOI: 10.2174/1389203715666140327111139.
[82] Sugawara, T.; Yamashita, K.; Sakai, S.; Asai, A.; Nagao, A.; Shiraishi, T.; Imai, I.; Hirata, T. Induction of Apoptosis in DLD-1 Human Colon Cancer Cells by Peridinin Isolated from the Dinoflagellate, Heterocapsa Triquetra. *Biosci. Biotechnol. Biochem*. 2007, *71*(4), 1069. DOI: 10.1271/bbb.60597.
[83] Onodera, K. -I.; Konishi, Y.; Taguchi, T.; Kiyoto, S.; Tominaga, A. Peridinin from the Marine Symbiotic Dinoflagellate, Symbiodinium Sp., Regulates Eosinophilia in Mice. *Mar. Drugs*. 2014, *12*(4), 1773. DOI: 10.3390/md12041773.
[84] Supasri, K. M.; Kumar, M.; Segečová, A.; McCauley, J. I.; Herdean, A.; Padula, M. P.; O'Meara, T.; Ralph, P. J. Characterisation and Bioactivity Analysis of Peridinin-Chlorophyll A-Protein (PCP) Isolated from *Symbiodinium Tridacnidorum* CS-73. *J. Mar. Sci. Eng*. 2021, *9*(12), 1387. DOI: 10.3390/jmse9121387.

[85] Supasri, K. M.; Kumar, M.; Mathew, M. J.; Signal, B.; Padula, M. P.; Suggett, D. J.; Ralph, P. J. Evaluation of Filter, Paramagnetic, and STAGETips Aided Workflows for Proteome Profiling of Symbiodiniaceae Dinoflagellate. *Processes*. 2021, *9*(6), 983. DOI: 10.3390/pr9060983.

[86] Wang, F.; Huang, L.; Gao, B.; Zhang, C. Optimum Production Conditions, Purification, Identification, and Antioxidant Activity of Violaxanthin from Microalga *Eustigmatos* Cf. *Polyphem (Eustigmatophyceae). Marine Drugs (Eustigmatophyceae). polyphem (Eustigmatophyceae). Marine Drugs*. 2018, *16*, 6. DOI: 10.3390/md16060190.

[87] Fu, H.; Xie, B.; Ma, S.; Zhu, X.; Fan, G.; Pan, S. Evaluation of Antioxidant Activities of Principal Carotenoids Available in Water Spinach (*Ipomoea Aquatica*). *J. Food Compost. Anal*. 2011, *24*(2), 288. DOI: 10.1016/j.jfca.2010.08.007.

[88] Soontornchaiboon, W.; Joo, S. S.; Kim, S. M. Anti-Inflammatory Effects of Violaxanthin Isolated from Microalga *Chlorella Ellipsoidea* in RAW 264.7 Macrophages. *Biol. Pharm. Bull*. 2012, *35*(7), 1137. DOI: 10.1248/bpb.b12-00187.

[89] Torregrosa-Crespo, J.; Montero, Z.; Fuentes, J. L.; Reig Garcia-Galbis, M.; Garbayo, I.; Vilchez, C.; Martinez-Espinosa, R. M. Exploring the Valuable Carotenoids for the Large-Scale Production by Marine Microorganisms. *Mar. Drugs*. 2018, *16*, 6. DOI: 10.3390/md16060203.

[90] Takemura, M.; Sahara, T.; Misawa, N. Violaxanthin: Natural Function and Occurrence, Biosynthesis, and Heterologous Production. *Appl. Microbiol. Biotechnol*. 2021, *105*(16), 6133. DOI: 10.1007/s00253-021-11452-2.

[91] Cha, K. H.; Koo, S. Y.; Lee, D. U. Antiproliferative Effects of Carotenoids Extracted from *Chlorella Ellipsoidea* and *Chlorella Vulgaris* on Human Colon Cancer Cells. *J. Agric. Food Chem*. 2008, *56*(22), 10521. DOI: 10.1021/jf802111x.

[92] Pasquet, V.; Morisset, P.; Ihammouine, S.; Chepied, A.; Aumailley, L.; Berard, J. B.; Serive, B.; Kaas, R.; Lanneluc, I.; Thiery, V., et al. Antiproliferative Activity of Violaxanthin Isolated from Bioguided Fractionation of *Dunaliella Tertiolecta* Extracts. *Mar. Drugs*. 2011, *9*(5), 819. DOI: 10.3390/md9050819.

[93] Park, S. -B.; Yun, J. -H.; Ryu, A. J.; Yun, J.; Kim, J. W.; Lee, S.; Choi, S.; Cho, D. -H.; Choi, D. -Y.; Lee, Y. J., et al. Development of a Novel *Nannochloropsis* Strain with Enhanced Violaxanthin Yield for Large□scale Production. *Microbial cell factories*. 2021, *20*(1), 43. DOI: 10.1186/s12934-021-01535-0.

[94] Krinsky, N. I.; Beecher, G.; Burk, R.; Chan, A.; Erdman, J.; Jacob, R.; Jialal, I.; Kolonel, L.; Marshall, J.; Taylor Mayne, P. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. *Institute of Medicine*. 2000, *19*, 95.

[95] Kopec, R. E.; Failla, M. L. Recent Advances in the Bioaccessibility and Bioavailability of Carotenoids and Effects of Other Dietary Lipophiles. *J. Food Compost. Anal*. 2018, *68*, 16. DOI: 10.1016/j.jfca.2017.06.008.

[96] Tyssandier, V.; Reboul, E.; Dumas, J. -F.; Bouteloup-Demange, C.; Armand, M.; Marcand, J.; Sallas, M.; Borel, P. Processing of Vegetable-Borne Carotenoids in the Human Stomach and Duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol*. 2003, *284*(6), G913. DOI: 10.1152/ajpgi.00410.2002.

[97] Mayer, C.; Côme, M.; Blanckaert, V.; Chini Zittelli, G.; Faraloni, C.; Nazih, H.; Ouguerram, K.; Mimouni, V.; Chénais, B. Effect of Carotenoids from Phaeodactylum Tricornutum on Palmitate-Treated HepG2 Cells. *Molecules*. 2020, *25*(12), 2845. DOI: 10.3390/molecules25122845.

[98] Liu, J.; Liu, D.; Bi, J.; Liu, X.; Lyu, Y.; Verkerk, R.; Dekker, M. Micelle Separation Conditions Based on Particle Size Strongly Affect Carotenoid Bioaccessibility Assessment from Juices After in vitro Digestion. *Food Res. Int*. 2022, *151*, 110891. DOI: 10.1016/j.foodres.2021.110891.

[99] Alapatt, P.; Guo, F.; Komanetsky, S. M.; Wang, S.; Cai, J.; Sargsyan, A.; Díaz, E. R.; Bacon, B. T.; Aryal, P.; Graham, T. E. Liver Retinol Transporter and Receptor for Serum Retinol-Binding Protein (RBP4). *J. Biol. Chem*. 2013, *288*(2), 1250. DOI: 10.1074/jbc.M112.369132.

[100] Bandara, S.; Ramkumar, S.; Imanishi, S.; Thomas, L. D.; Sawant, O. B.; Imanishi, Y.; von Lintig, J. Aster Proteins Mediate Carotenoid Transport in Mammalian Cells. *Proc. National Academy Sci*. 2022, *119*(15), e2200068119. DOI: 10.1073/pnas.2200068119.

[101] During, A.; Harrison, E. H. Mechanisms of Provitamin a (Carotenoid) and Vitamin a (Retinol) Transport into and Out of Intestinal Caco-2 Cells. *J. Lipid Res*. 2007, *48*(10), 2283. DOI: 10.1194/jlr.M700263-JLR200.

[102] Reboul, E. Absorption of Vitamin a and Carotenoids by the Enterocyte: Focus on Transport Proteins. *Nutrients*. 2013, *5*(9), 3563. DOI: 10.3390/nu5093563.

[103] Quadro, L.; Giordano, E.; Costabile, B. K.; Nargis, T.; Iqbal, J.; Kim, Y.; Wassef, L.; Hussain, M. M. Interplay Between β-Carotene and Lipoprotein Metabolism at the Maternal-Fetal Barrier. *Biochimica Et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2020, *1865*(11), 158591. DOI: 10.1016/j.bbalip.2019.158591.

[104] Desmarchelier, C.; Borel, P. Overview of Carotenoid Bioavailability Determinants: From Dietary Factors to Host Genetic Variations. *Trends in Food Science & Technology*. 2017, *69*, 270. DOI: 10.1016/j.tifs.2017.03.002.

[105] Lobo, G. P.; Amengual, J.; Palczewski, G.; Babino, D.; Von Lintig, J. Mammalian Carotenoid-Oxygenases: Key Players for Carotenoid Function and Homeostasis. *Biochimica Et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2012, *1821*(1), 78. DOI: 10.1016/j.bbalip.2011.04.010.

[106] Moran, N. E.; Mohn, E. S.; Hason, N.; Erdman, J. W., Jr; Johnson, E. J. Intrinsic and Extrinsic Factors Impacting Absorption, Metabolism, and Health Effects of Dietary Carotenoids. *Adv. Nutr.* 2018, *9*(4), 465. DOI: 10.1093/advances/nmy025.
[107] O'Byrne, S. M.; Wongsiriroj, N.; Libien, J.; Vogel, S.; Goldberg, I. J.; Baehr, W.; Palczewski, K.; Blaner, W. S. Retinoid Absorption and Storage is Impaired in Mice Lacking Lecithin: Retinol Acyltransferase (LRAT). *J. Biol. Chem.* 2005, *280*(42), 35647. DOI: 10.1074/jbc.M507924200.
[108] Daruwalla, A.; Kiser, P. D. Structural and Mechanistic Aspects of Carotenoid Cleavage Dioxygenases (CCDs). *Biochim Biophys Acta Mol Cell Biol Lipids.* 2020, *1865*(11), 158590. DOI: 10.1016/j.bbalip.2019.158590.
[109] Ford, N. A.; Elsen, A. C.; Erdman, J. W. Genetic Ablation of Carotene Oxygenases and Consumption of Lycopene or Tomato Powder Diets Modulate Carotenoid and Lipid Metabolism in Mice. *Nutr. Res.* 2013, *33*(9), 733. DOI: 10.1016/j.nutres.2013.07.007.
[110] Tan, H. -L.; Thomas-Ahner, J. M.; Moran, N. E.; Cooperstone, J. L.; Erdman, J. W., Jr; Young, G. S.; Clinton, S. K. β-Carotene 9′,10′ Oxygenase Modulates the Anticancer Activity of Dietary Tomato or Lycopene on Prostate Carcinogenesis in the TRAMP Model. *Cancer Prev. Res.* 2017, *10*(2), 161. DOI: 10.1158/1940-6207.CAPR-15-0402.
[111] Pérez-Gálvez, A.; Martin, H. D.; Sies, H.; Stahl, W. Incorporation of Carotenoids from Paprika Oleoresin into Human Chylomicrons. *Br. J. Nutr.* 2003, *89*(6), 787. DOI: 10.1079/BJN2003842.
[112] Breithaupt, D. E.; Weller, P.; Wolters, M.; Hahn, A. Comparison of Plasma Responses in Human Subjects After the Ingestion of 3R, 3R′-Zeaxanthin Dipalmitate from Wolfberry (Lycium Barbarum) and Non-Esterified 3R, 3R ′-Zeaxanthin Using Chiral High-Performance Liquid Chromatography. *Br. J. Nutr.* 2004, *91*(5), 707. DOI: 10.1079/BJN20041105.
[113] Breithaupt, D. E.; Wirt, U.; Bamedi, A. Differentiation Between Lutein Monoester Regioisomers and Detection of Lutein Diesters from Marigold Flowers (Tagetes Erecta L.) and Several Fruits by Liquid Chromatography– Mass Spectrometry. *J. Agric. Food Chem.* 2002, *50*(1), 66. DOI: 10.1021/jf010970l.
[114] Qin, X.; Zeevaart, J. A. The 9-Cis-Epoxycarotenoid Cleavage Reaction is the Key Regulatory Step of Abscisic Acid Biosynthesis in Water-Stressed Bean. *Proc. National Academy Sci.* 1999, *96*(26), 15354. DOI: 10.1073/pnas.96.26.15354.
[115] Chitchumroonchokchai, C.; Failla, M. L. Hydrolysis of Zeaxanthin Esters by Carboxyl Ester Lipase During Digestion Facilitates Micellarization and Uptake of the Xanthophyll by Caco-2 Human Intestinal Cells. *J. Nutr.* 2006, *136*(3), 588. DOI: 10.1093/jn/136.3.588.
[116] Failla, M. L.; Rodrigues, D. B.; Chitchumroonchokchai, C. CHAPTER 13 Bioavailability and Metabolism of Carotenoid Esters. In *Carotenoid Esters in Foods: Physical, Chemical and Biological Properties* ed. ; Mercadante, A. Z. Ed(s). ; The Royal Society of Chemistry: London, UK, 2019; pp 390. DOI: 10.1039/9781788015851-00390.
[117] Jewell, C.; O'Brien, N. M. Effect of Dietary Supplementation with Carotenoids on Xenobiotic Metabolizing Enzymes in the Liver, Lung, Kidney and Small Intestine of the Rat. *Br. J. Nutr.* 1999, *81*(3), 235. DOI: 10.1017/S0007114599000434.
[118] Gradelet, S.; Astorg, P.; Leclerc, J.; Chevalier, J.; Vernevaut, M. F.; Siess, M. H. Effects of Canthaxanthin, Astaxanthin, Lycopene and Lutein on Liver Xenobiotic-Metabolizing Enzymes in the Rat. *Xenobiotica.* 1996, *26*(1), 49. DOI: 10.3109/00498259609046688.
[119] Zheng, Y. F.; Bae, S. H.; Kwon, M. J.; Park, J. B.; Choi, H. D.; Shin, W. G.; Bae, S. K. Inhibitory Effects of Astaxanthin, β-Cryptoxanthin, Canthaxanthin, Lutein, and Zeaxanthin on Cytochrome P450 Enzyme Activities. *Food Chem. Toxicol.* 2013, *59*, 78. DOI: 10.1016/j.fct.2013.04.053.
[120] Ben-Dor, A.; Steiner, M.; Gheber, L.; Danilenko, M.; Dubi, N.; Linnewiel, K.; Zick, A.; Sharoni, Y.; Levy, J. Carotenoids Activate the Antioxidant Response Element Transcription System. *Mol. Cancer Ther.* 2005, *4*(1), 177. DOI: 10.1158/1535-7163.177.4.1.
[121] Bohn, T. Carotenoids and Markers of Oxidative Stress in Human Observational Studies and Intervention Trials: Implications for Chronic Diseases. *Antioxidants.* 2019, *8*(6), 179. DOI: 10.3390/antiox8060179.
[122] Do Nascimento, T. C.; Pinheiro, P. N.; Fernandes, A. S.; Murador, D. C.; Neves, B. V.; de Menezes, C. R.; de Rosso, V. V.; Jacob-Lopes, E.; Zepka, L. Q. Bioaccessibility and Intestinal Uptake of Carotenoids from Microalgae *Scenedesmus Obliquus*. *Learning with Technologies.* 2021, *140*, 110780. DOI: 10.1016/j.lwt.2020.110780.
[123] Page, S. W.; Maddison, J. E. Chapter 1 - Principles of Clinical Pharmacology. In *Small Animal Clinical Pharmacology*, 2nd ed.; Maddison, J.E., Page, S.W. and Church, D.B., Eds.; Edinburgh: W.B. Saunders, 2008; p. 1.
[124] Carbonell-Capella, J. M.; Buniowska, M.; Barba, F. J.; Esteve, M. J.; Frígola, A. Analytical Methods for Determining Bioavailability and Bioaccessibility of Bioactive Compounds from Fruits and Vegetables: A Review. *Compr. Rev. Food Sci. Food Saf.* 2014, *13*(2), 155. DOI: 10.1111/1541-4337.12049.
[125] Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Bohn, T.; Bourlieu, C.; Carrière, F.; Boutrou, R.; Corredig, M.; Dupont, D. A Standardised Static in vitro Digestion Method Suitable for Food–An International Consensus. *Food & Function.* 2014, *5*(6), 1113. DOI: 10.1039/C3FO60702J.
[126] Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F. INFOGEST Static in vitro Simulation of Gastrointestinal Food Digestion. *Nat. Protoc.* 2019, *14*(4), 991. DOI: 10.1038/s41596-018-0119-1.

[127] Rodrigues, D. B.; Mariutti, L. R. B.; Mercadante, A. Z. An in vitro Digestion Method Adapted for Carotenoids and Carotenoid Esters: Moving Forward Towards Standardization. *Food & Function*. 2016, *7*(12), 4992. DOI: 10.1039/C6FO01293K.
[128] Cha, K. H.; Koo, S. Y.; Song, D. G.; Pan, C. H. Effect of Microfluidization on Bioaccessibility of Carotenoids from *Chlorella Ellipsoidea* During Simulated Digestion. *J. Agric. Food Chem*. 2012, *60*(37), 9437. DOI: 10.1021/jf303207x.
[129] Gille, A.; Hollenbach, R.; Trautmann, A.; Posten, C.; Briviba, K. Effect of Sonication on Bioaccessibility and Cellular Uptake of Carotenoids from Preparations of Photoautotrophic *Phaeodactylum Tricornutum*. *Food Res. Int*. 2019, *118*, 40. DOI: 10.1016/j.foodres.2017.12.040.
[130] Bernaerts, T. M. M.; Verstreken, H.; Dejonghe, C.; Gheysen, L.; Foubert, I.; Grauwet, T.; Van Loey, A. M. Cell Disruption of *Nannochloropsis* Sp. Improves *In Vitro* Bioaccessibility of Carotenoids and ω3-LC-PUFA. *J. Funct. Foods*. 2020, *65*, 103770. DOI: 10.1016/j.jff.2019.103770.
[131] Viera, I.; Pérez-Gálvez, A.; Roca, M. Bioaccessibility of Marine Carotenoids. *Mar. Drugs*. 2018, *16*, 10. DOI: 10.3390/md16100397.
[132] Granado-Lorencio, F.; Herrero-Barbudo, C.; Acién-Fernández, G.; Molina-Grima, E.; Fernández-Sevilla, J. M.; Pérez-Sacristán, B.; Blanco-Navarro, I. *In Vitro* Bioaccesibility of Lutein and Zeaxanthin from the Microalgae *Scenedesmus Almeriensis*. *Food Chem*. 2009, *114*(2), 747. DOI: 10.1016/j.foodchem.2008.10.058.
[133] Akepach, P.; Ribeiro-Filho, N.; Wattanakul, J.; Darwish, R.; Gedi, M. A.; Gray, D. A. Bioaccessibility of Carotenoids (β-Carotene and Lutein) from Intact and Disrupted Microalgae (*Chlamydomonas Reinhardtii*). *LWT*. 2022, *160*, 113292. DOI: 10.1016/j.lwt.2022.113292.
[134] Guo, B.; Oliviero, T.; Fogliano, V.; Ma, Y.; Chen, F.; Capuano, E. Gastrointestinal Bioaccessibility and Colonic Fermentation of Fucoxanthin from the Extract of the Microalga *Nitzschia Laevis*. *J. Agric. Food Chem*. 2020, *68*(7), 1844. DOI: 10.1021/acs.jafc.9b02496.
[135] Huang, Z.; Xu, L.; Zhu, X.; Hu, J.; Peng, H.; Zeng, Z.; Xiong, H. Stability and Bioaccessibility of Fucoxanthin in *Nanoemulsions* Prepared from Pinolenic Acid-Contained Structured Lipid. *Int. J. Food Eng*. 2017, *13*, 1. DOI: 10.1515/ijfe-2016-0273.
[136] Fernandes, A. S.; Nascimento, T. C.; Pinheiro, P. N.; Vendruscolo, R. G.; Wagner, R.; de Rosso, V. V.; Jacob-Lopes, E.; Zepka, L. Q. Bioaccessibility of Microalgae-Based Carotenoids and Their Association with the Lipid Matrix. *Food Res. Int*. 2021, *148*, 110596. DOI: 10.1016/j.foodres.2021.110596.
[137] Tudor, C.; Pintea, A. A Brief Overview of Dietary Zeaxanthin Occurrence and Bioaccessibility. *Molecules*. 2020, *25*, 18. DOI: 10.3390/molecules25184067.
[138] Wang, L.; Liu, Z.; Jiang, H.; Mao, X. Biotechnology Advances in β-Carotene Production by Microorganisms. *Trends in Food Sci. Technol*. 2021, *111*, 322. DOI: 10.1016/j.tifs.2021.02.077.
[139] Yang, C.; Fischer, M.; Kirby, C.; Liu, R.; Zhu, H.; Zhang, H.; Chen, Y.; Sun, Y.; Zhang, L.; Tsao, R. Bioaccessibility, Cellular Uptake and Transport of Luteins and Assessment of Their Antioxidant Activities. *Food Chem*. 2018, *249*, 66. DOI: 10.1016/j.foodchem.2017.12.055.
[140] Odeberg, J. M.; Lignell, Å.; Pettersson, A.; Höglund, P. Oral Bioavailability of the Antioxidant Astaxanthin in Humans is Enhanced by Incorporation of Lipid Based Formulations. *Eur. J. Pharm. Sci*. 2003, *19*(4), 299. DOI: 10.1016/S0928-0987(03)00135-0.
[141] Tan, C.; Zhang, Y.; Abbas, S.; Feng, B.; Zhang, X.; Xia, S. Modulation of the Carotenoid Bioaccessibility Through Liposomal Encapsulation. *Colloids and Surfaces B: Biointerfaces*. 2014, *123*, 692. DOI: 10.1016/j.colsurfb.2014.10.011.
[142] Llansola-Portoles, M. J.; Litvin, R.; Ilioaia, C.; Pascal, A. A.; Bina, D.; Robert, B. Pigment Structure in the Violaxanthin–chlorophyll-A-Binding Protein VCP. *Photosynth. Res*. 2017, *134*(1), 51. DOI: 10.1007/s11120-017-0407-6.
[143] Rodríguez-Roque, M. J.; Sánchez-Vega, R.; Aguiló-Aguayo, I.; Medina-Antillón, A. E.; Soto-Caballero, M. C.; Salas-Salazar, N. A.; Valdivia-Nájar, C. G. Chapter 12 - Bioaccessibility and Bioavailability of Bioactive Compounds Delivered from Microalgae. In *Cultured Microalgae for the Food Industry*; Lafarga, T. and Acién, G., Eds.; Academic Press: Cambridge, MA, USA, 2021; p. 325.
[144] Mashurabad, P. C.; Palika, R.; Jyrwa, Y. W.; Bhaskarachary, K.; Pullakhandam, R. Dietary Fat Composition, Food Matrix and Relative Polarity Modulate the Micellarization and Intestinal Uptake of Carotenoids from Vegetables and Fruits. *J. Food Sci. Technol*. 2017, *54*(2), 333. DOI: 10.1007/s13197-016-2466-7.
[145] Jayarajan, P.; Reddy, V.; Mohanram, M. Effect of Dietary Fat on Absorption of β Carotene from Green Leafy Vegetables in Children. *Indian J. Med. Res*. 2013, *137*, 5.
[146] van Het Hof, K. H.; West, C. E.; Weststrate, J. A.; Hautvast, J. G. A. J. Dietary Factors That Affect the Bioavailability of Carotenoids. *J. Nutr*. 2000, *130*(3), 503. DOI: 10.1093/jn/130.3.503.
[147] Failla, M. L.; Chitchumronchokchai, C.; Ferruzzi, M. G.; Goltz, S. R.; Campbell, W. W. Unsaturated Fatty Acids Promote Bioaccessibility and Basolateral Secretion of Carotenoids and α-Tocopherol by Caco-2 Cells. *Food Function*. 2014, *5*(6), 1101. DOI: 10.1039/C3FO60599J.
[148] Reboul, E. Mechanisms of Carotenoid Intestinal Absorption: Where Do We Stand? *Nutrients*. 2019, *11*, 4. DOI: 10.3390/nu11040838.

[149] Broekmans, W. M. R.; Klöpping-Ketelaars, I. A. A.; Weststrate, J. A.; Tijburg, L. B. M.; van Poppel, G.; Vink, A. A.; Berendschot, T. T. J. M.; Bots, M. L.; Castenmiller, W. A. M.; Kardinaal, A. F. M. Decreased Carotenoid Concentrations Due to Dietary Sucrose Polyesters Do Not Affect Possible Markers of Disease Risk in Humans. *J. Nutr.* 2003, *133*(3), 720. DOI: 10.1093/jn/133.3.720.

[150] Tyssandier, V.; Lyan, B.; Borel, P. Main Factors Governing the Transfer of Carotenoids from Emulsion Lipid Droplets to Micelles. *Biochimica Et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2001, *1533*(3), 285. DOI: 10.1016/S1388-1981(01)00163-9.

[151] Goltz, S. R.; Ferruzzi, M. G. Carotenoid Bioavailability: Influence of Dietary Lipid and Fiber. In *Carotenoids and Human Health*; Tanumihardjo, S.A., Ed.; Humana Press: Totowa, NJ, 2013; p. 111.

[152] Canene-Adams, K.; Erdman, J. W. Absorption, Transport, Distribution in Tissues and Bioavailability. In *Carotenoids* eds.; George, B., Pfander, H., Liaaen-Jensen, S. Ed(s).; Birkhauser Verlag: Basel (Switzerland), 2009; pp 115.

[153] Yonekura, L.; Nagao, A. Soluble Fibers Inhibit Carotenoid Micellization in vitro and Uptake by Caco-2 Cells. *Biosci. Biotechnol. Biochem.* 2009, *73*(1), 196. DOI: 10.1271/bbb.80510.

[154] Soukoulis, C.; Tsevdou, M.; Yonekura, L.; Cambier, S.; Taoukis, P. S.; Hoffmann, L. Does Kappa-Carrageenan Thermoreversible Gelation Affect β-Carotene Oxidative Degradation and Bioaccessibility in O/W Emulsions? *Carbohydr. Polym.* 2017, *167*, 259. DOI: 10.1016/j.carbpol.2017.03.007.

[155] Corte-Real, J.; Bertucci, M.; Soukoulis, C.; Desmarchelier, C.; Borel, P.; Richling, E.; Hoffmann, L.; Bohn, T. Negative Effects of Divalent Mineral Cations on the Bioaccessibility of Carotenoids from Plant Food Matrices and Related Physical Properties of Gastro-Intestinal Fluids. *Food & Function*. 2017, *8*(3), 1008. DOI: 10.1039/C6FO01708H.

[156] Biehler, E.; Hoffmann, L.; Krause, E.; Bohn, T. Divalent Minerals Decrease Micellarization and Uptake of Carotenoids and Digestion Products into Caco-2 Cells. *J. Nutr.* 2011, *141*(10), 1769. DOI: 10.3945/jn.111.143388.

[157] Yang, L.; Tucker, I. G.; Østergaard, J. Effects of Bile Salts on Propranolol Distribution into Liposomes Studied by Capillary Electrophoresis. *J. Pharm. Biomed. Anal.* 2011, *56*(3), 553. DOI: 10.1016/j.jpba.2011.06.020.

[158] Kim, S. K.; Chojnacka, K., *Microalgal Carotenoids: Bioactive Roles, Health Foods, and Pharmaceuticals. Marine Algae Extracts: Processes, Products, and Applications*. 2015;

[159] Sugawara, T.; Matsubara, K.; Akagi, R.; Mori, M.; Hirata, T. Anti-Angiogenic Activity of Brown Algae Fucoxanthin and Its Deacetylated Product, Fucoxanthinol. *J. Agric. Food Chem.* 2006, *54*(26), 9805. DOI: 10.1021/jf062204q.

[160] Ganesan, P.; Matsubara, K.; Sugawara, T.; Hirata, T. Marine Algal Carotenoids Inhibit Angiogenesis by Down-Regulating FGF-2-Mediated Intracellular Signals in Vascular Endothelial Cells. *Mol. Cell. Biochem.* 2013, *380*(1–2), 1. DOI: 10.1007/s11010-013-1651-5.

[161] Liu, C. L.; Huang, Y. S.; Hosokawa, M.; Miyashita, K.; Hu, M. L. Inhibition of Proliferation of a Hepatoma Cell Line by Fucoxanthin in Relation to Cell Cycle Arrest and Enhanced Gap Junctional Intercellular Communication. *Chem.-Biol. Interact.* 2009, *182*(2–3), 165. DOI: 10.1016/j.cbi.2009.08.017.

[162] Heo, S. -J.; Yoon, W. -J.; Kim, K. -N.; Oh, C.; Choi, Y. -U.; Yoon, K. -T.; Kang, D. -H.; Qian, Z. -J.; Choi, I. -W.; Jung, W. -K. Anti-Inflammatory Effect of Fucoxanthin Derivatives Isolated from *Sargassum Siliquastrum* in Lipopolysaccharide-Stimulated RAW 264.7 Macrophage. *Food Chem. Toxicol.* 2012, *50*(9), 3336. DOI: 10.1016/j.fct.2012.06.025.

[163] Rodríguez-Luna, A.; Ávila-Román, J.; González-Rodríguez, M. L.; Cózar, M. J.; Rabasco, A. M.; Motilva, V.; Talero, E. Fucoxanthin-Containing Cream Prevents Epidermal Hyperplasia and UVB-Induced Skin Erythema in Mice. *Mar. Drugs*. 2018, *16*(10), 378. DOI: 10.3390/md16100378.

[164] Kawee-Ai, A.; Kuntiya, A.; Kim, S. M. Anticholinesterase and Antioxidant Activities of Fucoxanthin Purified from the Microalga *Phaeodactylum Tricornutum*. *Nat. Prod. Commun.* 2013, *8*(10), 1381. DOI: 10.1177/1934578X1300801010.

[165] Chang, P. M.; Li, K. L.; Lin, Y. C. Fucoidan– Fucoxanthin Ameliorated Cardiac Function via IRS1/GRB2/SOS1, GSK3β/CREB Pathways and Metabolic Pathways in Senescent Mice. *Mar. Drugs*. 2019, *17*, 1. DOI: 10.3390/md17010069.

[166] Radzun, K. A.; Rusmidi, M. H. H.; Norisam, I.; Iran, N.; Pardi, F.; Ismail, A.; Razak, W.; Hafid, S. R. A. Anti-Inflammatory Effects of Astaxanthin Extracted from Microalgae *Hematococcus Pluvialis* and Combinations with Palm Tocotrienol Rich-Fraction in RAW 264.7 Macrophages. *Pharmacogn. J.* 2022, *14*, 1. DOI: 10.5530/pj.2022.14.26.

[167] Hwang, S. H.; Kim, J. M.; Kim, S.; Yoon, M. J.; Park, K. S. Chemical Transformation of Astaxanthin from *Haematococcus Pluvialis* Improves Its Antioxidative and Anti-Inflammatory Activities. *ACS Omega*. 2020, *5*(30), 19120. DOI: 10.1021/acsomega.0c02479.

[168] Rao, A. R.; Sarada, R.; Shylaja, M. D.; Ravishankar, G. A. Evaluation of Hepatoprotective and Antioxidant Activity of Astaxanthin and Astaxanthin Esters from Microalga-*Haematococcus Pluvialis*. *J. Food Sci. Technol.* 2015, *52*(10), 6703. DOI: 10.1007/s13197-015-1775-6.

[169] Murthy, K. N.; Rajesha, J.; Swamy, M. M.; Ravishankar, G. A. Comparative Evaluation of Hepatoprotective Activity of Carotenoids of Microalgae. *J. Med. Food*. 2005, *8*(4), 523. DOI: 10.1089/jmf.2005.8.523.

[170] Zhang, X.; Pan, L.; Wei, X.; Gao, H.; Liu, J. Impact of Astaxanthin-Enriched Algal Powder of *Haematococcus Pluvialis* on Memory Improvement in BALB/C Mice. *Environ. Geochem. Health*. 2007, *29*(6), 483. DOI: 10.1007/s10653-007-9117-x.
[171] Fleischmann, C.; Shohami, E.; Trembovler, V.; Heled, Y.; Horowitz, M. Cognitive Effects of Astaxanthin Pretreatment on Recovery from Traumatic Brain Injury. *Front. neurol*. 2020, *11*. DOI: 10.3389/fneur.2020.00999.
[172] Lin, K. H.; Lin, K. C.; Lu, W. J.; Thomas, P. A.; Jayakumar, T.; Sheu, J. R. Astaxanthin, a Carotenoid, Stimulates Immune Responses by Enhancing IFN-γ and IL-2 Secretion in Primary Cultured Lymphocytes *In Vitro* and *Ex Vivo*. *Int. J. Mol. Sci*. 2015, *17*, 1. DOI: 10.3390/ijms17010044.
[173] Hussein, G.; Nakamura, M.; Zhao, Q.; Iguchi, T.; Goto, H.; Sankawa, U.; Watanabe, H. Antihypertensive and Neuroprotective Effects of Astaxanthin in Experimental Animals. *Biol. Pharm. Bull*. 2005, *28*(1), 47. DOI: 10.1248/bpb.28.47.
[174] Weinrich, T.; Xu, Y.; Wosu, C.; Harvey, P. J.; Jeffery, G. Mitochondrial Function, Mobility and Lifespan are Improved in Drosophila Melanogaster by Extracts of 9-Cis-β-Carotene from *Dunaliella Salina*. *Mar. Drugs*. 2019, *17*(5), 279. DOI: 10.3390/md17050279.
[175] Miranda, M. S.; Cintra, R. G.; Barros, S. B.; Mancini Filho, J. Antioxidant Activity of the Microalga *Spirulina Maxima*. *Braz. J. Med. Biol. Res*. 1998, *31*(8), 1075. DOI: 10.1590/S0100-879X1998000800007.
[176] El-Baz, F. K.; Salama, A. A. A.; Hussein, R. A. *Dunaliella Salina* Microalgae Oppose Thioacetamide-Induced Hepatic Fibrosis in Rats. *Toxicology Reports*. 2020, *7*, 36. DOI: 10.1016/j.toxrep.2019.10.017.
[177] Hickenbottom, S. J.; Follett, J. R.; Lin, Y.; Dueker, S. R.; Burri, B. J.; Neidlinger, T. R.; Clifford, A. J. Variability in Conversion of β-Carotene to Vitamin a in Men as Measured by Using a Double-Tracer Study Design. *Am. J. Clin. Nutr*. 2002, *75*(5), 900. DOI: 10.1093/ajcn/75.5.900.
[178] NIH. Lutein + Zeaxanthin and Omega-3 Fatty Acids for Age-Related Macular Degeneration: The Age-Related Eye Disease Study 2 (AREDS2) Randomized Clinical Trial. *JAMA*. 2013, *309*(19), 2005. doi:10.1001/jama.2013.4997.
[179] Qiao, Y. Q.; Jiang, P. F.; Gao, Y. Z. Lutein Prevents Osteoarthritis Through Nrf2 Activation and Downregulation of Inflammation. *Arch. Med. Sci*. 2018, *14*(3), 617. DOI: 10.5114/aoms.2016.59871.
[180] Uotsu, N.; Shen, M.; Yazawa, K.; Teramoto, S. Suppression of Arthritis Progression with Lutein Extracted from Tagetes Erecta in Collagen-Induced Arthritis Model Rats. *Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry (Under Re-Organization)*. 2016, *16*(2), 134. DOI: 10.2174/1871522216666161014155337.
[181] Rengarajan, T.; Rajendran, P.; Nandakumar, N.; Periyasamy Balasubramanian, M.; Nishigaki, I. Cancer Preventive Efficacy of Marine Carotenoid Fucoxanthin: Cell Cycle Arrest and Apoptosis. *Nutrients*. 2013, *5*(12), 4978. DOI: 10.3390/nu5124978.
[182] Jang, H.; Choi, J.; Park, J. -K.; Won, G.; Seol, J. -W. Fucoxanthin Exerts Anti-Tumor Activity on Canine Mammary Tumor Cells via Tumor Cell Apoptosis Induction and Angiogenesis Inhibition. *Animals*. 2021, *11*(6), 1512. DOI: 10.3390/ani11061512.
[183] Giannotta, M.; Trani, M.; Dejana, E. VE-Cadherin and Endothelial Adherens Junctions: Active Guardians of Vascular Integrity. *Dev. Cell*. 2013, *26*(5), 441. DOI: 10.1016/j.devcel.2013.08.020.
[184] Li, S.; Ren, X.; Wang, Y.; Hu, J.; Wu, H.; Song, S.; Yan, C. Fucoxanthin Alleviates Palmitate-Induced Inflammation in RAW 264.7 Cells Through Improving Lipid Metabolism and Attenuating Mitochondrial Dysfunction. *Food & Function*. 2020, *11*(4), 3361. DOI: 10.1039/D0FO00442A.
[185] Lee, A.; Shin, H. -Y.; Park, J. -H.; Koo, S. Y.; Kim, S. M.; Yang, S. -H. Fucoxanthin from Microalgae Phaeodactylum Tricornutum Inhibits Pro-Inflammatory Cytokines by Regulating Both NF-κB and NLRP3 Inflammasome Activation. *Sci. Rep*. 2021, *11*(1), 1. DOI: 10.1038/s41598-020-80748-6.
[186] Kim, K. -N.; Heo, S. -J.; Yoon, W. -J.; Kang, S. -M.; Ahn, G.; Yi, T. -H.; Jeon, Y. -J. Fucoxanthin Inhibits the Inflammatory Response by Suppressing the Activation of NF-κB and MAPKs in Lipopolysaccharide-Induced RAW 264.7 Macrophages. *Eur. J. Pharmacol*. 2010, *649*(1–3), 369. DOI: 10.1016/j.ejphar.2010.09.032.
[187] Su, J.; Guo, K.; Huang, M.; Liu, Y.; Zhang, J.; Sun, L.; Li, D.; Pang, K. -L.; Wang, G.; Chen, L. Fucoxanthin, a Marine Xanthophyll Isolated from Conticribra Weissflogii ND-8: Preventive Anti-Inflammatory Effect in a Mouse Model of Sepsis. *Front. Pharmacol*. 2019, *10*, 906. DOI: 10.3389/fphar.2019.00906.
[188] Pangestuti, R.; Vo, T. -S.; Ngo, D. -H.; Kim, S. -K. Fucoxanthin Ameliorates Inflammation and Oxidative Responses in Microglia. *J. Agric. Food Chem*. 2013, *61*(16), 3876. DOI: 10.1021/jf400015k.
[189] Lin, J.; Huang, L.; Yu, J.; Xiang, S.; Wang, J.; Zhang, J.; Yan, X.; Cui, W.; He, S.; Wang, Q. Fucoxanthin, a Marine Carotenoid, Reverses Scopolamine-Induced Cognitive Impairments in Mice and Inhibits Acetylcholinesterase in vitro. *Mar. Drugs*. 2016, *14*(4), 67. DOI: 10.3390/md14040067.
[190] Kawee-Ai, A.; Kuntiya, A.; Kim, S. M. Anticholinesterase and Antioxidant Activities of Fucoxanthin Purified from the Microalga Phaeodactylum Tricornutum. *Nat. prod. communicat*. 2013, *8*(10), 1934578X1300801010. DOI: 10.1177/1934578X1300801010.
[191] Chang, P. M.; Li, K. L.; Lin, Y. C. Fucoidan-Fucoxanthin Ameliorated Cardiac Function via IRS1/GRB2/SOS1, GSK3β/CREB Pathways and Metabolic Pathways in Senescent Mice. *Mar. Drugs*. 2019, *17*, 1. DOI: 10.3390/md17010069.

[192] Khaw, Y. S.; Yusoff, F. M.; Tan, H. T.; Noor Mazli, N. A. I.; Nazarudin, M. F.; Shaharuddin, N. A.; Omar, A. R. The Critical Studies of Fucoxanthin Research Trends from 1928 to June 2021: A Bibliometric Review. *Mar. Drugs*. 2021, *19*(11), 606. DOI: 10.3390/md19110606.

[193] Maeda, H.; Hosokawa, M.; Sashima, T.; Murakami-Funayama, K.; Miyashita, K. Anti-Obesity and Anti-Diabetic Effects of Fucoxanthin on Diet-Induced Obesity Conditions in a Murine Model. *Mol. Med. Rep*. 2009, *2*(6), 897. DOI: 10.3892/mmr_00000189.

[194] Urikura, I.; Sugawara, T.; Hirata, T. Protective Effect of Fucoxanthin Against UVB-Induced Skin Photoaging in Hairless Mice. *Biosci. Biotechnol. Biochem*. 2011, *75*(4), 757. DOI: 10.1271/bbb.110040.

[195] Zhang, L.; Wang, H.; Fan, Y.; Gao, Y.; Li, X.; Hu, Z.; Ding, K.; Wang, Y.; Wang, X. Fucoxanthin Provides Neuroprotection in Models of Traumatic Brain Injury via the Nrf2-ARE and Nrf2-Autophagy Pathways. *Sci. Rep*. 2017, *7*(1), 46763. DOI: 10.1038/srep46763.

[196] YuanPing, Y.; QiaoYun, T.; ShiHua, Z.; MingDong, Z.; YouMeng, Z.; TingTing, Z. Anti-Inflammatory Effect of Fucoxanthin on Dextran Sulfate Sodium-Induced Colitis in Mice. *Nat. Prod. Res*. 2020, *34*(12), 1791. DOI: 10.1080/14786419.2018.1528593.

[197] Ha, Y. J.; Choi, Y. S.; Oh, Y. R.; Kang, E. H.; Khang, G.; Park, Y. B.; Lee, Y. J. Fucoxanthin Suppresses Osteoclastogenesis via Modulation of MAP Kinase and Nrf2 Signaling. *Mar. Drugs*. 2021, *19*, 3. DOI: 10.3390/md19030132.

[198] Riccioni, G.; D'Orazio, N.; Franceschelli, S.; Speranza, L. Marine Carotenoids and Cardiovascular Risk Markers. *Mar. Drugs*. 2011, *9*(7), 1166. DOI: 10.3390/md9071166.

[199] Islam, M. A.; Al Mamun, M. A.; Faruk, M.; Ul Islam, M. T.; Rahman, M. M.; Alam, M. N.; Rahman, A.; Reza, H. M.; Alam, M. A. Astaxanthin Ameliorates Hepatic Damage and Oxidative Stress in Carbon Tetrachloride-Administered Rats. *Pharmacognosy. res*. 2017, *9*(Suppl 1), S84. DOI: 10.4103/pr.pr_26_17.

[200] Chiu, C. -H.; Chang, C. -C.; Lin, S. -T.; Chyau, C. -C.; Peng, R. Y. Improved Hepatoprotective Effect of Liposome-Encapsulated Astaxanthin in Lipopolysaccharide-Induced Acute Hepatotoxicity. *Int. J. Mol. Sci*. 2016, *17*(7), 1128. DOI: 10.3390/ijms17071128.

[201] Park, J. S.; Chyun, J. H.; Kim, Y. K.; Line, L. L.; Chew, B. P. Astaxanthin Decreased Oxidative Stress and Inflammation and Enhanced Immune Response in Humans. *Nutri. Metab*. 2010, *7*(1), 18. DOI: 10.1186/1743-7075-7-18.

[202] Maci, S.; Santos, R. The Beneficial Role of Lutein and Zeaxanthin in Cataracts. *Nutrafoods*. 2015, *14*, 63–69. DOI: 10.1007/s13749-015-0014-0.

[203] Schnebelen-Berthier, C.; Acar, N.; Simon, E.; Thabuis, C.; Bourdillon, A.; Mathiaud, A.; Dauchet, L.; Delcourt, C.; Benlian, P.; Crochet, M., et al. The ALGOVUE Clinical Trial: Effects of the Daily Consumption of Eggs Enriched with Lutein and Docosahexaenoic Acid on Plasma Composition and Macular Pigment Optical Density. *Nutrients*. 2021, *13*(10), 3347. DOI: 10.3390/nu13103347.

[204] Qiao, Y. Q.; Jiang, P. F.; Gao, Y. Z. Lutein Prevents Osteoarthritis Through Nrf2 Activation and Downregulation of Inflammation. *Archives of Medical Science*. 2018, *14*(3), 617. DOI: 10.5114/aoms.2016.59871.

[205] Kim, J. E.; Clark, R. M.; Park, Y.; Lee, J.; Fernandez, M. L. Lutein Decreases Oxidative Stress and Inflammation in Liver and Eyes of Guinea Pigs Fed a Hypercholesterolemic Diet. *Nutr. Res. Pract*. 2012, *6*(2), 113. DOI: 10.4162/nrp.2012.6.2.113.

[206] Chu, W. L. Biotechnological Applications of Microalgae. *International E-Journal of Science, Medicine & Education*, *international E-Journal of Science, Medicine & Education*. 2012, *6*(Suppl 1), 24. DOI: 10.56026/imu.6.Suppl1.S24.

[207] Anis Hamizah, H.; Noh, N. H.; Jamaludin, M. A.; Othman, R.; Fadzillah, N. A. Determination of Natural Carotenoid Pigments from Freshwater Green Algae as Potential Halal Food Colorants. *Int. Food Res. J*, *24* S468–S471, 2017.

[208] Cezare-Gomes, E. A.; Mejia-da-Silva, L. D. C.; Perez-Mora, L. S.; Matsudo, M. C.; Ferreira-Camargo, L. S.; Singh, A. K.; de Carvalho, J. C. M. Potential of Microalgae Carotenoids for Industrial Application. *Appl. Biochem. Biotechnol*. 2019, *188*(3), 602. DOI: 10.1007/s12010-018-02945-4.

[209] Novoveská, L.; Ross, M. E.; Stanley, M. S.; Pradelles, R.; Wasiolek, V.; Sassi, J. F. Microalgal Carotenoids: A Review of Production, Current Markets, Regulations, and Future Direction. *Mar. Drugs*. 2019, *17*(11), 640. DOI: 10.3390/md17110640.

[210] Kroll, H.; Friedrich, J.; Menzel, M.; Schreier, P. Carbon and Hydrogen Stable Isotope Ratios of Carotenoids and β-Carotene-Based Dietary Supplements. *J. Agric. Food Chem*. 2008, *56*(11), 4198. DOI: 10.1021/jf8002575.