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REVIEW

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Recent advances of natural pigments from algae

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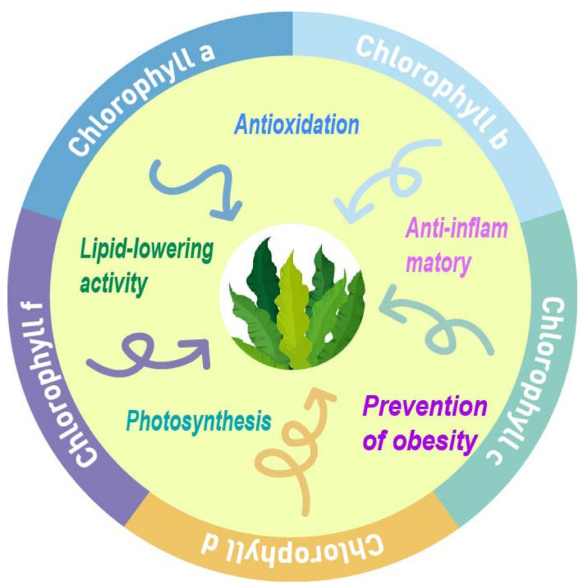
Abstract
Pigment is an important food additive that plays a major role in the sensory impact of food. And natural sources, healthy and non-toxic edible pigments are receiving a lot of attention. Algae is an important source of natural pigments, and contain chlorophyll, phycoerythrin, carotene, and other natural pigments. Besides staining, the pigment also has powerful physiological activities such as antioxidants, anti-inflammatory, anti-obesity, and lipid-lowering. In this paper, three pigments in seaweed were reviewed, and their main structural properties and functions are presented, filling the gap in the review of pigments with seaweed as the main object of introduction. This review provides research basis for the development of new health foods, a new direction for the use of seaweed chlorophyll in the food and pharmaceutical industries.

Keywords Algal pigment, Chlorophyll, Phycobiliprotein, Carotenoid

Graphical Abstract



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Introduction

Algae are a kind of heterogeneous flora with a long fossil history and are widely distributed in various areas of the earth. According to statistics, there are about 10, 000 species of algae in the world (Mac Monagail et al. 2017), and about 291 species are used by people (White & Wilson 2015). In recent years, with the development and utilization of marine resources, various components functions of marine algae have been explored by researchers. Marine algae are rich in active substances such as protein, carbohydrates, fiber, microorganisms, amino acids, and fatty acids, which have a positive effect on the human body. Both nutrient-rich seaweeds themselves and seaweed extracts with various bioactivities are becoming new materials for the development of functional and nutritional health foods (Ścieszka & Klewicka 2019; Charoensiddhi et al. 2020). For example, it can be used to produce foods rich in polyunsaturated fat, providing a healthy fat source (Marques et al. 2021). The rich functional active ingredients in algae are well known for their anti-cancer (Ouyang et al. 2021), anti-inflammatory (Olsthoorn et al. 2021), antibacterial (Karpiński & Adamczak 2019), preventive and therapeutic effects on diabetes (Lin et al. 2018), and other positive effects on living organisms (Zhao et al. 2020). These results provide data support and a theoretical basis for developing natural medicines for the prevention and treatment of diabetes and other diseases (Yan et al. 2019).

Algae are rich in pigments, which consist of three main species, chlorophylls, phycobiliprotein, and carotenoids. Different algae have different pigments and appear in different colors. Biologists classify algae into Chlorophyta, Rhodophyta, and Phaeophyta based on their color. Chlorophyta appears green because it contains a large amount of chlorophyll a, chlorophyll b, and part of carotene and lutein (Baweja et al. 2016). The pigment ratio of algae is similar to that of seed plants. Common Chlorophyta includes *Ulva*, *Spirogyra*, etc. Rhodophyta contains a large number of phycoerythrin and phycocyanin (Baweja et al. 2016), generally dominant in the number of phycoerythrins, hence the red or purplish red color of most Rhodophyta. *Porphyra haitanensis* and *Gelidium amansii* belong to the Rhodophyta, and are commonly used in the processing of food. The yellow-brown or dark brown algae are Phaeophyta, which contains chlorophyll a, chlorophyll c, carotene, and several kinds of lutein, mainly fucoxanthin (Baweja et al. 2016). A large amount of yellow pigment will cover the green so that they will have a different color from the other two types of algae. The pigment is widely used in the food industry, and is generally divided into natural pigments and synthetic pigments. Synthetic pigments are currently the most widely used pigments in the food industry. Although they have various types and good colorability advantages, their safety is still controversial (Shanmugasundaram & Rujaswini 2019). Therefore, there is still a great demand for natural pigments. Seaweed is not only rich in pigments but also diverse enough to meet the industrial coloring needs to some extent. With the increasing interest in seaweed in recent years, the utilization of seaweed pigments has gradually entered the public's vision.

There are many kinds of research on natural pigments, but few are basically free to describe the natural pigments of algae from the perspective of algae. At present, there is an increasing number of research on algal pigments (Fig. 1). In this paper, three pigments from algae are introduced, and their structures and physiological activities are also described.

Chlorophyll

Chlorophyll is a family of fat pigments that includes chlorophyll a, chlorophyll b, chlorophyll c, chlorophyll d, protochlorophyllide, etc. (Fig. 2). It can reflect green light well, so its overall green appearance, and it plays a central role in the light absorption process of photosynthesis. Chlorophyll is an unstable magnesium porphyrin compound with poor water solubility and high solubility in organic solvents, and is easily decomposed by light, acid, base, and oxidants. To make chlorophyll's green color persist during food processing, food developers, in order to maintain the stability of chlorophyll, often use chlorophyllide, a derivative of chlorophyll (Bednarczyk et al. 2021), or by adding sodium caseinate (He et al. 2019), antioxidants, or replace magnesium ions in the chlorophyll structure by using zinc and copper plasma. As an essential natural green pigment, chlorophyll has no toxic side effects (Solymosi & Mysliwa-Kurdziel 2017). With the maturity of green protection technology year by year, chlorophyll has become more and more widely used and favored by food processing (Solymosi & Mysliwa-Kurdziel 2017). In the food industry, it is often used to color cakes and beverages, or to protect the color of canned vegetables and fruits (Yasuda & Tabata 2021).

Chlorophyll is the most common pigment in algae, and the chlorophyll contained in algae mainly includes a, b, c, d, and f (Chen et al. 2017). The main food-related ones are chlorophyll a and b, both of which can be found and extracted in algae, especially green algae. Among them, *Spirulina* is one of the plants with the highest chlorophyll a content, even up to 2–3 times the content of other plants, and is considered a good source of chlorophyll a. Chlorophyll a extracted from *Spirulina* interacts with its C-phycocyanin and prevents cell death through BDNF

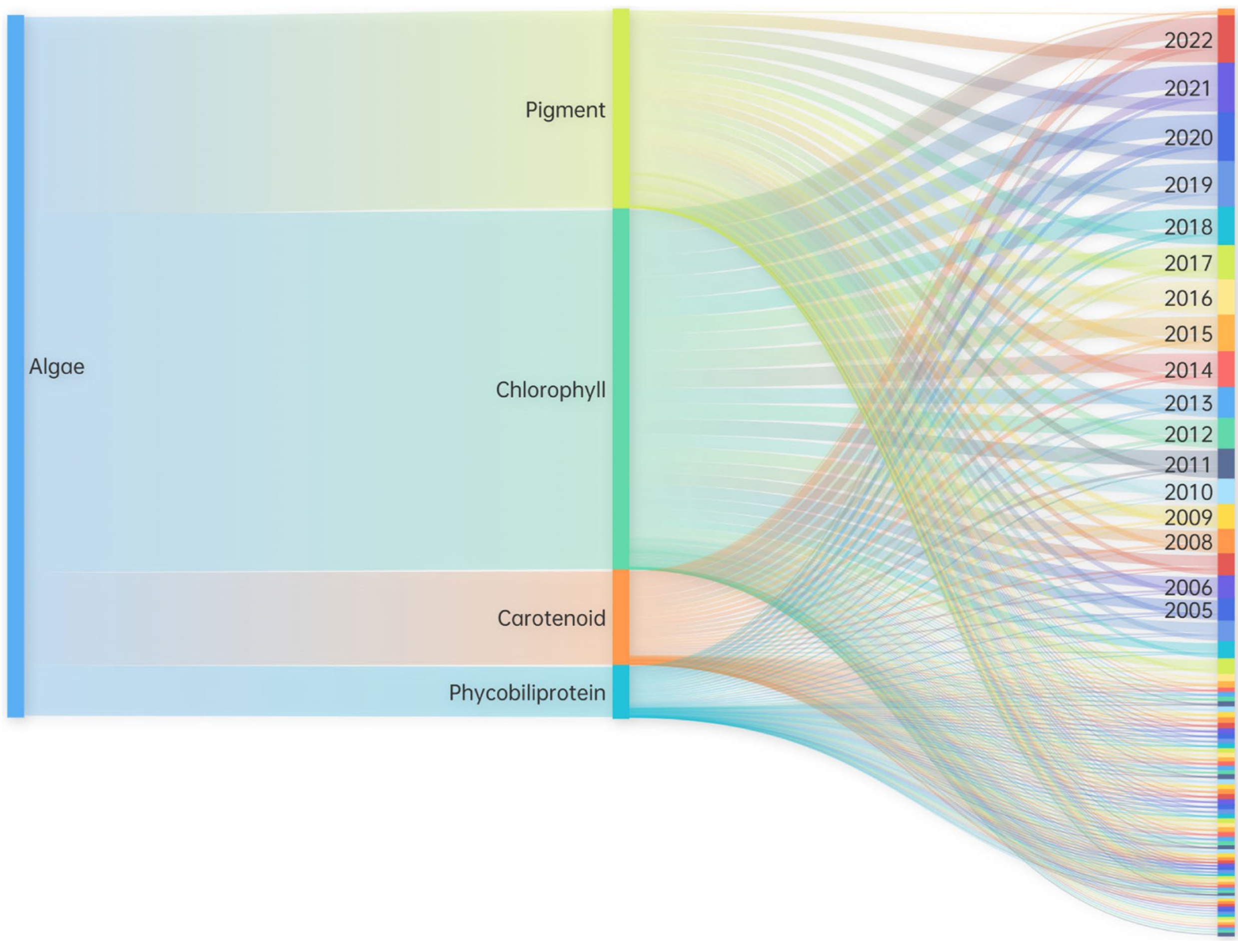

Fig. 1 Sankey map of the number of the literature related to the three pigments in algae. The number of the literature is based on the ISI Web of Science search engine search using the following topics, The Phycobiliprotein bar chart contains the search results of Phycobiliprotein, Phycoerythrin, and Phycocyanin. Lines of the same color represent keywords from the same type. The last row of columns with different colors represents different years, and the different thickness of the columns represents the number of the literature related to the keywords

activation against amyloid beta 1–42 (Aβ)-induced neurotoxicity in PC12 cells (Koh et al. 2018). Chlorophyll a can also act with C-phycocyanin to reduce fat production and prevent obesity (Seo et al. 2018). Chlorophyll c is most commonly found in various algae rather than terrestrial plants, mainly including diatoms, dinoflagellates, and brown algae (Bachvaroff et al. 2005). Most of the chlorophyll c required for experiments is extracted from diatoms. Chlorophyll b, d, and f are pretty important photosynthetic pigments (Loughlin et al. 2013; Hernández-Prieto et al. 2022), that can expand the absorption range of algae photo cooperation and improve the efficiency of photosynthesis, especially chlorophyll f (Follana-Berná et al. 2021; Nürnberg et al. 2018; Tomo et al. 2007). Chlorophyll d is mostly found in cyanobacteria because the cysteine subunit rich in cyanobacteria may be directly involved in its synthesis, or that far-red light bicylindrical cores stabilize far-red light Photosystem II to prevent loss of chlorophyll d (Bryant et al. 2020).

As an important source of marine natural products, algae have been proven to have be rich in active substances and these active substances have been shown to have antioxidant effects and can neutralize reactive oxygen species, as well as which have certain immune responses against cancer, diabetes, and inflammation (Pradhan et al. 2020). Osuna-Ruiz et al. (2016) compared the antioxidant ability of various bioactive substances of algae from the Mexican islands and found that chlorophyll is one of the main carriers of algal antioxidant ability. In addition to its use as a food coloring, algal chlorophyll is also used as an antioxidant (Zhang et al. 2022). Moreover, the chlorophyll extracted from diatoms and the decomposition product of lysophosphatidylcholine were related to the anti-inflammatory activity (Lauritano et al. 2020). Besides chlorophyll, chlorophyll derivatives, such as pheophytin and chlorophyll-derived, can also be obtained from algae. The chlorophyll derivatives extracted from *Grateloupia elliptica* by HPLC can significantly inhibit

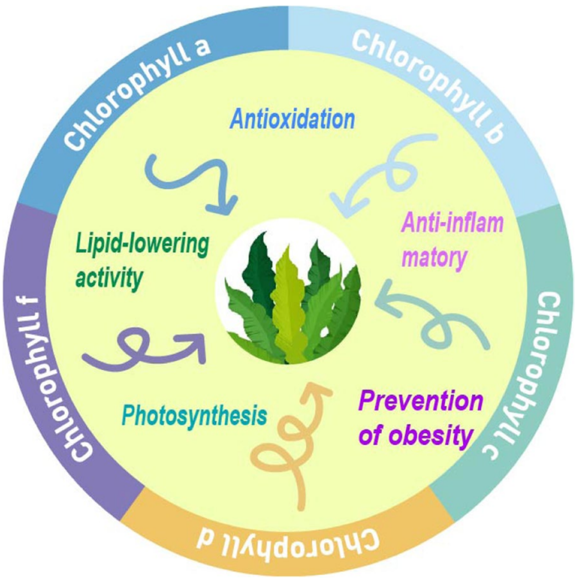

Fig. 2 Chlorophyll in algae and its biological activity

the accumulation of intracellular lipids and have beneficial effects on lipid metabolism (Huang et al. 2021; Lee et al. 2021). Two chlorophyll derivatives derived from *Spirulina*, 132-hydroxy-pheophytin and the new compound 132-hydroxy-pheofarnesin, are also involved in lipid-lowering activities and have obesity-preventive effects (Freitas et al. 2019). Chlorophyll extracted from the marine brown alga *Sargassum fulvellum* enhances the neural differentiation of PC12 cells and has the potential to treat neurodegenerative diseases, such as Alzheimer's disease (Ina et al. 2007). Nelson and Ferruzzi (2008) demonstrated that chlorophyll and its derivatives are degraded in the human body and are absorbed and utilized by intestinal cells through in vitro simulated digestion experiments (Zhong et al. 2021).

In addition to the *Spirulina* mentioned above, Chlorella, Microalgae, and other algae (Prasanna et al. 2007) have been gradually valued as important sources of chlorophyll by the food, medicine, cosmetics, and other industries due to their wide sources and high concentration of chlorophyll. Based on this, researchers have continued to optimize and innovate techniques for extracting chlorophyll from algae (Martins et al. 2021) and have attempted to grow algae with higher chlorophyll content (Nakanishi & Deuchi 2014). All these have laid a solid foundation for the wide applications of chlorophyll in algae.

Phycobiliprotein

Phycobilin is a class of photosynthetic pigments found only in algae, mainly includes phycoerythrobilin, phycourobilin, phycobiliviolin, and phycocyanobilin (Fig. 3). Their difference mainly lies in the number and position of their conjugated double bonds. Phycobilin is structurally similar to chlorophyll and consists of four pyrrole rings linked by methylene (Mysliwa-Kurdziel & Solymosi 2017), but unlike chlorophyll, the molecule is in a straight chain and does not contain magnesium atoms. Phycobilins are the chromophore of phycobiliprotein that not only absorbs normal light, it also collects light in the green gap of chlorophyll (Zhao et al. 2007), thus expanding the light absorption range of algae and improving the efficiency of photosynthesis.

Phycobilin is usually covalently bound to apoprotein via a thioether bond to produce phycobiliprotein, which exists in algae as phycobiliprotein, and one phycobiliprotein contains at least 8 phycobilins. Phycobiliprotein is an oligomeric protein composing of two polypeptide chains with α, and β subunits bound as a monomer (αβ), generally in the form of trimers or hexamers in phycobilisomes of algae (Tandeau de Marsac 2003), and each subunit carrying a covalently linked tetrapyrrole prosthetic groups related to the bile pigment biliverdin. Phycobiliprotein is an important protein source for algae, and in the case of cyanobacteria, for example, it can make up 50% of total protein in the cell (Pagels et al. 2019). As an important light-trapping color protein unique to algae, they only play the role of absorbing and transmitting light during photosynthesis, so they are also known as an auxiliary pigment of photosynthetic (Sui 2021). The type and proportion of phycobiliprotein, the auxiliary photosynthetic pigment vary depending on the location of growth and light absorption by the algae (Bogorad 1975). Phycobiliprotein mainly include phycoerythrocyanin, phycoerythrin, and phycocyanin, depending on the phycobilin to which it is linked. According to the literature, phycobilin is a natural protein with antioxidant (Kim et al. 2018), anti-inflammatory (Cervantes-Llanos et al. 2018), anti-aging, anti-cancer, and other properties (Pagels et al. 2019). Phycobiliprotein is not only an important natural pigment, but it also acts as a protein and contains a variety of essential amino acids that the human body needs. It can become a kind of bioactive substance with high nutritional value to the human body after proper treatment. As a unique natural pigment in algae, phycobiliprotein provides new options for products in the food, medicine, and cosmetics (Bleakley & Hayes 2017; Saini et al. 2018). In recent years, humanity has suffered another infectious disease crisis caused by the coronavirus, and drugs against such as viruses are urgently needed. The main protease and papain-like protease proteases of coronaviruses were studied as targets, and found that phycobilin has a specific affinity for them, indicating that it has some inhibitory potential on both proteins (Pendyala et al. 2021). The data shows that the

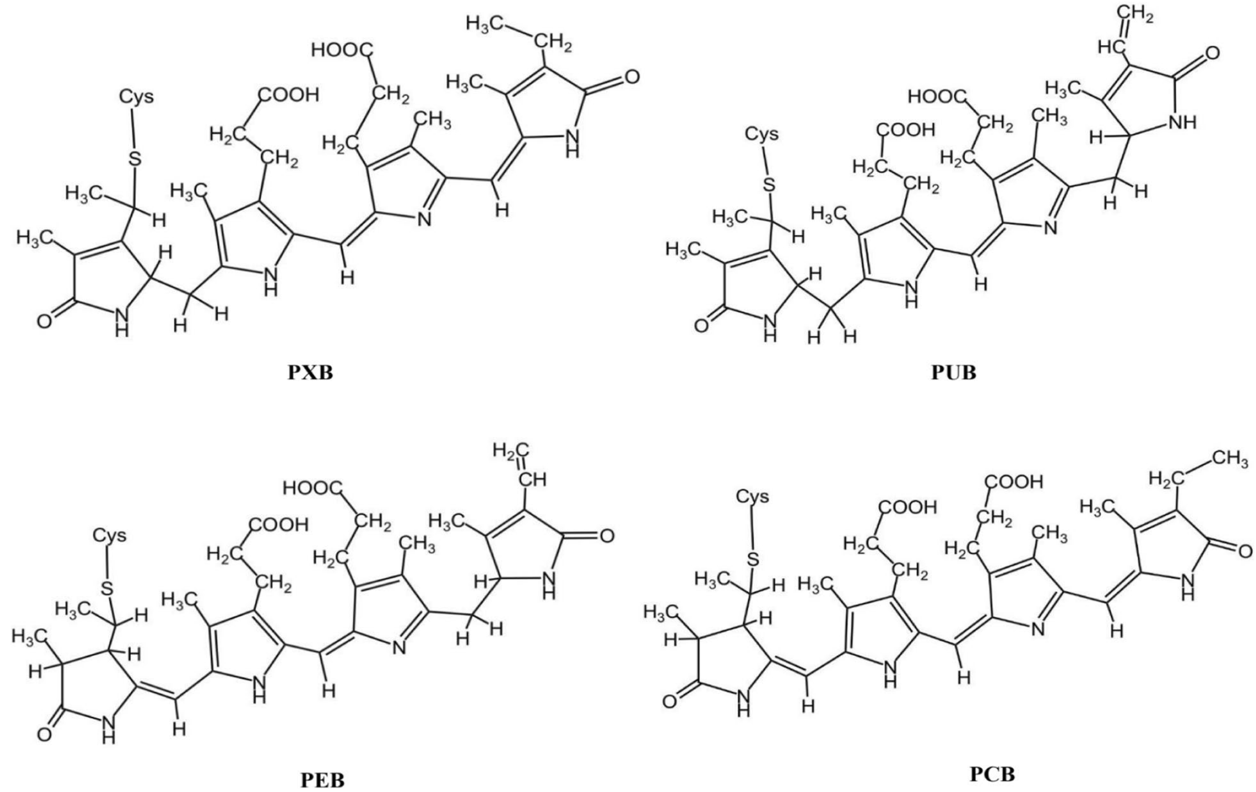

Fig. 3 The structures of phycobilins. PXB, phycobiliviolin; PUB, phycourobilin; PEB, phycoerythrobilin; PCB, phycocyanobilin

market value of phycobiliprotein in 2018 reached approximately 30 million US dollars, and the commercial value is increasing steadily at a compound annual growth rate of 21.3% (Patel et al. 2022).

Phycoerythrin

Phycoerythrin is a red-based phytochrome found in some algae that appears red or intense pink (Ramu Ganesan et al. 2022; Manirafasha et al. 2016). The color it displays depends on the phycobilin to which it is attached. Due to its molecular structure contains 75% α -helix, it is relatively stable (Sathuvan et al. 2022). Based on their origin and absorption spectra, phycoerythrins are classified into four types: R-phycoerythrin, C-phycoerythrin, b-phycoerythrin, and B-phycoerythrin (Munier et al. 2014) (Fig. 4). The difference in their absorption spectra is due to the existence of different types of *Bilin-prosthetic* groups (Glazer 1988). Algae generally contain a variety of phycoerythrin, with B- and R-phycoerythrin being the most common in algae.

The C-phycoerythrin absorption peak is at 565 nm (Galland-Irmouli et al. 2000). It has only two subunits, α and β, forming hexamers $(\alpha\beta)_6$ (Glazer 1977). Moreover, it binds to only one kind of phycobilin, phycoerythrobilin. C-phycoerythrin can prevent oxidative stress and cell damage, inhibit the generation of intracellular reactive oxygen species, and up-regulate the activities of superoxide dismutase and catalase (Sonani et al. 2017). Also, a previous study indicated that it has the potential to fight cancer and improve diabetes complications (Madamwar et al. 2015; Soni et al. 2009).

B-phycoerythrin is mainly found in *Porphyridium cruentum* (Gantt & Lipschultz 1974). The protein part of B-phycoerythrin is composed of three dissimilar subunits, α, β, and γ, with 17.5 kDa, 7.5 kDa, and 30.2 kDa (Glazer & Hixson 1977). B-phycoerythrin forms the same hexamer as R-phycoerythrin but has a different absorption spectrum (Munier et al. 2014). Its absorption peaks are at 545 and 565 nm, with a shoulder at 499 nm (Galland-Irmouli et al. 2000). This is because although B-phycoerythrin is also linked to phycoerythrobilin and phycourobilin, they contain two different types of tetrapyrrole prosthetic groups — the mesobilirhodin type (Glazer 1977). B-phycoerythrin has good structural stability at pH 4.0–10.0 (Gonzalez-Ramirez et al. 2014). it was discovered to be a new natural pigment for milk that gives it a pink color and does not change color during milk processing and preservation (García et al. 2021).

The protein part of b-phycoerythrin also has only two subunits, α and β, each of which is 17.5 kDa and forms

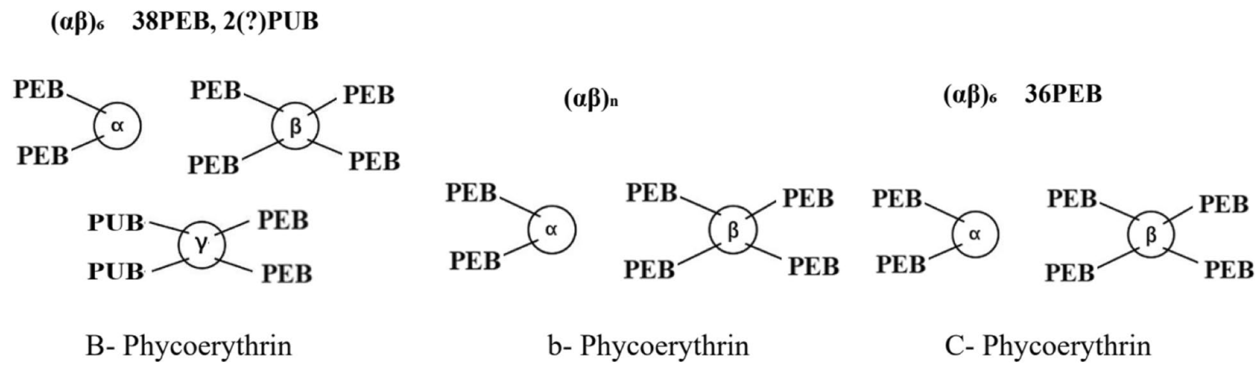

Fig. 4 Distribution and quantity of phycobilin in subunits of phycocyanin (Glazer 1977)

a complex $(\alpha\beta)_n$ (n = 1–6) (Glazer 1977). The value of n depends on the pH value, ionic strength, and other factors in the environment, with absorption spectral maxima are at 543 and 563 nm (Glazer & Hixson 1977). It has a similar amino acid composition, phycoerythrin content, and the same NH_2 terminal sequence as B-phycoerythrin (Glazer & Hixson 1977). It has been speculated that b-phycoerythrin may be a part of B-phycoerythrin (Glazer 1977), but until now, there is no clear evidence to confirm this conjecture.

Compared with other species, R-phycoerythrin is more widely distributed and more abundant. It is the most abundant phycoerythrin in red algae, accounting for about 1.3–1.5% of the dry cell weight (Babu Balaraman et al. 2021). According to research, R-phycoerythrin has physiological activities such as regulating immunity, anticancer, and anti-tumor (Pan et al. 2013; Tan et al. 2016; Wang et al. 2020), so its application is pretty extensive. The protein part of R-phycoerythrin is a 240 kDa oligomeric protein composed of three subunit types, namely α (18 kDa), β (21 kDa), and γ (21 kDa) (Sathuvan et al. 2022), where the γ subunit is a linker polypeptide containing a chromophore that joins two $(\alpha\beta)_3$ trimers to form a complex $(\alpha\beta)_6\gamma$ (Galland-Irmouli et al. 2000). According to the research by Ulagesan et al. (2021), its α subunit has excellent antioxidant activity, and is a promising natural antioxidant and anti-cancer agent. The R-phycoerythrin-linked phycobilins are phycoerythrin and phycourobilin, which have absorption peaks at 499 and 565 nm and a shoulder at 545 nm (Galland-Irmouli et al. 2000). These optical properties are determined by the number of conjugated double bonds or the delocalization of their conjugated π-electrons (Sun et al. 2009).

The purity of R-phycoerythrin is generally obtained by calculating the absorbance with the following calculation formula: Purity index = A_{565}/A_{280} (A_{565}, absorbance of the sample at 565 nm; A_{280}, absorbance of the sample at 280 nm) (Gu et al. 2018). Depending on the increase in purity, it can be priced as high as US$180–250/mg (Xu et al. 2020). And with its rich physiological activity, R-phycoerythrin is considered as a bioactive molecule with high value. Based on its high utilization value, there are various methods for the extraction and purification of R-phycoerythrin. Commonly includes ammonium sulfate precipitation in combination with diethylaminoethyl-Sepharose fast flow column chromatography (Munier et al. 2015), the combination of precipitation with ammonium sulfate and Q-Sepharose column chromatography (Senthilkumar et al. 2013), hydroxyapatite column chromatography (Niu et al. 2006), etc. To improve the extraction efficiency and purity of R-phycoerythrin and reduce the acquisition cost, there are several techniques for R-phycoerythrin extraction and purification based on two water-based systems (Gu et al. 2018; Xu et al. 2020), or combination and upgrade of existing techniques. However, due to the similarity of several phycobiliproteins, it is still necessary to improve the techniques and methods to obtain high purity R-phycoerythrin.

Phycocyanin

Like phycoerythrin, phycocyanin is a phytochrome found in algae, but it is usually found in large quantities in cyanobacteria and exhibits an intense blue color with a light absorption range is about 560–600 nm (Manirafasha et al. 2016). When algae photosynthesize, the light captured by phycoerythrin is first transmitted to phycocyanin and then chlorophyll, so phycocyanin plays a significant role in this process (Bogorad 1975). The structure of phycocyanin is similar to that of phycoerythrin, including chromophore (phycobilin) and two protein parts, where the protein part also includes two subunits, α and β, which are abundant in *Spirulina* and some microalgae. Phycocyanins are classifiedinto four groups: C-phycocyanin, R-phycocyanin, allophycocyanin, and allophycocyanin B (Kuddus et al. 2013). And, *Spirulina* is the most common source of various phycocyanin.

The protein part of R-phycocyanin is composed of only α and β subunits. There is one α subunit with a molecular weight of 17.5 kDa, and two β subunits with a molecular weight of 21.3 kDa and 22.6 kDa, respectively (Wang et al. 2014). They exist as complexes $(\alpha\beta)_3$ (Glazer & Hixson 1977). The phycobilin connected to its α subunit is phycocyanobilin, and the β subunit is linked to two phycobilins, phycoerythrobilin, and phycocyanobilin (Glazer and Hixson 1977). Both the α and β subunits of R-phycocyanin show a certain antioxidant activity, which can inhibit the generation of free radicals, and provide a new direction for the prevention of aging (Feng et al. 2022). Also, R-phycocyanins have positive anti-allergic effects (Liu et al. 2015), and their physiological activity is gaining receiving attention.

Allophycocyanin is the simplest type of phycocyanin. The α and β subunits bind to form αβ complexes, with each of the two subunits linked to a phycocyanobilin (Dagnino-Leone et al. 2020). The α-subunit of allophycocyanin can be combined with streptavidin to form a fusion protein with similar spectral properties to native allophycocyanin (Wu et al. 2018). However, after processing, the fusion protein will have higher sensitivity for immunofluorescence detection and a more comprehensive range of application (Chen & Jiang 2018). Allophycocyanin also has some antioxidant activity, making it a better agent for peroxyl clearance than C-phycocyanin. However, it is a much lower scavenging agent of hydroxyl groups (Cherdkiatikul & Suwanwong 2014).

The structure of allophycocyanin B is similar to that of allophycocyanin in that their protein parts are composed of two subunits, α and β, a complex $(\alpha\beta)_3$ (Fig. 5), but they have different absorption spectral maxima, 650 nm for allophycocyanin, but 670 nm for allophycocyanin B (Lundell & Glazer 1981). Similar to Allophycocyanin, the protein portion of allophycocyanin B does not contain histidine or tryptophan, but in other respects the two proteins are completely different (Glazer & Bryant 1975).

The protein part of C-phycocyanin is a trimeric $(\alpha\beta)_6$ or a hexameric $(\alpha\beta)_3$ consisting of an α subunit with a molecular weight of 18 kDa and a β subunit of 20 kDa (Patil et al. 2006; Galland-Irmouli et al. 2000). The only phycobilin to which it is attached is phycocyanobilin. The data from Dong et al. (2022) confirmed that C-phycocyanin has an antioxidant effect, especially its β subunit has a protective effect against cell damage induced by hydrogen peroxide (Cherdkiatikul & Suwanwong 2014). In recent years, C-phycocyanin has become the focus of phycobilin research and has been proven to have strong physiological activities, such as anti-inflammatory (Blas-Valdivia et al. 2022), antioxidant, antibacterial (Pourhajibagher & Bahador 2021), etc. (Piovan et al. 2022).

The calculation method of phycocyanin purity is similar to that of phycoerythrin, which is also obtained by the absorbance ratio. The calculation formula is as follows: Purity Index = A_{620}/A_{280} (A_{620}, Absorbance of the sample at 620 nm; A_{280}, Absorbance of the sample at 280 nm) (Kuddus et al. 2013). When the ratio of the two reaches 0.7, it can be regarded as food grade. C-phycocyanin is relatively stable at pH 5.0–7.0 and extremely unstable at pH 3.0; During the extraction of C-phycocyanin from *Spirulina spirulina*, impurities can also affect the stability of the pigment (Zhang et al. 2021), which increased its extraction difficulty to a certain extent. Therefore, phycocyanin extraction is usually carried out at pH 7.0 or 0.5 M $(NH_4)_2SO_4$ (Kuddus et al. 2013). The properties of C-phycocyanin are similar to phycoerythrin, so the extraction and purification are also similar. However, C-phycocyanin is usually extracted from *Spirulina platensis* because the cell shape of *Spirulina platensis* is cylindrical and spiral, the cell wall is hard, and it is difficult to destroy (Yu 2017). Therefore, to efficiently extract C-phycocyanin with high purity, it is necessary to improve the cell disruption technology and minimize the generation of impurities that can affect its stability during the extraction process.

Phycobilin also has specific optical properties and physiological activities, but it is less stable and more expensive to extract than phycobiliprotein, which is why phycobiliprotein is more commonly used in industry (Li et al. 2019). Phycobiliprotein is a natural and healthy pigment. In addition to its dyeing function and various physiological activities that are beneficial to the human body, it is also a high-quality protein rich in various essential amino acids required by the human body, with extremely high nutritional value (Brown et al. 2014). In recent years, more and more physiological activities of phycobiliproteins have been explored, expanding its prospects and making its application more extensive.

Carotenoids

Carotenoids are a general term for a class of hydrocarbons and their derivatives (Fig. 6). They are isoprenoid polymers (tetraterpenoids) containing 40 carbons and are the primary source of vitamin A. Algae, higher plants, fungi, fish, and other organisms contain a variety of carotenoids. More than 850 carotenoids have been found in nature, 250 of which come from algae (Christaki et al. 2013). Carotenoids are one of the three major pigments in algae and are widely found in most algae. It can be found in Chlorophyta, Rhodophyta, Phaeophyta, Dinoflagellate, and other photosynthetic algae (Takaichi 2011). Carotenoids account for an average of 0.1% of the dry weight of algae, but some algae may reach 14% under certain growth conditions, such as *Dunaliella salina*, which grows under high salt, light conditions, and nutrients (Prasanna et al. 2007). Some carotenoids are found only in algae, or only in large quantities of algae

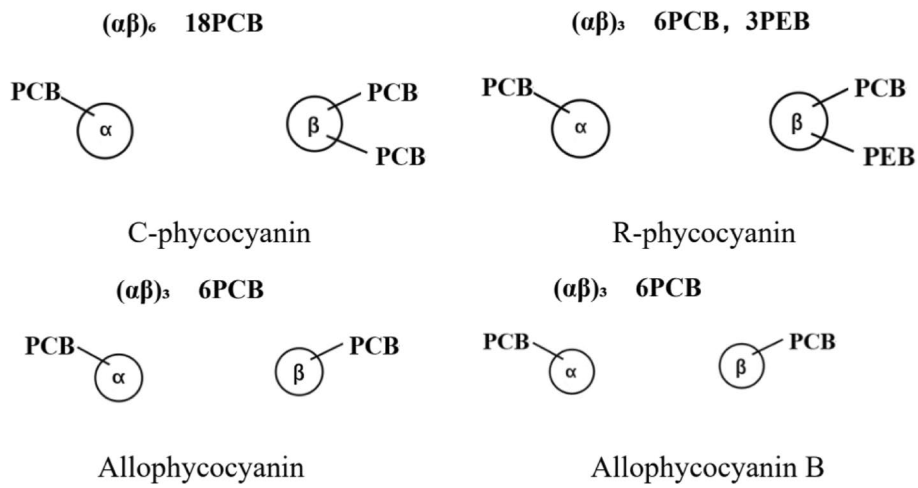

Fig. 5 Distribution and quantity of phycobilin in subunits of phycocyanin (Glazer 1977)

(Takaichi 2011). Carotenoids in algae mainly include two major categories, namely carotene, and xanthophyll.

Carotene is a class of unsaturated hydrocarbons with the general formula $C_{40}H_{56}$, insoluble in water, but soluble in organic solvents such as benzene and acetone (Zhang et al. 2022). The carotenoids in algae are mainly lycopene, α-carotene, and β-carotene, among which β-carotene is the most common (Blatt et al. 2015). The halotolerant green microalgae *Dunaliella salina* is one of the richest algae in β-carotene content found so far, with up to 10% dry weight (Pereira et al. 2021). In algae, phytoene is converted to lycopene by the action of three enzymes: phytoene desaturase, ζ-carotene desaturase, and cis-carotene isomerase (Takaichi 2011). Lycopene is used as a precursor to producing β-carotene and α-carotene after different cyclizations (Blatt et al. 2015; Deng et al. 2020). The content of the two carotenes depends on the content of their related genes and enzymes in the algae (Takaichi 2011). The carotenoid synthesis pathways in land plants and green algae are very similar as they have some homologous genes (Blatt et al. 2015), but some specific synthesis pathways of carotenoids in algae are yet to be investigated. Green algae are an important new source of carotenoids.

Xanthophyll is the most important part of algal carotenoids and is a derivative of carotenoids after oxidation. After oxidation, xanthophyll has several more oxygen-containing groups than carotenes, so it is more stable. The sources of lutein are extensive, and various kinds of lutein are widely distributed in algae, such as *Spirulina platensis*, *Dunaliella salina*, *Dinoflagellates*, *Chlorella* spp., *Haematococcus lacustris*, and *Scenedesmus* spp. (Patel et al. 2022). There are many types of xanthophylls in algae, including violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, lutein, loroxanthin, diadinoxanthin, and fucoxanthin. (Christaki et al. 2013). Lutein, the most typical type of xanthophyll, is a polyisoprene compound with a backbone of unsaturated polyene chains (Xie et al. 2021). Multiple double bonds in the carbon chain endow lutein with a certain antioxidant capacity. Zeaxanthin, which is structurally similar to lutein (Pereira et al. 2021), is an isomer of lutein and one of the most common xanthophylls in algae. Humans are unable to synthesize zeaxanthin and can only ingest it from food. Compared with other plants, zeaxanthin in algae is mostly free, which is more beneficial for people to extract and utilize (Pereira et al. 2021). Zeaxanthin is one of the most important classes of xanthophyll in the human eye, and in addition to the antioxidant capacity, it can effectively reduce the risk of eye-related diseases such as cataracts. *Spirulina* is not low in zeaxanthin and is now considered to be a good source of zeaxanthin (Yu et al. 2012).

Fucoxanthin accounts for about 10% of the total production of natural lutein (Pangestuti & Kim 2011). The structure of fucoxanthin is rather special because its molecule has a less common allene bond and a 5,6-monoepoxide (Pangestuti & Kim 2011). Among carotenoids, Allene (C=C=C), a structure present in natural substances, is relatively rare, and only about 40 carotenoids contain this Allene bond, of which fucoxanthin is the most typical (Christaki et al. 2013). Based on its unique structure, fucoxanthin can act as an antioxidant under hypoxic conditions, a special function that other carotenoids do not possess (Nomura et al. 1997). Another special structure in fucoxanthin, Acetylated, is also a significant feature of some unique carotenoids in algae. Carotenoids containing this structure are mostly found in dinoflagellates and other algae. Besides fucoxanthin, such

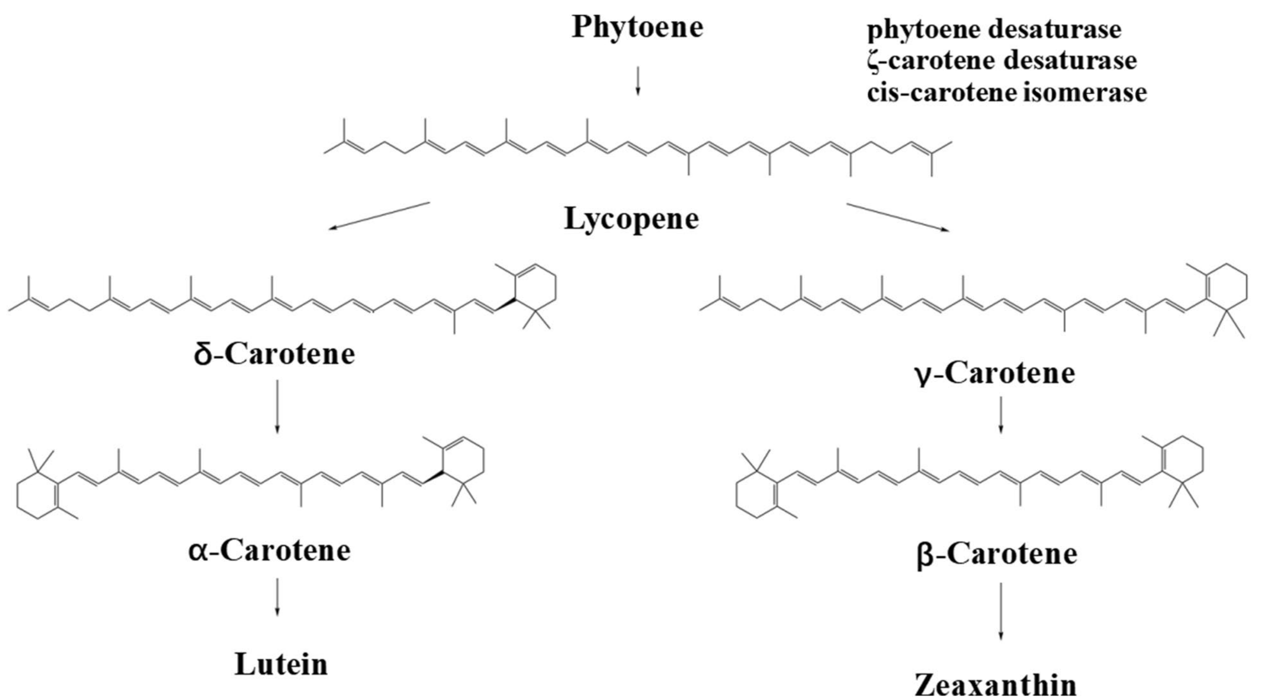

Fig. 6 Possible production pathways of carotenoids

carotenoids include peridinin and dinoxanthin (Takaichi 2011). In addition to Allene, a special structure in carotenoids is Acetylene (C≡C). Similar to the structure of Allene, carotenoids with this structure are only found in algae, such as alloxanthin, crocoxanthin, and diadinoxanthin (Takaichi 2011).

As a carotenoid with a unique structure that exists only in algae. Fucoxanthin has physiological activities such as preventing diabetes (Yang et al. 2021), anti-inflammatory, anti-cancer (Mohibbullah et al. 2022), regulating lipid metabolism, and preventing obesity (Ye et al. 2022). Additionally, it also has certain effects in preventing some chronic diseases (Bae et al. 2020), and has a vast application prospect. The price of fucoxanthin can reach US $40,000-80,000/kg according to market demand and purity (Wang et al. 2021). According to estimates, the market for fucoxanthin could expand to US$120 million by the end of this year (Lourenço-Lopes et al. 2020). There are already some techniques to synthesize fucoxanthin in the laboratory, but this operation makes the synthesis very expensive, so obtaining fucoxanthin from algae by extraction methods is still the most preferred way (Zhao, Chen, et al. 2022; Leong et al. 2022).

Carotenoid is a natural substance that cannot be synthesized by the human body but has a positive effect on the human body (Fig. 7). It is a natural pigment widely distributed in algae, assisting photosynthesis and giving color to algae. It is also a biomolecule with potent physiological activity. Besides the most prominent antioxidant function, it also has the effects of preventing obesity (Bonet et al. 2020), anti-inflammatory (Patel et al. 2022), and so on (Takaichi 2011). There are many kinds of carotenoids in algae, widely distributed and high in content, and some carotenoids are found only in algae. Therefore, algae are an ideal and irreplaceable important source of carotenoids in modern industry.

Status of algal pigments

The extraction of algal pigment is the first step of utilization. It can be roughly divided into five stages, including macro pretreatment, separation of macromolecules and micromolecules, extraction, purification, and product generation (Galanakis 2012). Traditionally, organic solvents such as acetone, ethanol, and chloroform are used to extract algal pigments. However, most of the reagents used are toxic or expensive and usually take a long time, and the recovery rate of pigment has a certain limit (Warkoyo & Saati 2011). Therefore, new technologies such as microwave-assisted extraction, pulsed electric field, high hydrostatic pressure assisted extraction, and ultrasonic assisted extraction have been developed to avoid the harm and limitation of traditional technologies (Poojary et al. 2016). These methods are very effective for extracting chlorophyll and carotenoids from algae. During the extraction process, the temperature has a great impact on extraction efficiency (Saravana et al. 2016). When the experimental temperature rises

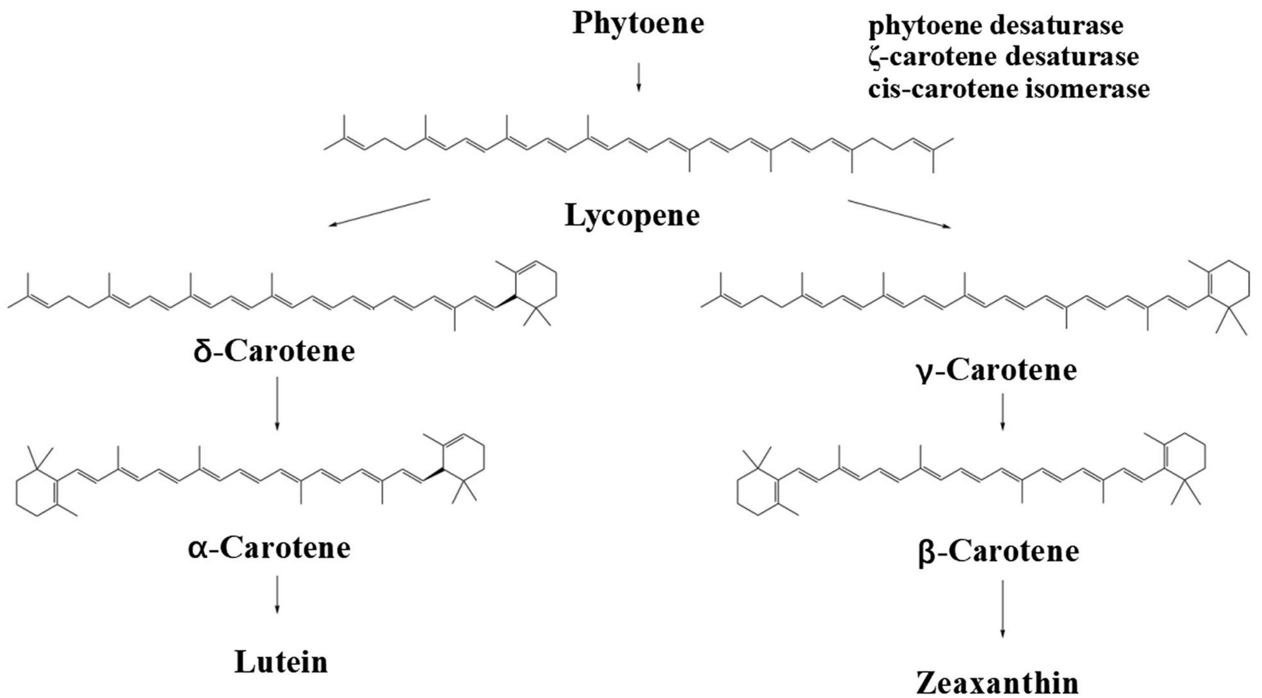

Fig. 7 The structures of the common carotenoids

from 46 °F to 122 °F, the extraction amount of carotenoids increases significantly (Fratianni et al. 2010). However, when the temperature rises further, the loss of some volatile and unstable carotenoids that are easy to decompose will be affected. Chlorophyll extraction efficiency also showed a positive correlation with temperature in the range of 122 °F to 158 °F (Putnik et al. 2016). At the same time, the filtration membrane with a molecular weight of 10 kDa had a good retention effect on algal pigments, and the ultrasonic-assisted extraction in the new technology could well improve the efficiency of pigment recovery (Zhu et al. 2017).

Synthetic pigments have the advantages of better stability and a wider selection range, so they still occupy the main market of industrial pigments in the past decades. However, with the pursuit of health and green concepts, the safety controversy of synthetic pigments has been mentioned again (Shanmugasundaram & Rujaswini 2019), so the advantages of high-quality natural pigments from algae have been highlighted. These natural pigments are considered safer, relatively free of side effects (Burrows 2009), and have the biological activity characteristic of natural compounds. Despite these advantages, pigments from algae have not yet been widely used because they have some limitations. For example, chlorophyll is an unstable magnesium porphyrin compound, which is easily decomposed by light, acid, base, and oxidant. To make it more widely used, food developers typically use chlorophyllide directly (Bednarczyk et al. 2021) or stabilize chlorophyll as a good natural green pigment by adding sodium caseinate (He et al. 2019), adding antioxidants, or replacing its magnesium ions with zinc and copper plasma. In addition, the extraction rate of pigment is not yet able to fully meet the industrial demand, but with the update in technology (such as the ultrasonic-assisted technology mentioned above), this limit will be broken shortly.

Conclusion

As the importance of natural pigments continues to grow, the demand for natural pigments is increasing in various industries, and algae, as an economical and widely sourced organism, has been used as an essential source of natural pigments. The unique pigments in algae, phycobiliprotein, fucoxanthin, etc., can also be synthesized in the laboratory, but their cost is too expensive compared with direct extraction from algae. At present, purification and cell wall-breaking technologies are two major technical barriers affecting algal pigment extraction. To further promote the commercial application of natural pigments in algae, more and more researchers are studying algae with higher pigment content or developing more efficient algal pigment extraction methods. At the same time, cracking the pigment structure, also provides a new basis for the artificial synthesis of these pigments. As a class of natural biological macromolecules with high economic benefits and nutritional value, more and more studies have proven their various biological and physiological activities.

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Authors' contributions
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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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