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Review article

A Review about Using Bioactive Compounds-Rich Microalgae as Pigments

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Abstract

Microalgae are photosynthetic microorganisms with different morphological, physiological, and genetic characteristics. Microalgae, which grow in fresh and salt water, have an important role in the aquatic ecosystem due to their photosynthetic properties. Microalgae-derived bioactive components are produced as primary metabolism sources, such as proteins, various fatty acids, vitamins, or secondary metabolism products. Various microalgae can produce different bioactive compounds. Frequently studied microalgae can be listed as; *Spirulina* sp. (phycocyanin, tocopherols, phenolic acids), *Haematococcus pluvialis* (lutein, oleic acid, beta carotene), *Chlorella* sp. (carotenoids, eicosapentaenoic acid) and *Dunaliella* (trans-betacarotene, oleic acid, linolenic acid). It has been reported that these microalgae can be used in a wide variety of areas and can gain new uses day by day. They have antioxidative, antimicrobial, antihypertensive, immunomodulatory, and anticarcinogenic effects with their important bioactive components. Their antioxidant properties are of great interest in industrial applications. Microalgae have different colors due to their pigment contents and gain coloring properties. These properties emerge through various pigments called chlorophyll Chlorophyll is the green pigment and plays an important role in the photosynthesis of microalgae. microalgae species can produce different chlorophyll variants, making them appear in different colors. In addition to chlorophyll, the coloring properties of microalgae emerge through other pigments. For example, Microalgae also contain carotenoids with a red, orange, or yellow color and phycobilins with a blue, green, or red color. These various pigments and their coloring properties allow microalgae to be used in industrial, agricultural, and biotechnological applications.

Keywords: Microalgae, Bioactive Compound, Pigment, Chlorophyll, Carotenoid.

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INTRODUCTION

Algae are living organisms in multicellular or unicellular form, accounting for half of the world's primary food chain production. They are divided into macroalgae and microalgae according to their size. Microalgae are simple microscopic heterotrophic and/or autotrophic photosynthetic organisms that thrive in the aquatic environment, can be in unicellular or multicellular structures, and are among the oldest forms of life on earth. Microalgae, which can be found in prokaryotic or eukaryotic structures, can reproduce rapidly and can live even under adverse conditions thanks to their single-celled or simple multicellular structures (Elcik & Çakmakcı, 2017; Uzuner & Haznedar, 2020). Microalgae, consumed as food by humans since ancient times,it has been rich in carbohydrates, proteins, lipids, and nutritional value. Although microalgae are affected by many different factors in terms of nutritional values, they generally contain 30-40% protein, 10-20% lipids, and 5-15% carbohydrates in the last logarithmic growth stage (Özçiçek et al., 2017; Anonymous, 2022b). Depending on the species, their size can range from a few micrometers (µm) to several hundred micrometers. Roots, stems and leaves in higher plants are absent in microalgae. Microalgae capable of photosynthesis are essential to life on Earth; They use the greenhouse gas carbon dioxide and produce about half of atmospheric oxygen (Anonymous, n.d.). The use of microalgae as a food source and food additive has been known for centuries, and its large-scale commercial production dates back to the 1950s. Microalgae, which are considered to be very interesting and important biological resources in the development of new food products, can be used to increase the nutritional value of traditional foods due to their biochemical composition, and they affect human health positively compared to conventional products with their rich content (Sasa et al., 2020). Microalgae, which enable innovative agricultural production without the need for fertile soils, are referred to as functional foods and are used in many fields, such as human foods, animal feeds, cosmetics, and pharmacy today (Anonymous, 2017; Oğur, 2016).

Important Bioactive Compounds of Microalgae

Bioactive compounds or bioproducts are physiologically active substances that have functional properties in the human body. Microalgae have been identified as natural reactors for the production of bioactive natural compounds and are excellent alternatives for synthesizing some commercially interesting bioactive natural compounds (Mobin et al., 2019). Microalgae, as a source of bioactive compounds, can result directly from primary metabolisms, such as proteins, fatty acids, vitamins, and pigments or from secondary metabolism. Such compounds may show antifungal, antiviral, antialgal, anti-enzymatic, or antibiotic effects. Many of these compounds (cyanovirin, oleic acid, linolenic acid, palmitoleic acid, vitamin E, B12, β-carotene, phycocyanin, lutein, and zeaxanthin) have antimicrobial, antioxidant, and anti-inflammatory capacities with potential to reduce and prevent diseases. In most microalgae, bioactive compounds accumulate in the biomass; however, in some cases, these metabolites are secreted into the environment as exometabolites. Much of this biomass has been an attractive source for producing various high-value products such as polyunsaturated fatty acids, carotenoids, phycobiliproteins, polysaccharides, and phytotoxins. (Morais et al., 2015; Uzuner & Haznedar, 2020). Primary bioactive compounds extracted from microalgae are shown in Table 1.

Table 1. Major bioactive compounds extracted from microalgae (Morais et al., 2015)

Microalgae	Bioactive compounds
Spirulina sp.	Polysaccharides, Phycocyanin, C-phycocyanin, Phenolic acids, Tocopherols
Spirulina platensis	(vitamin E), neophytadiene, phytol, PUFAs (n-3), fatty acids, oleic acid, linolenic acid, palmitoleic acid
Spirulina fusiformis	Diacylglycerols
Haematococcus pluvialis	Astaxanthin, lutein, zeaxanthin, canthaxanthin , lutein, β-carotene, oleic acid
Chlorella sp.	Carotenoids, sulfated polysaccharides, sterols, PUFAs, (n-3) fatty acids
Chlorella vulgaris	Canthaxanthin, astaxanthin, peptide, oleic acid
Chlorella minutissima	Eicosapentanoic acid (EPA)
Chlorella ellipsoidea	Zeaxanthin, violaxanthin
Dunaliella salina	trans-Beta carotene, cis-beta carotene, β-carotene, oleic acid, linolenic acid, palmitic acid
Dunaliella	Diacylglycerols
Botryococcus braunii	Linear alkadienes (C25, C27, C29, and C31), triene (C29)
Chlorella zofingiensis	Astaxanthin
Chlorella protothecoides	Lutein, zeaxanthin, canthaxanthin
Chlorella pyrenoidosa	Lutein, sulfated polysaccharide
Nostoc linckia and *Nostoc spongiaeforme*	Borofisin
Nostoc sp.	Cryptophycin

Spirulina

Spirulina, which has a high protein value and digestibility, contains significant amounts of essential polyunsaturated fatty acids and phenolic compounds. Due to its high nutritional value and active biocompounds, this microorganism is one of the most studied microalgae worldwide. The protein content of *Spirulina* varies between 50-70% of its dry weight; the carbohydrate content is between 10-

20% (w/w), and the lipid content is between 5-10% (w/w) (Morais et al., 2015). *Spirulina* is rich in vitamins A, B1, B2, B12 and phytopigments, including carotenoids and xanthophyll. It is one of the richest algae sources containing γ-linolenic acid (GLA). GLA is a polyunsaturated fatty acid and a powerful nutraceutical (Uzuner & Haznedar, 2020) *Spirulina* is an excellent source of phycobiliprotein. Due to their high free radical scavenging capacity, these compounds are also used as potential antitumor and anticancer supplements. *Spirulina* can be used with noodles, cookies, and other functional food products. (Uzuner & Haznedar, 2020). Some bioactive compounds extracted from *Spirulina* are shown in Table 2.

Table 2. Bioactive compounds extracted from *Spirulina* (Morais et al., 2015)

Microalgae	Bioactive compound	Concentration (% w/w)
Spirulina fusiformis	C-phycocyanin	46.0
Spirulina platensis	C-phycocyanin	9.6
Spirulina platensis	Allophycocyanin	9.5
Spirulina sp.	C-phycocyanin	17.5
Spirulina sp.	Allophycocyanin	20.0
Spirulina platensis	Phenolics	0.71
Spirulina platensis	Terpenoids	0.14
Spirulina platensis	Alkaloids	3.02
Spirulina maxima	Phenolics	1.29
Spirulina maxima	Flavonoids	0.46

Dunaliella

It is a unicellular, halophilic green bi-flagellate microalgae with no hard cell wall structure. *Dunaliella* species are responsible for β-carotene production and produce more than 14% of their dry weight is β-carotene. β-carotene was the first product commercially produced by *Dunaliella salina*. Since *D. salina* contains abundant β-carotene, it can be used as a food coloring agent, a provitamin A supplement for food and animal feed, a food and cosmetic additive, and an antioxidant-rich food product (Hu et al., 2008; Akyıl et al., 2016). Some bioactive compounds extracted from *Dunaliella* are shown in Table 3.

Table 3. Bioactive compounds extracted from *Dunaliella* (Morais et al., 2015)

Microalgae	Bioactive compound	Concentration (% w/w)
Dunaliella salina	β-Carotene	12
Dunaliella salina	All-trans-β-carotene	13.8
Dunaliella salina	All-trans-zeaxanthin	1.1
Dunaliella salina	All-trans-lutein	0.66
Dunaliella tertiolecta	Sterols	1.3
Dunaliella salina	Sterols	0.89

Chlorella

Chlorella, a species rich in chlorophyll, proteins, polysaccharides, vitamins, minerals, and essential amino acids, contains 53% protein, 23% carbohydrates, 9% lipids, and 5% minerals and oligo-elements in its composition. This microalgae biomass is rich in B-complex vitamins, especially B12, vital for forming and regenerating blood cells. The bioactive substances contained in Chlorella have medicinal properties. Experimental studies have shown the antitumor, anticoagulant, antibacterial, antioxidant, and antihyperlipidemic effects of enzymatic protein hydrolyzate and hepatoprotective properties and immunostimulatory activity. Many antioxidant compounds may be responsible for the functional activities of Chlorella. Antioxidants such as lutein, α-carotene, β-carotene, ascorbic acid, and α-tocopherol, active against free radicals, have been identified. Some of these compounds are not only important as natural colorants or additives but may also be useful in reducing the incidence of cancer and preventing macular degeneration (Uzuner & Haznedar, 2020; Gouveia et al., 2006; Gouveia et al., 2007). The bioactive substances in Chlorella are shown in Table 4.

Table 4. Bioactive compounds extracted from *Chlorella* (Morais et al., 2015)

Microalgae	Bioactive compound	Concentration (% w/w)
Chlorella protothecoides	Lutein	4.60
Chlorella zofingiensis	Astaxanthin	1.50
Chlorella vulgaris	Phenolics	0.20
Chlorella vulgaris	Terpenoids	0.09
Chlorella vulgaris	Alkaloids	2.45
Chlorella minutissima	Phytol	2.70
Chlorella minutissima	Phenol	1.81

Nostoc

Microalgae biomass is used in medicine and as a nutritional supplement due to its protein, vitamin, and fatty acid content. The medicinal value of this microalgae has been proven by its use in fistula treatment and some types of cancer. Historically, the biomass of this microorganism has been described as anti-inflammatory and aids digestion, immunity boosting, and blood pressure control. Studies have shown that Nostoc produces antimicrobial, antiviral, and anticancer activity compounds. These results encouraged its cultivation at a high rate. Nostoc has great economic potential due to its nutritional and pharmaceutical importance (Morais et al., 2015). Some bioactive compounds extracted from Nostoc microalgae are shown in Table 5.

Table 5. Bioactive compounds extracted from *Nostoc* (Morais et al., 2015)

Microalgae	Bioactive compound	Concentration (%)
Nostoc sp.	Phycocyanin	20.0 (p/p)
Nostoc muscorum	Phenolics	0.61 (p/p)
Nostoc muscorum	Terpenoids	0.10 (p/p)
Nostoc muscorum	Alkaloids	2.30 (p/p)
Nostoc muscorum	Phycobilins	0.0229 (p/v)
Nostoc humifusum	Phenolics	0.34 (p/p)
Nostoc humifusum	Terpenoids	0.10 (p/p)
Nostoc humifusum	Alkaloids	1.65 (p/p)
Nostoc humifusum	Phycobilins	0.0031 (p/v)

Usage Areas of Bioactive Components Obtained from Microalgae

Bioactive compounds obtained from microalgae are rich in polyunsaturated fatty acids, polysaccharides, pigments, sterols, and vitamins, allowing them to be used in many areas (Akyıl et al., 2016).

The use of microalgae in human nutrition is currently marketed in different forms, such as tablets, capsules, and liquids. They can be added to various snack foods, candy bars, pasta, chewing gums, and drinks. Thanks to their different chemical properties, they can act as nutritional supplements or be a source of natural food colorants. The most common species in commercial applications are *Arthrospira*, *Chlorella*, *D. salina*, and *Aphanizomenon flos-aquae* (Nuwanthi, 2018; Spolaore et al., 2006).

In addition to human nutrition, microalgae are used as animal food and organic fertilizers in agriculture. Also, in recent years, microalgae, which has been used in biomass production for fuel production, is particularly important. The successful commercial use of microalgae has been in the production of nutritional additives, antioxidants, natural dyes, and polyunsaturated fatty acids. Due to the pigments, antibiotics, and vitamins they contain are used as additives in medicine, pharmacy, and cosmetic products (Özçiçek et al., 2017).

COLORIZING PROPERTIES OF MICROALGAE

Microalgae are a source of microorganisms capable of accumulating some commercially valuable metabolites. Algae are called *Cholophyceae* (green algae), *Rhodophyceae* (red algae), *Cyanophyceae* (blue-green algae), and *Pheophyceae* (brown algae) according to their color. They produce essential pigments chlorophyll a, b, and c, β-carotene, astaxanthin, phycocyanin, lutein, and phytoerythrin (Çelikel et al. 2006). These pigments are available in various algae species and are obtained by different extraction methods using solvents suitable for their structures (İlter et al., 2017). The main pigments obtained from various algae species and the extraction methods used can be seen in Table 6.

Table 6. Pigments obtained from algae, their source and extraction methods (İlter et al., 2017).

Pigment	The algae from which it is obtained	Extraction method
Phycocyanin Phycoerythrin	*Spirulina platensis* *Spirulina maxima* *Spirulina fusiformis* *Anabaena* sp. *Synechococcus* sp. *Aphanothece halophytica* *Nostoc* sp., *Oscillatoria quadripunctulata* and *Phormidium ceylanicum* *Porphyridium* species	Freeze-thaw Homogenization High-pressure application Sonication Acid application Lysozyme application Extraction by microorganisms Nitrogen cavitation method
Astaxanthin	*Haematococcus pluvialis* spp.	Solvent extraction Ultrasonic-assisted microwave extraction Enzyme-assisted solvent extraction Soxhlet extraction
Canthaxanthin	*Haematococcus lacustris*	Solvent extraction

	Bradyrhizobium *halobacterium*	Ultrasonic assisted extraction Acid treatment Supercritical CO_2 extraction
β-carotene	*Dunaliella salina,* *Dunaliella bardawil*	Supercritical extraction Pressurized liquid extraction Ultrasonic assisted extraction Pulsed electrical field extraction
Lutein	*Chlorella pyrenoidosa* *Scenedesmus obliquus* *Chlorella ellipsoidea*	Microwave-assisted extraction Ultrasonic assisted extraction Classical extraction
Fucoxanthin	*Undaria pinnatifida* *Hijikia fusiformis* *Sargassum fullvellum* *Chaetoseros* sp. *Eisenia bicyclis,* *Kjellmaniella crassifolia* *Alaria crassifolia* *Sargassum horneri* *Cystoseira hakodatensis* *Laminaria japonica* *Undaria pinnatifida* and *Sargassum fusiforme*	Microwave-assisted extraction Classical extraction Pressurized liquid extraction

Phycocyanin and Phycoerythrin

Phycocyanin and phycoerythrin are pigments known as protein-structured phycobiliproteins (phycobilins). They are known as water soluble, dark colored, and fluorescent. Phycocyanins are obtained from blue-green algae, and phycoerythrin is obtained from red microalgae (İlter et al., 2017; Sasa et al., 2020). Phycobilins are used as fluorescent agents in the pharmaceutical industry. It has pharmacological potential; It contains antioxidant, anti-inflammatory, nerve cells, and liver protective substances. The main species used in phycocyanin production are *Arthrospira platensis* and *Spirulina platensis* and in the production of phycoerythrin, it is *Porphyridium* (Akyıl et al., 2016). The most crucial phycocyanin is C-phycocyanin. This is because it is odorless, water-soluble, has high antioxidant properties, and has a fluorescent effect. The dose of blue pigment, produced 60% from the *Porphyridium aeugineum* biome, in foods and beverages varies between 140-180 mg/kg. It does not change with pH and remains stable at 60 ºC for 40 minutes. The use of the pigment, which is frequently used especially in acidic beverages and ice cream, in foods has not reached a sufficient level since it is not produced commercially (Çelikel et al., 2006; Kargın Yılmaz et al., 2016).

Astaxanthin

Astaxanthin is a natural pigment obtained from the unicellular green microalgae *Haematococcus pluvialis*. *Haematococcus pluvialis* is known for accumulating large amounts of the potent antioxidant astaxanthin (up to 2-3% dry weight) without specific requirements. Astaxanthin is a more potent and potent antioxidant than other carotenoids such as lycopene, lutein, and zeaxanthin. Astaxanthin is known to have many benefits in humans and animals, such as its anti-inflammatory and immune-boosting properties. It is widely used, especially in salmon feeds (Arpacı & Ayaz, 2011; Uzuner & Haznedar, 2020). *Haematococcus pluvialis* stores high astaxanthin content under stress conditions such as high salinity, nitrogen deficiency, high temperature, and light. At this stage, green algae turn red. Astaxanthin protects algae's DNA, lipids, and cell structure during the entire dormancy period from UV radiation and other accessible radical sources. The microalgae can survive for months or even years until environmental conditions are restored. This reveals the power of astaxanthin (Anonymous, 2022a). The most common use of commercially produced astaxanthin is in the fish farming, food, and pharmaceutical industries. It is preferred in fish farming because it gives trout and crustaceans a natural pink-red color. In addition, this powerful natural ingredient is used in domestic animals, competitive sports animals, and agricultural livestock (Duru & Kargın Yılmaz, 2013; Anonymous, 2022).

Canthaxanthin

Canthaxanthin is a red-orange type of xanthophyll, a subgroup of carotenoids. It is obtained from *Haematococcus lacustris* and *Bradyrhizobium* species. Chemical groups provide free radical scavenging and antioxidant effects such as β-carotene in its chemical structure. Canthaxanthin is fat and partially water soluble (Tanaka et al., 2012). Canthaxanthin is used in foods and animal feeds. It has wide applications in the pharmaceutical, cosmetic, fish farming, poultry, and food industries. It is used in food products such as poultry, cosmetics, fish and meat products, fruit products, beverages, snacks, beer, and wine to impart its yellowish hue. Among them, Brevibacterium linens is a red layer covering the outer surface of cheeses in soft cheese production (İlter et al. 2017; Galaup et al. 2005). The highest amount of canthaxanthin allowed for use in foods is 200 mg/kg in products such as gel and marmalade, while the lowest amount of use is 5 mg/kg in alcohol, carbonated and uncarbonated water-based beverages (Erdal & Ökmen, 2013).

Beta Carotene

β-carotene is an oil-soluble yellow-orange pigment that emulsifies in aqueous solutions. The chromophores forming the highly unsaturated chain provide the yellow-orange color of the structure. In addition to being found in the structure of many living things in nature, β-carotene is also present in the chloroplasts of algal carotenoids. *Dunaliella salina* and *Dunaliella bardawil* of the genus *Dunaliella*, known as the best source of carotenoids, are the species used to create pigment. *Dunaliella* is a green algae in the *Chlorophyceae* family. These species can collect high levels of β-carotene in their structures. The examinations determined that *Dunaliella salina* can produce 400 mg of β-carotene in culture medium under ideal conditions. *Dunaliella* makes more than 14% of its dry weight β-carotene (İlter et al., 2017).

β-carotene shows a photoprotective effect against harmful light during photosynthesis (Kahyaoğlu and Kıvanç 2007). In terms of health, it is stated that it has properties such as antioxidants, protection against cancer, increasing the immune system, and inhibiting tumor development (Yaakob et al., 2014). It is used in different products with its precursor of vitamin A and its high antioxidant properties (Yaakob et al., 2014). It is a type of carotenoid widely studied with its many benefits and should be present in the diet. β-carotene contributes to the development of color by adding to the structure of some cheese products and margarines. It is used in poultry feed and sometimes to improve the color of meat and egg yolk (Lee et al., 2010). The highest amount of β-carotene in foods is 1000 mg/kg in daily beverages (milk drinks, fruit juices), milk and milk powders, yoghurt gels, and marmalades. The lowest usage amount is 20 mg/kg in fresh meat and cream derivatives (Codex, 2006).

Lutein

Lutein is a xanthophyll in the carotenoid group and accumulates in the retina. A natural colorant, lutein is found in green leafy vegetables (spinach, kale, etc.), egg yolk, and some flowers (marigolds). Lutein gives a bright yellow color to foods as a pigment (Duru & Yılmaz, 2013). Lutein reduces the risk of eye diseases such as age-related cataracts, retinitis pigmentosa, and cancer thanks to its high antioxidant properties. Lutein is widely used in instant soups, alcoholic and non-alcoholic beverages, biscuits, sauces, cakes, and confectionery industries. Lutein is added to chicken feeds to increase yolk yellowness in the feed industry. It is used for pet foods, animal and fish feeds, and pharmaceutical purposes (İlter et al., 2017; Duru & Yılmaz, 2013).

Fucoxanthin

Fucoxanthin is a member of the carotenoid family. It accounts for more than 10% of carotenoid production. Fucoxanthin is a brown-orange pigment with chlorophyll-a, chlorophyll-c, and β-carotene found in brown seaweeds (*Phaeophyceae*), diatoms (*Bacillariophyta*), and *Chromophyta* (*Heterokontophyta* or *Ochrophyta*). Studies have shown that fucoxanthin has anticancer, antihypertensive, antipyretic, high antioxidant activity, and antiobesity effects. Fucoxanthin is not widely used as a food additive in our country and is a weight loss drug. In addition, fucoxanthin protects the blood vessels of the liver, brain, bone, skin, and eyes (Akyıl et al., 2016; İlter et al., 2017).

Conclusion

Despite their small size, microalgae are organisms that have a significant impact. With their photosynthetic abilities, pigment diversity, and various uses, microalgae play an essential role in the functioning of nature and human life. It is known that pigments obtained from microalgae are of great importance for human health in preventing the development of many diseases and in treating some conditions. They are also necessary for maintaining normal life functions. Unlike synthetic dyes, the use of microalgae, known for its health and environmental benefits, is increasing daily. The effects of the beneficial components contained in its biomass in the use as pigment have been revealed as a result of the studies. The use of microalgae-derived products containing pigment components such as phycocyanin, phycoerythrin, astaxanthin, canthaxanthin , beta-carotene, lutein, and fucoxanthin as nutritional supplements and food coloring is of great importance. In this study, the bioactive components of microalgae, the primary pigments, their properties, and main application areas are discussed in the light of current literature data.

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