Biotech Studies 32(2), 59-64
http://doi.org/10.38042/biotechstudies.1310572

BIOTECH STUDIES

REVIEW

Food grade microalgae-based biopigments and their production technique versus synthetic colorants

Didar Üçüncüoğlu[1,2]

[1]Department of Food Engineering, Faculty of Engineering, Çankırı Karatekin University, 18100, Çankırı, Türkiye.
[2]Yoka Functional Food, İnd. Trade. Co. Ltd., 18260, Korgun, Çankırı, Türkiye.

How to cite:
Üçüncüoğlu, D. (2023). Food grade microalgae-based biopigments and their production technique versus synthetic colorants. *Biotech Studies*, *32*(2), 59-64. http://doi.org/10.38042/biotechstudies.1310572

Article History
Received 11 January 2023
Accepted 29 May 2023
First Online 05 June 2023

Corresponding Author
Tel.: +90 376 21895008358
E-mail: didaru@karatekin.edu.tr

Keywords
Biotechnology
Microalgae
Biopigment
Synthetic colorants
Food additive

Abstract

In the food industry, synthetic color-active compounds can be added as additives to replace natural colors that are damaged during processing. This addition reduces the batch-to-batch fluctuation and increases the development of new or desired products that are appealing to consumers where natural colors are absent. Synthetic colorants cannot be produced by any bioprocess. In contrast, the Food and Drug Administration declared that algae such as *Chlorella*, *Cryptothecodinium*, *Dunaliella Nannochloropsis*, *Nitzschia*, *Phaeodactylum*, *Schizochytrium*, and *Spirulina* are trustable sources of food pigments as natural sources. These microalgae are photoautotrophic species and can be found on the "Generally Recognized as Safe-GRAS" list of food additives. Microalgae-derived pigments, which are also known as nutraceutical supplements, have been recently used in functional food products. Some of them are used as health and color supporters because of their excellent antioxidant properties that block oxidative reactions in lipid-rich food products. Their unique properties of being harmless to the environment were scientifically proven as well. As a result, the demand for their commercial use is increasing gradually. However, the bioprocess of algae on a huge scale is very limited due to some environmental factors and is hard to produce continuously. The scope of this review was to provide concise knowledge about biopigments extracted from microalgae and their production methods and to clarify the current implementations in the industry. Additionally, food-grade biopigments were compared with synthetic ones. The primary issues with bioprocesses used to produce colorants were highlighted, and as a result, the expected studies were discussed that would be conducted soon.

Introduction

Since color is one of the most valued features by customers, natural or synthetic (artificial) colorants are widely used in the food industry as essential elements in many products. Moreover, colorant usage is also being expanded to include food packaging innovations. Food manufacturers have been using synthetic food colors more frequently than natural food colors to achieve certain features including low cost, improved look, high color intensity, more color stability, and consistency. Several food products on the market may include some unexpected synthetic colors or excessive numbers of the permitted colorants by food regulations. It has been considered that some serious health issues such as mutations, malignancies, decreased hemoglobin concentrations, and allergic reactions could result from this usage (Olas et al., 2021). Therefore, law and education campaigns about food colors applied to consumers and food producers are strongly recommended (Malabadi et al., 2022).

Algae are major elements in the flora of marine and humid environments. Microalgae, which are phytoplankton, are the main source of ω-3 fatty acids that come from marine and/or aquaculture, in particular (Adarme-Vega et al., 2012). Further implementations have been developed to obtain high-quality and high-value bioactive products for the cosmetic and food supplement industries (Chatterjee et al., 2017). Algal biomass could also be applied for biofuels, biomonitoring, bioremediation, bioplastic production (Zhang et al., 2019), and feeding aquatic organisms (Wang et al., 2020). According to Ruggiero et al. (2015) *Chlorophyta*, *Cryptista*, *Cyanobacteria*, *Euglenozoa*, *Haptophyta*, *Heterokontophyta*, *Glaucophyta*, and *Rhodophyta* are the major phyla which are commonly used in bio-based industrial applications.

Recently, algal outputs have become more popular in many commercial applications such as natural pigment bioproduction. They have been reported as a natural and sustainable food source or as a superfood (Jung et al., 2019; Mouritsen et al., 2020). Moreover, microalgae have been widely evaluated in the production of nutraceuticals (Niccolai et al., 2019) and pharmaceuticals (Debnath & Ghosh, 2023) because of their antioxidant properties and pigment, oil, and vitamin contents.

The objective of this paper is to provide an overview of the biotechnological production of biopigments derived from microalgae and to illustrate how these pigments are now used in the food industry. Moreover, synthetic and food-grade biopigments were contrasted. Also, the anticipated research topics in the near future are discussed for improving algae-based sustainable economy.

To reach these goals, brief information about the biological and taxonomic properties of algae is firstly specified. Then, fundamental algae-based pigment compounds, which were certified as natural food additives, are described chemically and their most common microalgae sources are given. In the second section, bioproduction steps of microalgae are described. Limitations, advantages and disadvantages of different cultivation procedures are discussed. After all, these explanations with the current scientific and industrial background, synthetic colorants are defined with actual European Union legislation. Their ups and downs are also discussed with the food-grade bio-based pigments. Finally, a conclusion has been provided to navigate further research for industrial development.

Algae and algal pigments

Even though the current taxonomic systems for the classification of algae are based on morphological and cytological characteristics such as cell wall constituents and chemical nature of storage products in general, microalgae could also be classified according to their photosynthetic pigments (Levasseur et al., 2020). Microalgae contain pigments produced after photosynthetic reactions, such as chlorophylls (green), carotenoids (yellow to orange), and phycobiliproteins (red to blue) (Siqueira et al., 2018). The microalgae source of these natural colorants was given in **Table 1**. Among them, *Chlorella*, *Cryptothecodinium*, *Dunaliella*, *Nannochloropsis*, *Nitzschia*, *Phaeodactylum*, *Schizochytrium*, and *Spirulina* was declared in the "Generally Recognized as Safe" list as a natural food additive source by the Food and Drug Administration (de Oliveira & Arisseto-Bragotto, 2022).

Table 1. Algal biopigments and their common algae sources

Pigment type (Colorants)	Algal groups	
Chlorophylls		
Chlorophyll a	Charophyta	
Chlorophyll b	Chlorophyta	Heterokontophyta
Chlorophyll c_1	Cryptophyta	Phaeophyta
Chlorophyll c_2	Cyanobacteria	Rhodophyta
Chlorophyll d	Dinophyta	Xanthophyta
Chlorophyll e	Euglenophyta	
Chlorophyll f		
Carotenoids (Carotenes and Xanthophylls)	Bacillariophyta Charophyta Chlorophyta Chrysophyta	Euglenophyta Phaeophyta Rhodophyta Xanthophyta
Phycobiliproteins	Rhodophyta Dinophyta	Cryptophyta Cyanobacteria

Chlorophylls are responsible for the exact green and are in almost all photoautotrophic organisms. Chlorophylls are gaining great importance not only as a food additive but also as a colorant in the field of pharmaceuticals and cosmetics. *Arthrospira, Chlorella, Gloeothece, Monoraphidium* and *Scenedesmus* are the main microalgae for producing green colorants. Among these, the most well-known microalgae are from the genus *Chlorella*, whose chlorophyll content is about 7% of its biomass (Khanra et al., 2018).

Carotenoid compounds are the most common class of pigments. Most of them share similar chemical structure. Basically, they comprise an eighteen-carbon chain with a conjugated double-bond and two hexacarbonyl rings at each terminal. In this class, carotenoids are divided into carotenes and xanthophylls. The first (carotenes) are oxygen-free hydrocarbons such as α-carotene and β-carotene, and the others (xanthophylls) are oxygenated derivatives of carotene compounds such as astaxanthin, fucoxanthin, lutein, violaxanthin, and zeaxanthin (Haoujar et al., 2019). The main carotenoid sources are the *Chlorophyceae* class. These microalgae can produce both carotenes namely β-carotene and lycopene, and xanthophylls such as antheraxanthin, astaxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin. However, currently, β-carotene and astaxanthin from the genera *Dunaliella*, *Haematococcus*, and *Scenedesmus* have the highest demand in the global carotenoid market, respectively (Berthon et al., 2017).

Phycobiliproteins, hydrophilic protein pigment complexes present only in Cyanobacteria and microalgae of the *Rhodophyta* phylum as well as in some *Cryptophytes* and *Glaucophytes,* are the last class of pigments found in photosynthetic ones. On an industrial scale, these pigments are produced from several species of *Arthrospira*, *Aphanizomenon, Porphyridium,* and *Spirulina* (Bhalamur et al., 2018).

For several years, it has been accepted that color-active compounds have beneficial health properties such as oxidation inhibitors, precursors of vitamins, activators of the immune system, and inflammation blockers (Imchen & Singh, 2023). Therefore, they are commonly used in food, pharmaceutical and cosmetic industries as a natural color, food supplement, and a bioactive molecule (García et al., 2017; Hamed, 2016).

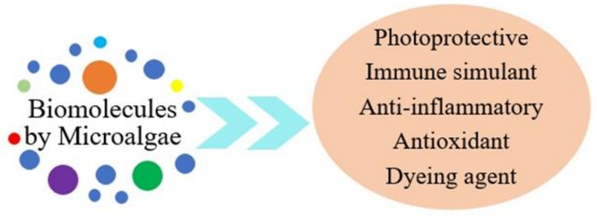

Figure 1. Industrial uses of biomolecules derived from microalgae.

Bio-pigment production

Biopigment production includes cell growth, cultivation and harvest, cell membrane damage, the extraction of pigments and separation, identification, and toxicologic testing for marketing process steps. The primary limitations and bottlenecks for these processes are outlined below along with an explanation.

Cell growth: Microalgae consume natural organic materials and CO_2 as energy and carbon sources. Because of their photoautotrophic structure, they can also produce new carbon sources under sunlight. Therefore, illumination and nutrient supply are the most important steps to start the biomass production during algal cultivation. Optimal light supply differs according to the type of microalgae and the type of end product. Moreover, photosynthesis increases with light supply until photoinhibition is reached. Higher illumination may cause cell death. Photosynthetic organisms naturally contain pigments such as several carotenes, chlorophylls, phycobilin, and xanthophylls for light collection and photoprotection. Photoprotective pigments are astaxanthin, carotene, and other carotenoids (Kratzer & Murkovic, 2021).

Cultivation and harvesting: Microalgae have complex pathways to cultivate. Photoautotrophic cultivation is commonly implemented in open systems (i.e. pools or more commonly ‘ponds’) on an industrial scale. These media should contain well-balanced salinity or pH to protect the strains from contaminants. Algal cultivation systems are divided into two major groups: indoor photobioreactors and outdoor open pools (ponds). In ponds, mixing, illumination, sterility, and operational costs are very low compared with stirred tank reactors. Even with the high cost of photobioreactors, producers feel comfortable about preferring this reactor type due to the complete sterility. In open systems (large scale), contamination, which is the main bottleneck, is unavoidable (Hu et al., 2018).

Damaging cell membrane and extracting biopigments: High pressure and pulsed electric field processes which are hard to apply to big areas and very expensive have been preferred in industry because of the higher yield. It might be faced with several struggles in separation steps (centrifugation, coagulation, filtration, flocculation, flotation, sedimentation, etc.) from the total bulk product such as cell size, gravity settling, and cell density (Grima et al., 2013). Especially for dye extraction, cell rupture, centrifugation, and solvent extraction usage are common and proper (Monte et al., 2018).

The other major concern is whether microalgal products are rentable or not. Accordingly, algal strains should be optimized genetically to improve the extracted pigment yield. The well-known struggles are the high production costs of cultivation, harvesting (dewatering), and stabilization in product quality (Kratzer & Murkovic, 2021; Lafarga, 2020).

Comparing bio-based pigments and synthetic colorants

Throughout the past two decades, the food sector has been influenced by several sociological, technological, and economic variables. Therefore, with a significant amount of food products being processed to satisfy the demands of brand-new customer categories, the food sector has undergone a quick transformation (Carocho et al., 2014). Hence, food engineers and scientists work hard to produce aesthetically pleasing foods that taste greatly and satisfy consumer demands for quality and cost.

Current statistics are unknown regarding the size of the color market; however, reports show that synthetic, natural, and caramel colors could be implemented in the food industry as food additives on a global scale (da Rosa et al., 2023). Synthetic colors are obtained by chemical synthesis, most often by introducing sulfonic or carboxyl groups into the natural dye molecule. Further, they do not occur naturally in nature. Their main sources are coal or petroleum which could be dangerous (Lis et al., 2020).

In other words, synthetic food colors are created through full chemical synthesis or alteration of several precursor molecules, as opposed to natural food colors which are typically taken from various natural sources and purified. Moreover, their chemical structure does not break down during processing and can be used directly. Due to their more stable colors, bigger synthesis scales, and more affordable manufacturing processes, synthetic colorants have largely replaced natural colorants. However, the negative effects on the environment and human health caused by the excessive use of these chemicals have increased the demand for natural colorants. Thus, intense gradual growth in the ‘natural color’ market is anticipated (Souza Mesquita et al., 2021).

The European Union has authorized 43 colorants as food additives. Seventeen of them are highly concentrated synthetic pigments. They have no declared limits on their daily intake levels. Moreover, they are widely available and reachable. However, only some synthetic colors are officially approved by the US and EU, such as Allura red, Brilliant Blue, Erythrosine, Indigo Carmine, Tartrazine, and Sunset Yellow (Olas et al., 2021).

Pigments from nature vary widely in physical and chemical properties. Many of them are extremely sensitive to process and storage conditions such as pH change and light concentration, oxidation, and spontaneous solubility. Initially, natural colors were considered less stable, more difficult to use, and more expensive than synthetic ones. However, considering the consumer's health, it is reasonable to evaluate it for foods and beverages (Downham & Collins, 2000). As a result, it can be accepted that natural colorants or algae-based pigments are preferable to all synthetic colorants due to their superior biocompatibility and lower toxicity (Debnath & Ghosh, 2023).

Table 2. Comparison of using biopigments and synthetic pigments in industry

Parameter	Natural pigments derived from algae	Synthetic dyes and pigments	Reference
Properties	Sensitive to processes	Stable to processes	Malabadi et al., 2022 Olas et al., 2021
Health	Promoting health	allergic, mutagenic, carcinogenic cases may happen	Downham & Collins, 2000 Sharma et al., 2021
Synthesis	Difficult Needs high-quality biotechnological processes	Easy, Semihard Needs chemical reactions in exact conditions	Debnath & Ghosh, 2023 Olas et al., 2021
Costs	Very expensive	Cheap to expensive	Malabadi et al., 2022
Consumer preference	Reasonable to consume	Questionable to consume	da Rosa et al., 2023 Souza Mesquita et al., 2021

Because of all these factors, scientists have focused on research and development activities to find a cost- and environment- effective way and sustainable source for producing natural pigments such as algal bioproducts. Synthetic pigments and biopigments were compared in **Table 2** based on several advantages and disadvantages of them such as process sensitivity, health effects, synthesis type, costs, and consumer preferences. As understood from the table microalgae-based pigment production gains great importance as a natural source.

A growing variety of natural colorants are now being produced on a larger scale in a more environmentally friendly and sustainable way thanks to recent developments in microbial metabolic engineering (Olas et al., 2021; da Rosa et al., 2023).

Conclusion

The use of artificial food colors is still debatable. Current literature clearly shows that using artificial colors might have hazardous and negative impacts on health. Although there are some official requirements for producers, there is not enough information on the possible issues caused by these substances to consumers. The behavior of synthetic dyes should be better understood, which may allow for their incorporation into food products as healthier substitutes.

Microalgae are confirmed crucial resources for innovative food and feed products as superfoods. Up to now, the most cultured species are *Arthrospira*, *Chlorella*, *Dunaliella*, and *Haematococcus*, which can also be cultivated as an energy source under sunlight. Microalgal biomass, as a natural source, includes valuable bioproducts such as pigments, functional food components, and nutraceuticals. The fatty acid-, protein-, and antioxidant-rich extracts can be used as feed and food supplement for daily intake. Microalgae biomass has become a basic component of powders, tablets, and capsules nowadays. Besides, many new products occur in the production of bakeries, meals, and chocolates.

Pigments such as astaxanthin red, lutein yellow, chlorophyll green, or phycocyanin bright blue are natural food dyes used as isolated pigments from microalgae. Biotechnological strategies worldwide, particularly the European Commission Green Deal, target sustainable growth and development. Therefore, algal bioproducts could be used in several areas, especially in food industry. This study described the importance of natural sources to develop a more effective and functional way of obtaining innovative bio-based foods as an example. Despite deep scientific knowledge about microalgae behavior, some gaps are still lacking in optimizing algal bioproducts. Strain selection and growing, harvesting, drying and damaging cell membrane methods are key points that need to be considered when isolating valuable compounds from microalgae.

In conclusion, to overcome the industrial limitations of biotechnological production, researchers and engineers in the industry should concentrate on eliminating the bottlenecks of large-scale harvest and extraction methodologies. It is also crucial to underline the necessity for additional industrial-scale research on algae. Even though several sectors use algae biomass, it is not too much. This is because processing biomass is challenging, yields are low, and product profit margins are therefore low. There is an eminent need to design future studies for the industry using this approach.

Acknowledgements

The author, DU, wants to thank the organizing committee of 1st ICBIOS Congress, held in Turkiye in November 2022, because of the opportunity of presenting the abstract of this study.

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