Food and Humanity 2 (2024) 100235

Contents lists available at ScienceDirect

Food and Humanity

journal homepage: www.editorialmanager.com/foohum/journal_overview.html

Phycocyanin from *Spirulina*: A comprehensive review on cultivation, extraction, purification, and its application in food and allied industries

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ARTICLE INFO

Keywords:
Phycocyanin
Spirulina
Extraction
Applications
Market value

ABSTRACT

Phycocyanin is a blue accessory protein pigment that is abundant in *Spirulina sp.* and has gained popularity due to its diverse applications in various industries. Phycocyanin is rich in natural properties that can be used as a nutraceutical, combining food and pharmaceutical sectors. It has been explored in the pharmaceutical industry by combining with disease-specific drugs and has been experimented against various conditions such as cancer, anemia, inflammation, diabetes, obesity, and neurodegenerative disorders. Additionally, phycocyanin has been used as a natural alternative to artificial food colorants, which imparts color to food and boosts the nutrient value of the food. The market value of phycocyanin is projected to reach $279.6 million by 2030, with a CAGR of 28.1%. Despite its various benefits, phycocyanin has some drawbacks, including instability towards light, pH, and temperature, lower yield, and higher production costs, which limit the expansion of the industry. This comprehensive review provides an overview of different aspects of phycocyanin production and utilization, including *Spirulina* cultivation, various extraction and purification strategies, and its applications predominantly in food industries and other allied sectors.

1. Introduction

As early as the 16th century, people living near alkaline lakes have used *Spirulina* as a dietary supplement. The Aztecs and other Meso-americans used it as a food source in Mexico. As described by one of Hernan Cortes' soldiers, algae were harvested at Lake Texcoco and turned into cakes called "tecuitlatl" (AlFadhly et al., 2022). Historically, it has been used as food by the Kanembu ethnic group in the Lake Chad region of the Republic of Chad to make and sell dried bread called "dihe". After being rediscovered by a European scientific mission in Chad, this traditional food has gained popularity in the human health food industry around the world. A 1967 conference by the International Association of Applied Microbiology highlighted *Spirulina* as a "wonderful future food source"(Jung et al., 2019).

Spirulina is recognized as a single-cell protein, which has gained many researchers' interest due to the presence of high concentrations of protein (about 60–70%), carbohydrates, lipids, and pigments (Kameshwari et al., 2020). It is also known as the "Best food for the future" and has been identified as "Space food" by NASA and European space energy as it is rich in nutrients and can be consumed for a longer duration (Priyanka et al., 2023). It has a widespread application in various fields including the pharmaceutical, nutraceutical, and food industries, and can also be used as a feed for poultry, swine, and fish, either directly or formulated with other feed ingredients (Altmann & Rosenau, 2022). *Spirulina* can be marketed in many forms' capsules, and powder, it can be combined with other food products such as *Spirulina* noodles, pasta, yogurt, and shakes, and with other cosmetic products (Lafarga et al., 2020). The CAGR of *Spirulina* is estimated to be 9.4% between the years 2023 to 2030 with a market value of $1.10 billion by 2030 (www.meticulousresearch.com). The market can further be enhanced by the extraction of pigments from *Spirulina*, which is also of high market value. Phycocyanin is one such pigment present in *Spirulina sp.* with an abundance of natural properties such as antioxidant, anti-microbial, anti-anaemic, etc., It has a market value of 9.6% and is projected to reach $279.6 million by the year 2030 (www.alliedmarketresearch.com). Phycocyanin comprises a diverse blend of monomers, trimers, hexamers, and various other oligomers. Phycocyanin is a member of the phycobiliprotein family, which includes two other proteins, namely phycoerythrin and allophycocyanin (**see Fig. 1**). These proteins are distinguished by their optical characteristics, with phycoerythrin exhibiting a λ_{max} value of 540–570 nm, phycocyanin at 610–620 nm, and allophycocyanin at 650–655 nm (Ashaolu et al.,

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https://doi.org/10.1016/j.foohum.2024.100235
Received 13 October 2023; Received in revised form 10 January 2024; Accepted 12 January 2024
Available online 17 January 2024

2021).

The extraction and purification of phycocyanin from the *Spirulina* sp. has been challenging and has been explicitly explored. Generally, phycocyanin has been extracted using the freeze-thaw method, ultrasonication, homogenization, and pulse-electric field (Pez Jaeschke et al., 2021). Phosphate buffer (PB) is the commonly used buffer for the extraction of phycocyanin. Purification is generally done by a combination of dialysis using ammonium sulfate precipitation combined with column chromatography (using different column materials) (Prabakaran et al., 2020; Puzorjov et al., 2022). The extracted phycocyanin finds application in various industries such as food, pharmaceutical, nutraceutical, and cosmetic industries due to the abundance of natural properties or so-called bioactivity within it. Some of the food products derived from phycocyanin include ice cream (Rodrigues et al., 2020), yogurts (Mohammadi-Gouraji et al., 2019; Sangian et al., 2022), candies (Dewi et al., 2018), and cosmetic products including lip balm (www.thelipbalmco.in) face and hair masks, and facial kits (www.beautyrelay.com).

Despite possessing an abundance of natural attributes, the phycocyanin industry is struggling to tackle a few problems in the establishment of the industry which include sensitivity towards temperature, pH, and light, high overall production and processing costs compared to that cost of *Spirulina* and produces low yield (Yuan et al., 2022). Tackling these problems would be helpful in the establishment of the phycocyanin industry worldwide in the near future.

This review throws a spotlight on optimum conditions for the cultivation of *Spirulina* (a major source of phycocyanin), extraction and purification methods available so far to enhance the yield of phycocyanin and the application of phycocyanin in various industries such as food, pharmaceutical, and cosmetics. The readers will gain knowledge about the initial process of cultivation of *Spirulina* in an optimal environment to the enhanced methods of extraction. This review will also provide a brief idea on enhancement in the extraction and purification of phycocyanin and its various applications which will help the readers in understanding the concepts based on real-time application.

2. Progression of phycocyanin research over the past two decades

From Scopus (a scientific repository), a total of 3516 relevant scientific publications have been found by searching with the keyword "Phycocyanin", within the period of 2001 to 2022. The exponential increase in the number of publications related to phycocyanin over time is depicted in the graphical representation presented in **Fig 2 . (a)**. The relative growth rate (RGR), which serves as an indicator for the increase in the total number of manuscripts or articles over a given period, was calculated using Eq. (1)**,** where (Δt) studied period and W_1 and W_2 are the initial and final publication count in the studied period (Tundup et al., 2021).

$$RGR = \frac{\ln(W_2) - \ln(W_1)}{\Delta t} \quad (1)$$

RGR data demonstrated a constant growth in the number of publications, which suggests that there are expanding frontiers in the field of phycocyanin research.

The numerous publications in the studied period were distributed among different subject categories (see **Fig 2 . (b)**). Among the different subject categories "Agricultural and Biological Sciences" has shown the highest contribution (accounting for 18% of the total contribution) followed by "Biochemistry, Genetics and Molecular Biology" (17%), "Environmental Science" (10%), "Environmental science" and "Medicine" (6%).

The phycocyanin content of *Spirulina sp.* contains approximately 25% of its total biomass, making it one of the most important sources of phycocyanin among blue-green algae.Marzorati et al. (2020)). The present review focuses on the different extraction, purification, and application of phycocyanin from *Spirulina sp.*

3. *Spirulina* morphology and nutrient profiling

Spirulina is a spiral-shaped saltwater alga that belongs to the family of cyanobacterium (blue-green algae). Though it is a saltwater organism it can also be grown in freshwater **(see Fig. 3).** The average size of the *Spirulina* is 8 μm in diameter and the length varies from 100–200 μm. The optimal pH range for *Spirulina* is 8.8–11 (highly alkaline) and the optimal temperature range is 25–37 °C (Masojídek & Torzillo, 2014). It is blue-green in color due to the presence of pigments like chlorophyll, carotenoids, phycocyanin, and phycoerythrin.

Spirulina is also known as a "single-cell protein" with a protein content of 50–70% (depending upon the strain used in cultivation) of its total biomass followed by 15–25% carbohydrates and 9% of fat as its major components due to which the World Health Organisation has recognized *Spirulina* as the "best food for the future" (Soni et al., 2017). The nutrient profile of *Spirulina* compared to other staple foods is given in **Table 1** . It is claimed that the protein in *Spirulina* is about 55%– 70% which is 6 times higher than egg, and 2 times higher than soybean, the fiber in *Spirulina* is about 8%– 10% which is 4 times higher than oats or corn which is about 1–2%, iron in *Spirulina* is 15 mg which is 9 times higher than spinach and 5 times higher than soybean, and potassium in *Spirulina* is 140 mg which is 4 times higher than a banana (Soni et al., 2021).

The protein content in the *Spirulina sp.* is far greater than the other staple crops like rice, and wheat, which are about 70–75% **(Table 1).** It is not only rich in proteins, carbohydrates, and fats but also has

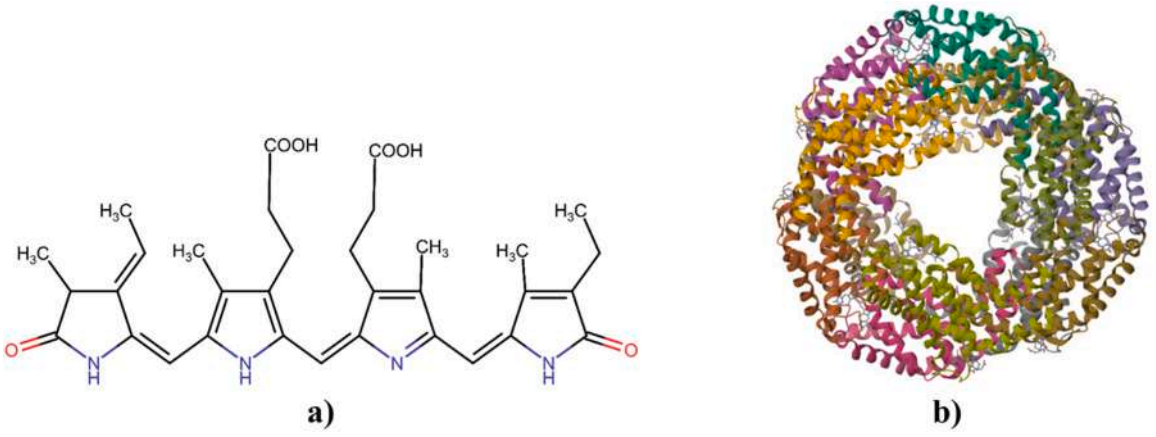

Fig. 1. a) Chemical structure of phycobiliprotein and, b) Three dimension structure of phycocyanin (downloaded from PDB database (PDB ID- IHA7)).

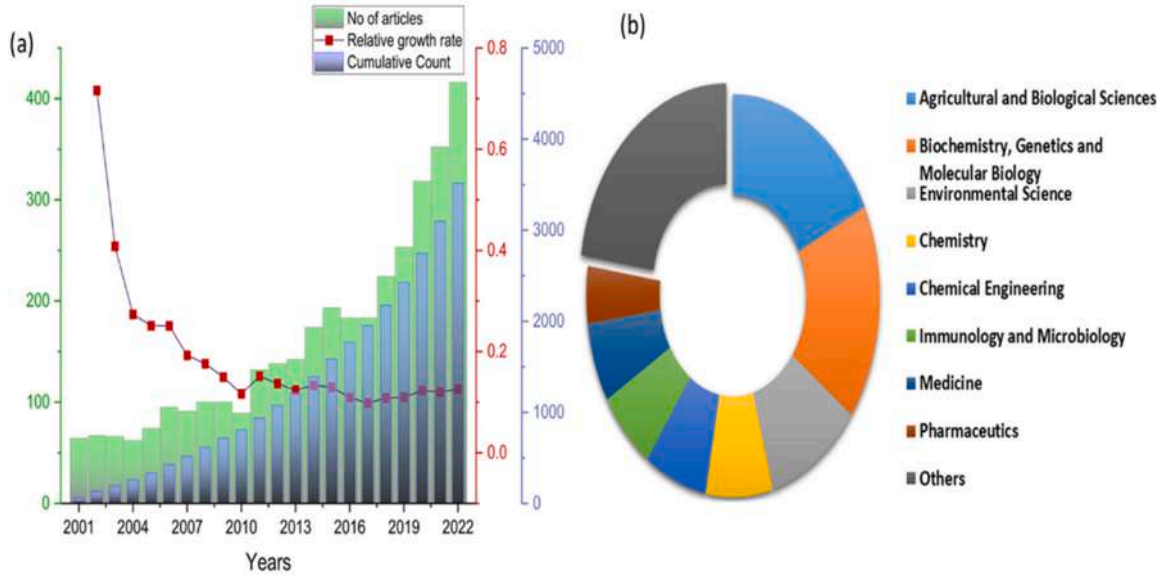

Fig 2. Field growth and publication distribution in the field of phycocyanin (a) over the years (2001- 2022) and (b) in different subject categories.

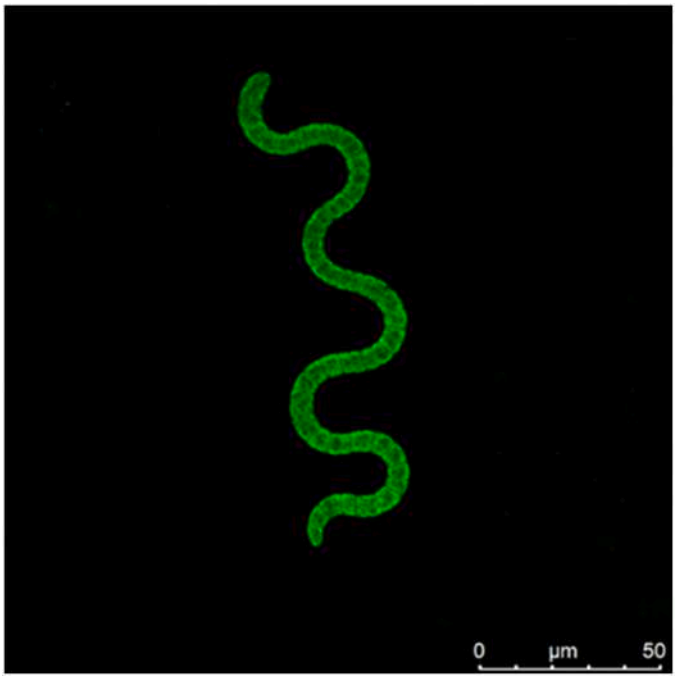

Fig. 3. Confocal microscopic image of 5-day-old *Spirulina sp.*

abundant vitamins and minerals (Liestianty et al., 2019; Soni et al., 2021) illustrated in (**Fig.4**).

3.1. Culture media

Generally, Zarrouk's media is preferred for the cultivation of *Spirulina sp.* The basic requirement of the culture is a carbon and nitrogen source which is provided by sodium bicarbonate and urea. The high alkaline pH is balanced using sodium chloride (Ragaza et al., 2020). There are a few more media that are explored as an alternative to the existing *Spirulina* cultivation media (**Table 2**).

3.2. Inoculum for the cultivation

A concentrated *Spirulina* culture that has fully grown is required for the preparation of inoculum (starter) for cultivation and culture maintenance. The chosen *Spirulina* strain must be highly coiled (at least 25% straight filaments, or none) and must consist of at least 1% gamma-linolenic acid (GLA) by dry weight. The seed culture of *Spirulina* can be obtained by diluting the freshly filtered biomass or from the floating layer of the composed culture, the culture color must be vivid green (Afroz & Singh, 2021). Generally, 10% (v/v) of *Spirulina* culture would be taken as inoculum for optimal growth of *Spirulina* (Rajasekaran et al., 2016).

3.3. Mixing and agitation

Aeration and agitation are two crucial parameters in *Spirulina* growth; the culture must be agitated to homogenize and create a homogeneous distribution of illumination throughout the *Spirulina*

Table 1
Nutrient profile of *Spirulina*, other algae, staple crops, and food.

Species	Nutrient value (per 100 g)					
	Protein (g)	Carbohydrate (g)	Fats/Lipids (g)	Dietary fibers (g)	Energy (kcal)	References
Spirulina sp.	70.2	19.4	1.2	11.7	338	Rajasekaran et al. (2016)
Chlorella vulgaris	60.6	3.7	12.8	13.0	372	Lee et al. (2008)
Rice	2.7	79	0.66	1.3	354	Nur et al. (2015)
Wheat	13.7	72.60	1.9	12.2	339	Murtaugh et al. (2003)
Boiled egg	12.58	1.12	10.6	0	155	Kuang et al. (2018)
Milk	3.15	4.8	3.25	0	61 s	Kuang et al. (2018)

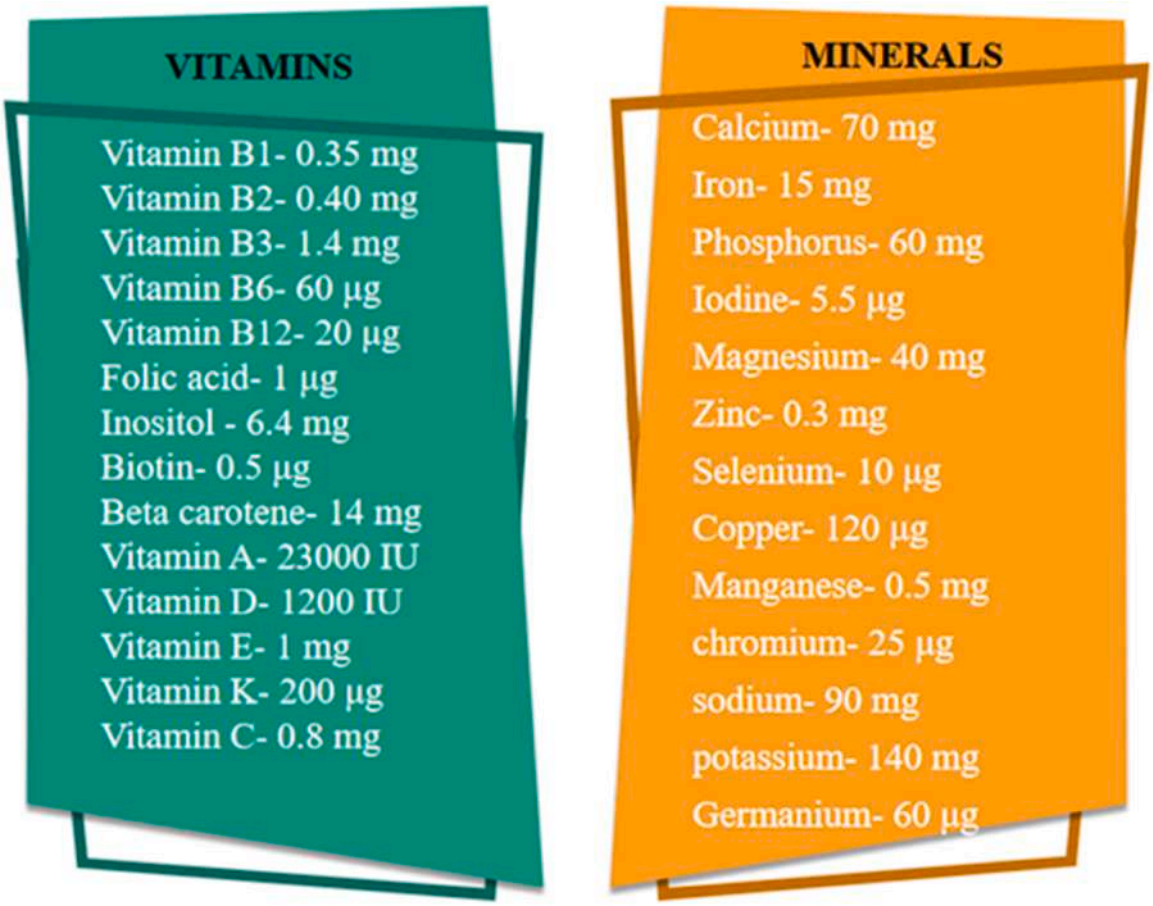

Fig 4. Vitamins and minerals content in *Spirulina* per g**4. Parameters for the Growth of *Spirulina*.**

filaments. The productivity of ultra-high-density cultures is greatly influenced by mixing. Rotators or agitators that gently agitate (bubble column reactor) the developing cells to keep them suspended influence the growth and yield of the culture. (Zhang et al., 2015). Aeration ensures uniform dispersion of *Spirulina* filaments throughout the growth media, ensuring appropriate illumination inside the system. Furthermore, it facilitates the even dispersion of carbon dioxide levels and the elimination of hindering substances and gases, such as oxygen. Inadequate aeration can lead to inefficiencies in energy consumption and biomass generation. Similarly, the absence of proper aeration within the medium results in the buoyancy of cells atop the surface because of the existence of vacuoles filled with air Therefore, the agitation must be maintained within 20 rpm for proper mixing without creating shear stress in the cells (Soni et al., 2019).

3.4. pH and temperature

Spirulina grows at temperatures ranging from 20 ºC to 37 ºC, although the most ideal temperature for the growth of *Spirulina* is between 29 ºC to 35 °C. The variations in atmospheric conditions such as change in temperature, humidity, and pressure are the fundamental determinants of biomass yield and quality. *Spirulina* cultures can withstand temperatures above 35 ºC, and bleaching of culture occurs which can be seen visually, but the culture cannot tolerate temperatures below 20 °C (Kumar et al., 2011; AlFadhly et al., 2022). To avoid contamination, the pH level of *Spirulina* media must be higher than 9. Carbonate salts are added to the culture to increase the level of carbon dioxide in the atmosphere. A healthy culture must have a pH between 9 and 11 (alkaline pH), pH has a direct impact on algae growth and pigment production (Soni et al., 2017). When growth is affected then it will directly affect the nutrient composition of the culture, which may decrease the concentration of protein in the culture. A decrease in protein affects the antioxidant function of the culture.

3.5. Light intensity

The ideal light intensity for cultivating *Spirulina* is 200 µmol $m^{-2}{\cdot}s^{-1}$ (Niangoran et al., 2021). High light intensity promotes growth factors like maximal specific growth rate, but low light intensity produces biomass rich in pigments and proteins. Outdoor algal cultures are subjected to two different light and dark cycles. The physiological regime imposed by light cycles governs the adjustment or acclimation of algal cells in outdoor environments. Increasing the cellular density within the culture medium results in increasing self-shading and dropping the rate of growth of *Spirulina* (Soni et al., 2017). A study proved that by increasing the light intensity, biomass production also increases which proves that light intensity and biomass production are directly proportional (Chaiklahan et al., 2022).

3.6. Cultivation system in Spirulina cultivation

There are three cultivation systems that are widely used for the cultivation of *Spirulina* which is open systems, closed systems, and hybrid systems (Solovchenko & Chekanov, 2014; Cuellar-Bermudez et al., 2015; Jeevanandam et al., 2020). A comparative description is given in **Fig 5** . Considering the benefits and drawbacks of all the cultivation systems of *Spirulina*, open and hybrid systems are widely used for large-scale cultivation of *Spirulina*. Several advantages can be derived from the use of these systems, including their low investment and ease of handling. Hence, a common man can cultivate *Spirulina*

Table 2
Different media used for the cultivation of *Spirulina* (Rajasekaran et al., 2016; Soni et al., 2017).

Ingredient	Zarrouk's media (g/L)	Modified Zarrouk's media (g/L)	Rao's media (g/L)	F/2 media (g/L)	CFTRI media (g/L)	OFERR media (g/L)	George's media (g/L)	Conventional growth media (g/L)	Reduced cost media (g/L)
$NaHCO_3$	16.80	16.8	15	75	4.5	8.0	-	16	16.8
K_2HPO_4	0.50	0.5	0.50	-	0.5	-	0.02	-	0.235
$NaNO_3$	2.50	-	2.50	-	1.5	-	-	-	-
K_2SO_4	1.00	1.00	0.60	-	1.0	0.5	-	0.5	0.353
NaCl	1.00	1.00	0.20	-	1.0	5.0	-	1.00	0.471
$MgSO_4{\cdot}7H_2O$	0.20	0.20	0.04	-	1.2	0.16	0.02	0.1	-
EDTA	0.08	0.08	-	-	-	-	-	-	0.353
$CaCl_2{\cdot}2H_2O$	0.04	0.04	0.008	-	0.04	-	-	0.1	0.176
$FeSO_4{\cdot}2H_2O$	0.01	0.01	-	-	0.01	0.05	-	-	0.265
H_3BO_3	2.86	-	-	-	-	0.052	-	-	2.86
$MnCl_2{\cdot}\ 4H_2O$	1.180	-	-	18	-	-	-	-	1.81
$ZnSO_4{\cdot}7H_2O$	0.222	-	-	22	-	-	-	-	0.222
Na_2MoO_3	0.015	-	-	-	-	-	-	-	0.0177
$CuSO_4.5H_2O$	0.074	-	-	-	-	-	-	-	0.079
NH_4VO_3	22.9	-	-	-	-	-	-	-	-
$NiSO_4.7H_2O$	47.8	-	-	-	-	-	-	-	-
$NaWO_2$	17.9	-	-	-	-	-	-	-	-
Na2MO4.2H2O	-	-	-	6.3	-	-	-	-	-
Na2EDTA·2H2O	-	-	-	4.35	-	-	-	-	-
$Ti2(SO_4)_3{\cdot}6H_2O$	4.4	-	-	-	-	-	-	-	-
Co $(NO_3)_2.6H_2O$	4.4	-	-	10	-	-	-	-	-
Ferric citrate	-	-	-	-	-	-	0.035	-	-
Peptone	-	-	-	-	-	-	1.00	-	-
KNO_3	-	2.5	-	-	-	-	-	2.00	-
$(NH_4)2HPO_4$	-	-	-	-	-	-	-	0.1	-
Chelated iron	-	-	-	-	-	-	-	2 Squeezes(1/4 teaspoon)	-
Lime	-	-	-	-	-	-	-	0.1	-
NH_4NO_3	-	-	-	-	-	-	-	-	0.118
CO $(NH_2)_2$	-	-	-	-	-	0.2	-	-	0.088
Fe EDTA	-	-	-	0.20	-	-	-	-	-
FeCl·6H2O	-	-	-	5	-	-	-	-	-
Na2SIO3.9H2O	-	-	-	30	-	-	-	-	-
Biotin	-	-	-	Trace	-	-	-	-	-
Cyanocobalamin	-	-	-	Trace	-	-	-	-	-

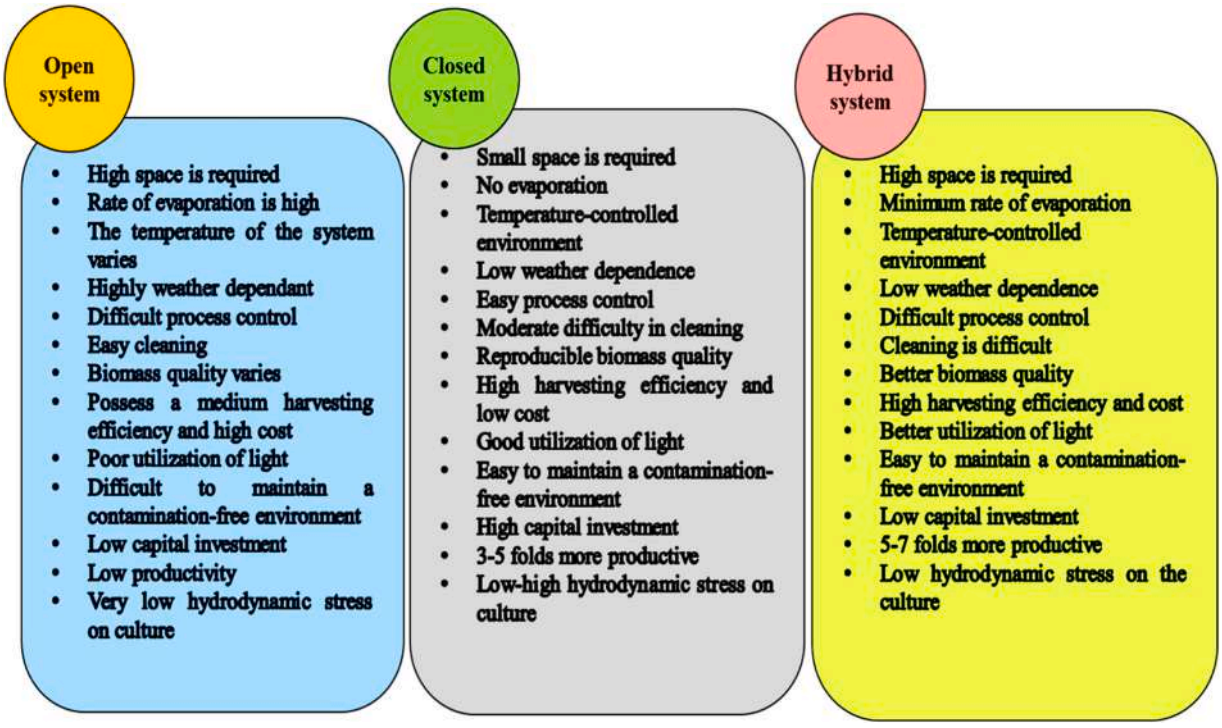

Fig 5. Comparison between *Spirulina* production in an open system, closed system, and hybrid system (combination of open raceway pond and photobioreactor).

without requiring much technical expertise.

4. Extraction and purification of phycocyanin from *Spirulina*

The pigmentation system of *Spirulina sp.* comprises various components, including chlorophyll, phycobilisomes consisting of pigments like phycoerythrin, phycocyanin, phycobilins, and lipophilic pigments such as carotenoids (beta-carotene, cryptoxanthin). *Spirulina* is well known for its noteworthy abundance of phycocyanin (PC), constituting approximately 20–25% of the total biomass (Vernès et al., 2015; Li et al., 2020). The pigment structure consists of a heterodimer: α (alpha) and β (beta) subunits of molecular weight 18 and 20 kDa, respectively (Glazer, 1989; de Morais et al., 2018) and it is present as a super-molecular protein complex consisting of phycoerythrin and allophycocyanin. The primary function of this complex is to harvest the sunlight in the photosystem II reaction center (Pradeep & Nayak, 2019).

Phycocyanin possesses a variety of natural properties that can be used in various industries, therefore, extraction of phycocyanin has increased in recent years. The phycocyanin extraction has been done using various methods, the most used method is freeze-thaw (Saran et al., 2016), followed by homogenization (Puzorjov et al., 2022), ultrasonication (Pan-utai et al., 2022). A combination of extraction methods can also be used to improve extraction, such as soaking *Spirulina* biomass in the solvent followed by ultrafine shearing and ultrasonication (Yu, 2017). Complete exhaustive data regarding the different extraction methods and the efficiency of each method has been detailed in **Table 3** . There are certain benefits as well as drawbacks for all the methods used in phycocyanin extraction and it is been tabulated in Table 4.

5. Purification of phycocyanin

In general, phycocyanin is purified using ammonium sulfate precipitation and dialysis combined with column chromatography (Prabakaran et al., 2020), ultrafiltration (Pan-utai et al., 2022), microfiltration and ultrafiltration (Li et al., 2020) and activated charcoal (Aoki et al., 2021). A summary of the purification methods used by various research groups has been provided in **Table 5** . Recently, a study by Lauceri et al. (2022) proposed an innovative approach that combined both the extraction and purification into a single step where both these crucial steps were done using ammonium sulphate solution and reached a purity > 2.5. All the purification methods involving ammonium sulfate precipitation produces high purity phycocyanin but serves as an expensive method. Recently developed purification method using activated charcoal is a less expensive and a sustainable alternative to the existing purification techniques.

6. Yield enhancement strategy

Enhancement in the phycocyanin yield can be done in two ways, one is by improving the extraction strategy and another is by subjecting the *Spirulina sp.* to abiotic stresses. When the *Spirulina* biomass was subjected to the freeze-thaw method combined with a pulse electric field the yield of phycocyanin was increased up to 147.33 ± 2.45 mg/g (Käferböck et al., 2020). Another study revealed that combining pulse electric field and bead milling increased the phycocyanin yield by 76% $_{w/w}$ (Jaeschke et al., 2019). The yield was further increased by 98.85% w/w when the biomass was subjected to homogenization followed by aqueous two-phase extraction (Patil et al., 2008).

Another strategy for increasing the yield is by subjecting the algal biomass to various stress-inducing conditions during the time of growth. Some examples of abiotic stresses to the algae are stress induced through temperature (Chentir, Doumandji et al., 2018), light (Bachchhav et al., 2017), and media alteration (Chentir et al., 2017). Research by Sang Hyo Lee (2015) found that the phycocyanin yield was increased by 1.28 mg/mL when the *Spirulina* was cultivated through two-stage cultivation, during the first stage the culture was subjected to red and blue LED light, and in the second stage, only blue light was used. Another study by (Chentir et al., 2017) used a two-stage cultivation system with multiple stress factors to enhance phycocyanin production. The study reported a 16% increase in the phycobiliprotein yield when cultivated under a low luminous intensity of 10 μmol photons m^{-2} s^{-1} along with media alteration (11.76 g/L of sodium chloride, 0.5 g/L of sodium nitrate, and 2.68 g/L of potassium hydrogen phosphate). There is very limited research done on the enhancement of phycocyanin using metabolic and genetic engineering strategies. More research has to be done particularly in yield enhancement of phycocyanin using these techniques to lower the production cost and also to reduce the production time. This might be an eco-friendly, reusable, and sustainable alternative to the existing techniques.

7. Application of phycocyanin

Phycocyanin has been explored as a potential functional food due to the demonstration of innumerable natural properties. It has been used as a natural food colorant in confectionery such as candies and gummies, ice cream, dessert coatings, and yogurts (Li et al., 2022). It has been used as a biofluorescent marker (due to its natural fluorescent property) to stain DNA, RBCs, WBCs, and platelets (Ashaolu et al., 2021). It has also been explored for nanoparticle synthesis during photothermal and photodynamic therapy for carcinogenesis treatment (Lee et al., 2016). Phycocyanin has been explored as a pharmaceutical agent, for example, a study by (Blas-Valdivia et al., 2022) observed that phycocyanin reduced the action of enzymes causing acute myocardial infarction in the animal model. The application of phycocyanin in various industries has been depicted in **Fig. 6** .

7.1. Phycocyanin in the food industry

Phycocyanin is generally used as a food colorant in the food industry. Food colorants or coloring additives are substances that impart color the food and beverages. One of the most important parameters considered in the food industry is the color of the food, which has an everlasting impact on the customers (Luzardo-Ocampo et al., 2021). Food colorants are generally divided into two categories: natural and synthetic. Sundown Yellow FCF E110, Tarrazine E102, Allura Red AC E129, Carmoisine E122, and Brilliant Blue are some of the commonly used synthetic food colorants (Li et al., 2022). One of the important advantages of synthetic food colorants is that it is cheap, easily available, and more stable than natural food colorants. Despite possessing plenty of advantages, synthetic food colorants can cause an ample of diseases and disorders including hypersensitivity reactions (caused by blue, red, and yellow dyes), carcinogenic effects (red and yellow dyes), and genotoxicity (red and orange dyes) (Kobylewski & Jacobson, 2012). Therefore, the shift to natural food colorants is occurring, these colorants not only impart color but also fortify the food by adding their physicochemical and bioactive functionalities.

Natural food colorants are extracted generally from various sources such as plants, animals as well as microorganisms. The colorants extracted from plant or animal sources have certain limitations such as lack of availability throughout the year, higher product cost, and lack of scalability (Rodriguez-Amaya, 2016). Microorganisms, especially microalgae are the best alternative source of natural food colorants as they can easily surpass the drawbacks of the natural pigments derived from animal and plant sources, they can be cultivated throughout the year, have low production costs, and are easy to scale up (Alam et al., 2020). Phycocyanin is one such pigment found extensively in *Spirulina sp.*, it has abundant beneficial properties (due to which it can be called a nutraceutical), and its splendid blue hue highly attracts customers. Phycocyanin has been experimentally incorporated in many food products such as jelly candy (Dewi et al., 2018), dairy products such as yogurt (Moreira et al., 2012), skim milk (Galetovic et al., 2020), ice

Table 3
Different extraction methods involved in phycocyanin extraction.

S. No	Species	Extraction method	State of biomass	Extraction parameters	Extraction duration	Solvent used	Yield or concentration of phycocyanin	Reference
1	*Spirulina platensis (CCC540)*	Freeze-thaw	Dried	Freezing temperature-(−20 °C), Thawing-room temperature	Not mentioned	20 mM AB (50 mM sodium chloride and 0.002 M sodium azide) (pH 5.10)	77.4 μg/mL	Kumar et al. (2014)
2	*Spirulina maxima*	NA	Dried	No pre-treatment, Incubated at room temperature	240 min	Calcium chloride	3.4 g /100 g	Herrera et al. (1989)
					280 min	Sodium nitrate	13.2 g/100 g	
3	*Spirulina* sp.	Water extraction	Wet	Biomass was suspended in solvent and incubated at 4 °C	1440 min	Water	13.40 ± 1.1 mg/g	Doke (2005)
		Homogenization		Biomass was suspended in solvent and homogenised	5 min	PB (pH 7)	82.10 ± 0.8 mg/g	
		Freeze-thaw		Freezing temperature-(−20 °C), Thawing-room temperature	240 min	PB (pH 7)	86.30 ± 1.1 mg/g	
		Buffer extraction	Dried	Biomass was suspended in buffer and incubated at 4 °C	1440 min	PB (pH 7)	80 ± 1.9 mg/g	
4	*Spirulina platensis LEB 52*	Water extraction	Dried	Biomass was suspended in solvent at 25 °C	240 min	Water	11.86 and 2.75 mg/mL	Moraes & Kalil (2009)
5	*Nostoc commune TUBT05*	Freeze-thaw	Wet	Freezing temperature-(−20 °C), Thawing-room temperature for 3 cycles	240 min	PB (pH 7)	29.66 ± 0.52 mg/g	Chittapun et al. (2020)
	Oscillatoria okeni TISTR8549			Freezing temperature-(−20 °C), Thawing-room temperature for 18 cycles	1080 min	Tris HCl buffer (pH 8)	39.93 ± 0.90 mg/g	
	Nostoc commune TUBT05	Pulse electric field		PEF treatment – 1500 pulses at 5 kV cm-1	Not mentioned	Distilled water	543.7 ± 28.78 mg/g	
6	*Arthronema africanum*	Freeze-thaw	Wet	Freezing and heated at 30 °C	60 min	0.001 M PB (pH 6.7) and 0.15 M NaCl	100 mg/500 mg	Minkova et al. (2007)
7	*Calothrix sp.*	Enzymatic extraction	Wet	Biomass was suspended in a solvent with enzymes	1440 min	0.1 M PB (pH 7) with EDTA and lyzozyme	0.03 mg/mL	Santiago-Santos et al. (2004)
8	*Oscillatoria quadripunctulata*	Freeze-thaw	Wet	Biomass was suspended in the solvent and 2 cycles of freeze-thaw were conducted	Not mentioned	1 M tris-Cl buffer (pH 8.1)	27.43 mg/mL	Soni et al. (2006)
9	*Phormidium sp.* *Spirulina* sp. *Lyngbya sp.*	Freeze-thaw	Dry	Biomass was suspended in the solvent, sonicated, the freeze-thaw cycle was followed (freezing-20 °C and thaw- room temperature)	Sonication-60 s	0.1 M PB (pH 7) with 1 mM sodium azide	4.1% w/w 17.5% w/w 3.9% w/w	Patel et al. (2005)
10	*Spirulina* fusiformis	Freeze-thaw	Wet	Freezing and heated at 30 °C	60 min	0.001 M PB (pH 6.7) and 0.15 M NaCl	1.28 mg/mL	Minkova et al. (2003)
11	*Spirulina platensis* UTEX 1926	Repeated freeze-thaw+sonication	Wet	Biomass was suspended in solvent and freeze-thaw cycles were conducted followed by sonication for 3 min	60 min	0.05 M PB (pH 7)	NA	Zhang & Chen (1999)
12	*Spirulina platensis (CFTRI)*	Freeze-thaw	Wet	Biomass was suspended in the solvent and 2 cycles of freeze-thaw was conducted	Not mentioned	50 mM PB (pH 6.8)	19.47 mg/ 100 mg	Sarada et al. (1999)
13	*Spirulina platensis Geitler*	Enzymatic extraction	Wet	Biomass was suspended in the solvent containing enzyme	Not mentioned	0.05 M PB (pH 7) + lysis buffer	0.0013 mg/mL	Bhaskar et al. (2005)
14	*Spirulina platensis*	Solvent extraction	Dry	Biomass was suspended in solvent	Not mentioned	Water	3.73 ± 0.12 mg/ mL	Silveira et al. (2007)

(*continued on next page*)

Table 3 (*continued*)

S. No	Species	Extraction method	State of biomass	Extraction parameters	Extraction duration	Solvent used	Yield or concentration of phycocyanin	Reference
						PB (pH 7)	4.20 ± 0.72 mg/mL	
15	*Spirulina platensis*	Freeze-thaw	Dry	Biomass was suspended in solvent and 3 freeze-thaw cycles was conducted (freezing-20 °C and thawing 4 °C)	Freezing − 180 min, thawing- 5 min	0.1 M PB (pH 7)	146 ± 0.265 mg/g	Saran et al. (2016)
16	*Spirulina platensis*	Freeze-thaw	Dry	Biomass was suspended in the solvent and 4 cycles of freeze-thaw were conducted	1800 min	0.1 PB (pH 6.8)	73.73 ± 1 mg/g	Tavanandi et al. (2018)
		Ultrasound		Biomass was suspended in solvent and ultrasound was performed at 20 kHz	2.5 min		51.51 ± 2 mg/g	
18	*Synechococcus sp. IO9201*	Freeze-thaw	Wet	Biomass was suspended in solvent and 3 freeze-thaw cycles was conducted	Not mentioned	Distilled water	0.0134 mg/mL	Abalde et al. (1998)
		Sonication		Sonication was done at 4 °C	5 min		0.0074 mg/mL	
19	*Spirulina platensis*	High-pressure processing	Dry	Biomass was suspended in the solvent and subjected to a pressure ranging from 100 Mpa	3.5 min	PB (pH 7)	The yield was given as absorbance (620 nm) = 0.35	Li et al. (2020)
		Pulse electric field		Biomass was suspended in the solvent and subjected to 50 to 200 pulses at 20 kV	Not mentioned		The yield was given as absorbance (620 nm) = 0.25	
		Ultrasonication		Biomass was suspended in the solvent and subjected to ultrasonication for 6 min	Not mentioned		Yield was given as absorbance (620 nm)= 1.25	
20	*Spirulina platensis*	Repeated freeze-thaw cycles	Wet	Biomass was suspended in the solvent and subjected to the freeze-thaw cycle (freezing at −20 °C, thaw-room temperature)	Not mentioned	20 mM AB with 50 mM sodium chloride and 0.002 M sodium azide (pH 5.10)	52.82% w/w	Prabakaran et al. (2020)
21	*Spirulina platensis*	High-pressure processing	Dry	Biomass was subjected to high pressure in the presence of hexane	720 min	Distilled water	8.40%	Seo et al. (2013)
22	*Spirulina platensis*	Soaking	Dry	Biomass was suspended in the solvent and soaked	1440 min	PB (pH 6.5)	8.91%	Yu (2017)
		Ultrasonication		Biomass was suspended in the solvent and ultrasonicated	8 min		7.97%	
		Freeze-thaw		Biomass was suspended in the solvent and 4 cycles of freeze-thaw was conducted (freezing at −20 °C and thawing at 25 °C)	Not mentioned		8.26%	
		Soaking-ultrafine shearing		Biomass was suspended in the solvent, soaked and ultrafine shearing is done	480 min-soaking, 8 min-ultrafine shearing		8.89%	
		Soaking-ultrafine shearing-ultrasonication		Biomass was suspended in the solvent, soaked, ultrafine sheared, and ultrasonicated	1440 min-soaking, 10 min-ultrafine shearing, 10 min-ultrasonication		9.07%	
23	*Spirulina platensis*	Homogenization	Wet	Biomass was suspended in the	5 min	Distilled water	80%	Chethana et al. (2015)

(*continued on next page*)

Table 3 (*continued*)

S. No	Species	Extraction method	State of biomass	Extraction parameters	Extraction duration	Solvent used	Yield or concentration of phycocyanin	Reference
				solvent and homogenized at 200-400 kg/cm2				
24	*Cyanidium caldarium*	Freeze-thaw	Dry	Biomass was suspended in solvent and freeze-thaw cycles was conducted (freezing-70 °C and 4 °C thawing)	Freezing-1440 min and thaw-1440 min	Distilled water	0.12 mg/g	Sommer et al. (2021)
		Sonication		Biomass was suspended in solvent and ultrasonication was performed	1 min and 44 s		53 mg/g	
		Spark discharges		Biomass was suspended in a solvent and spark discharges were ignited	30 min		4 mg/g	
25	*Spirulina maxima*	Aqueous extraction	Dry	Biomass was suspended in the solvent and incubated at room temperature	1440 min	Ultrapure water	1.174 ± 0.0244 mg/mL	Nisticò et al. (2022)
26	*Spirulina maxima*	Ultrasonication	Dry	Biomass was suspended in a solvent and ultrasonication was performed	1440 min	Distilled water	11.3 ± 0.06 mg/mL	Choi & Lee (2018)
27	*Synechocystis sp. PCC 6803*	High-pressure homogenization	Dry	Biomass was suspended in a solvent and homogenized at 10,000-25,000 psi	20 min	PBS (pH 8.8)	75.3 ± 1.7 mg/g	
28	*Pseudanabaena sp. ABRG5-3* *Limnothrix sp. SK1-2-1* *Spirulina platensis* (NIES-39)	Water extraction	Wet	Biomass was suspended in a solvent and incubated at room temperature	Not mentioned	Distilled water	30.4% w/w 28.9% w/w 7.8% w/w	Puzorjov et al. (2022)
29	*Spirulina platensis*	Ultrasonication	Dry	Biomass was suspended in a solvent and ultrasonication was performed	30 min	0.01 M PB (pH 7) Distilled water	56.091 ± 0.73 mg/g 47.703 ± 0.94 mg/g	Aoki et al. (2021)
30	*Spirulina platensis*	Ultrasonication	Wet	Biomass was suspended in a solvent and ultrasonication was performed	8 min	PBS (pH 6)	1.142 ± 0.1869 mg/g	Pan-utai et al. (2022)
31	*Spirulina platensis*	Ultrasonication	Dry	Biomass was suspended in solvent and ultrasonication and liquid biphase floatation was performed	5 min	PB (pH 7)	81.2 ± 0.28% w/w	Kunte & Desai (2017)
32	*Spirulina platensis* 21.99	Pulse electric field+bead milling	Dry	Biomass was suspended in a solvent, pulse-electric field pre-treatment is performed and bead milling was done	PEF treatment for 15 min and bead milling for 1.6 min at the 20 s for 4 cycles	PB (pH 7.2)	76% w/w	Chew et al. (2019)
33	*Desertifilum tharensde UAM-C/S02*	Homogenisation	Wet	Biomass was suspended in a solvent and homogenized at 3000 rpm and a freeze-thaw cycle was performed	1 min and 30 s	20 mM Tris HCl buffer (pH 8)	12.78 ± 0.64% w/w	Jaeschke et al. (2019)
34	*Spirulina platensis*	Microwave-assisted extraction	Dry	Biomass was suspended in two types of solvent system-polar and non-polar solvents	Polar solvents-55 min Non-polar solvent-55 min	10 mM Ammonium acetate and ethanol Limonene and ethyl acetate	1.13 ± 0.09% w/w 5.26 ± 0.11% w/w	Hernández, Martínez et al. (2022)
35	*Cyanidioschyzon merolae*	Sonication	Wet	Biomass was suspended in the solvent and incubated at 40 °C and the sample was sonicated	Sonication for 10 s	0.5 M $MgCl_2$	112 mg/g	Esquivel-Hernández et al. (2017)
36	*Spirulina* sp.	Ultrasonication	Dry	Biomass was suspended in the	15 min	1% Calcium chloride	19.5 ± 0.5 mg/g	Yoshida et al. (2021)

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Table 3 (*continued*)

S. No	Species	Extraction method	State of biomass	Extraction parameters	Extraction duration	Solvent used	Yield or concentration of phycocyanin	Reference
				solvent and the ultrasonication was performed at 50 kHz, 300 W at 30 °C				
37	*Spirulina* sp.	Solvent extraction	Dry	Biomass was suspended in a solvent and incubated	NA	100 mM PB (pH 7)	14.98 ± 0.87	Hadiyanto et al. (2021)
38	*Spirulina* sp.	Solvent extraction	Dry	Biomass was suspended in a solvent and incubated	1440 min	0.856 mol/L NaCl	102.4 mg/g	Chaiklahan et al. (2018)
39	*Spirulina* sp.	Solvent extraction	Dry	Biomass was suspended in a solvent and incubated at room temperature	240 min	100 mM PB (pH 7)	5.81 ± 2.71 mg/mL	Wang et al., 2022
40	*Spirulina platensis* MK343101	Solvent extraction	Wet	Biomass was suspended in a solvent and incubated at 35 °C	10 min	Distilled water	45.4 ± 0.01 mg/g	Sivasankari et al. (2021)

PB-Phosphate buffer, PBS-Phosphate buffered saline, and AB- Sodium acetate buffer

Table 4
Benefits and drawbacks of different phycocyanin extraction methods.

S. No	Extraction methods	Benefits	Drawbacks	References
1.	Freeze-Thaw cycle	• Easy to operate • Easily available equipment • Less technical expertise required • High yield	• Highly energy and time-consuming process • Increases production cost • Suitable only for laboratory scale and not for industrial use	Doke (2005),Kumar et al. (2014),Chittapun et al. (2020), Prabakaran et al. (2020)
2.	Enzyme extraction	• Efficient technique • Eco-friendly	• This technique yields more when combined with other extraction method rather than being standalone • Combining techniques rises production cost • Technical expertise needed • Stability maybe affected	Santiago-Santos et al. (2004),Bhaskar et al. (2005), (Jung et al., 2022), (Tavanandi & Raghavarao, 2020)
3.	Mixing or homogenisation	• Easy to operate • Easily available equipment • Less technical expertise required	• Might cause change in the temperature • Time consuming • Suitable only for laboratory scale and not for industrial use	Chethana et al. (2015),Choi & Lee (2018),Li et al. (2020)
4.	Ultrasonication	• High purity phycocyanin can be produced • Easy to scale up • Suitable for industrial scale	• Might damage the phycocyanin upon continous exposure to ultrasound • Might cause change in the temperature • Technical expertise needed • Increases production cost	Esquivel-Hernández et al. (2017),Aoki et al. (2021), Pan-utai et al. (2022)
5.	Pulse electric field	• High yield and purity of phycocyanin • Easy to scale up • Suitable for industrial scale • Reusable technique	• High production cost • High technical expertise required	Chittapun et al. (2020),Li et al. (2020), (Käferböck et al., 2020), (Knappert et al., 2022)
6.	Solvent extraction	• Low production cost • No complicated equipments required • Less technical expertise required • Easy to operate • Suitable for industrial scale	• Inconsistent yield • High solvent requirement	Chaiklahan et al. (2018),Sivasankari et al. (2021), Wang et al., 2022

creams and beverages (García et al., 2021).

Phycocyanin was added as a colorant in three types of beverages such as wine, isotonic and tonic beverages, and the color stability was analyzed for a period of 15 days. The study results revealed that the color of the beverage was stable and phycocyanin can be used as an alternative to the existing synthetic colorants (García et al., 2021). Galetović et al. (2020) formulated a phycobiliprotein-fortified fortified skim milk, this milk has immense properties one of which is anti-oxidant properties. The fortified milk was stable at a high temperature around 138 °C for 4 s. Phycocyanin was incorporated with icecream and the color stability and anti-oxidant activity were studied. The results revealed that the color of the was stable until 182 days and insignificant color loss was observed after that period and the anti-oxidant activity was 13 folds higher than the control (Campos Assumpção de Amarante et al., 2020). During a study, Chentir et al. (2019) incorporated phycocyanin into gelatin to increase the bioactivity (such as antioxidant and antibacterial activity) of the film, which could be potentially applied in food and in the pharmaceutical industry as a food packaging material

Table 5
Various purification techniques used in phycocyanin purification.

S.No	Purification techniques	Purity ratio	Grade of phycocyanin	References
1.	Ammonium sulfate precipitation combined with size exclusion and ion exchange chromatography	4.59	Analytical and reagent	Patel et al. (2005)
2.	Ion exchange chromatography	5.1 ± 0.08	Analytical and reagent	Patil et al. (2006)
3.	Microfiltration	1.09 ± 0.08	Food	Zhu et al. (2007)
4.	Ammonium sulfate precipitation combined with ion exchange chromatography	4.0	Analytical and reagent	Moraes & Kalil (2009)
5.	Microfiltration and ultrafiltration	1.11 ± 0.32	Food	Chaiklahan et al., 2011
6.	Ammonium sulfate precipitation and ion exchange chromatography	4.58	Analytical and reagent	Kumar et al. (2014)
7.	Ammonium sulfate precipitation and column chromatography	3.2	Food	Saran et al. (2016)
8.	Ultrafiltration	1.62 ± 0.04	Food	Sala et al. (2018)
9.	Ammonium sulfate precipitation and column chromatography	6.17 ± 0.075	Reagent	Purohit et al. (2019)
10.	Liquid biphasic flotation technique	2.6	Food	Chew et al. (2019)
11.	Activated charcoal	0.67 ± 0.03	Food	Pan-utai & Iamtham (2019)
12.	Ultrafiltration	0.8	Food	Campos Assumpção de Amarante et al. (2020)
13.	Ammonium sulfate precipitation and column chromatography	5.72	Analytical	Sivasankari et al. (2021)
14.	Column chromatography	1.7	Food	Yoshida et al. (2021)
15.	Activated charcoal	3.1	Food	Aoki et al. (2021)
16.	Ultrafiltration and diafiltration	1.16 ± 0.010	Food	Nisticò et al. (2022)
17.	Ammonium sulfate precipitation and size-exclusion chromatography	2.9 ± 0.7	Food	Puzorjov et al. (2022)
18.	Ammonium sulfate precipitation and size-exclusion chromatography	4.5	Analytical	Chen et al. (2022)

The grade of phycocyanin is determined using the purity ratio values, $\geq$ 0.7 is food grade phycocyanin, $\geq$ 3.9 is reagent grade phycocyanin, and $\geq$ 4.0 is analytical grade phycocyanin (Patil et al., 2006).

for dehydrated food products and capsules having a soft or hard shell. The exploration of phycocyanin as a functional food or food colorant has been less extensive in comparison to studies focusing on its nutraceutical properties. To unlock its full potential as a food colorant, further research must be conducted effectively.

Though phycocyanin has been experimented with as a food additive in a variety of food products, it is commercially available in powder form which can be added to the food of interest as a food colorant by the consumer. Some of the commercial producers of phycocyanin are listed in **Table 6** .

7.2. Phycocyanin in other applications

7.2.1. Phycocyanin in the cosmetic industry

Phycocyanin has also been explored in the cosmetic industry for its vibrant hue and abundant natural properties. Phycocyanin lip balm has been a huge success among other cosmetic products, as it is being manufactured by various companies and has also reached many customers (Sowndarya, 2021). When used with the proper developer and mordant, phycocyanin showed the potential to be an active ingredient in hair dye as 50% of the hair color remained intact after 5 shampoo washes. It also demonstrated a steady color deterioration when subjected to a stability test by adjusting heating/cooling cycles (Kraseasintra et al., 2022).

Phycocyanin can be formulated in anti-aging creams due to the abundance of antioxidant properties to scavenge the reactive oxygen species (ROS) which is a major causative factor for aging. A study was conducted by (Feng et al., 2022) where the anti-aging property of the phycocyanin was studied by using *in vivo* (*Drosophila melanogaster*) and *in vitro* (H_2O_2 induced HUVEC cells) models. The *in vivo* model showed a decreased level of ROS generation, and lipid peroxidation was inhibited which was monitored using the stress markers such as SOD1, SOD2, and CAT. There was an inhibition of free radicle generation as well as protection against the H2O2-induced HUVEC cell to apoptotic death. Wu et al. (2011) conducted a study on the anti-melanogenic property of phycocyanin, revealing that it inhibits tyrosine gene expression, leading to the suppression of melanin synthesis. This property of phycocyanin can find a potential application in the cosmetic industry in the form of sunscreen.

The anti-acne property of phycocyanin has also been reported by Nihal et al. (2018), in which the phycocyanin showed its activity against two strains of bacteria causing acne which were *Propionibacterium acne* and *Staphylococcus epidermidis.* There are a few companies that have established using phycocyanin as an ingredient in their products. Beauty Relay London is a cosmetic company that produces face cream, facial kits, and hair masks which were formulated with phycocyanin as a key ingredient (https://www.beautyrelay.com/), similarly, The Lipbalm company produces), balm incorporated with phycocyanin (https://thelipbalmco.in/).

7.2.2. Phycocyanin as a nutraceutical

Phycocyanin has an antioxidant capacity to scavenge the ROS in the *Spirulina* cells as a defense mechanism against abiotic stress factors. This property of phycocyanin has been used against various diseases which are produced due to the overproduction of ROS, the cells undergo oxidative stress which is a pathological characteristic of several diseases such as diabetes, cancer, neurodegenerative disorders, atherosclerosis, hyperpigmentation, and inflammation (Fernandes Raquel, Campos, Serra, Fidalgo Javier, & Almeida Hugo, 2023). The beneficial properties possessed by phycocyanin against various physiological diseases have been illustrated in **Fig. 7** .

Cancer is defined as a medical condition in which cells proliferate and disseminate uncontrollably (Dranseikienė et al., 2022). Phycocyanin has been used to treat cancer as it naturally has anti-cancer properties against various cancers such as liver cancer (Jiang et al., 2017), breast cancer (Heisnam et al., 2022), colon cancer (Wen et al., 2020), leukemia (Yu et al., 2022), and lung cancer (Hao et al., 2021). The possible mechanisms by which phycocyanin is exhibits its anticancer activity against cancer cells are by suppressing the cell cycle at specific phases, modifying the redox state of the cells, and promoting the expression of various genes and receptors responsible for necrosis and apoptosis (Dranseikienė et al., 2022). The activation of genes such as caspase-9 and − 3, which are responsible for DNA fragmentation and cell shrinkage, was initiated by phycocyanin, indicating its significant role in apoptotic pathways. Additionally, it elicits cleavage of poly [ADP-ribose] polymerase 1 (PARP-1) and alters the ratio of Bcl-2/Bax (Fernandes e Silva et al., 2018) The most documented apoptotic

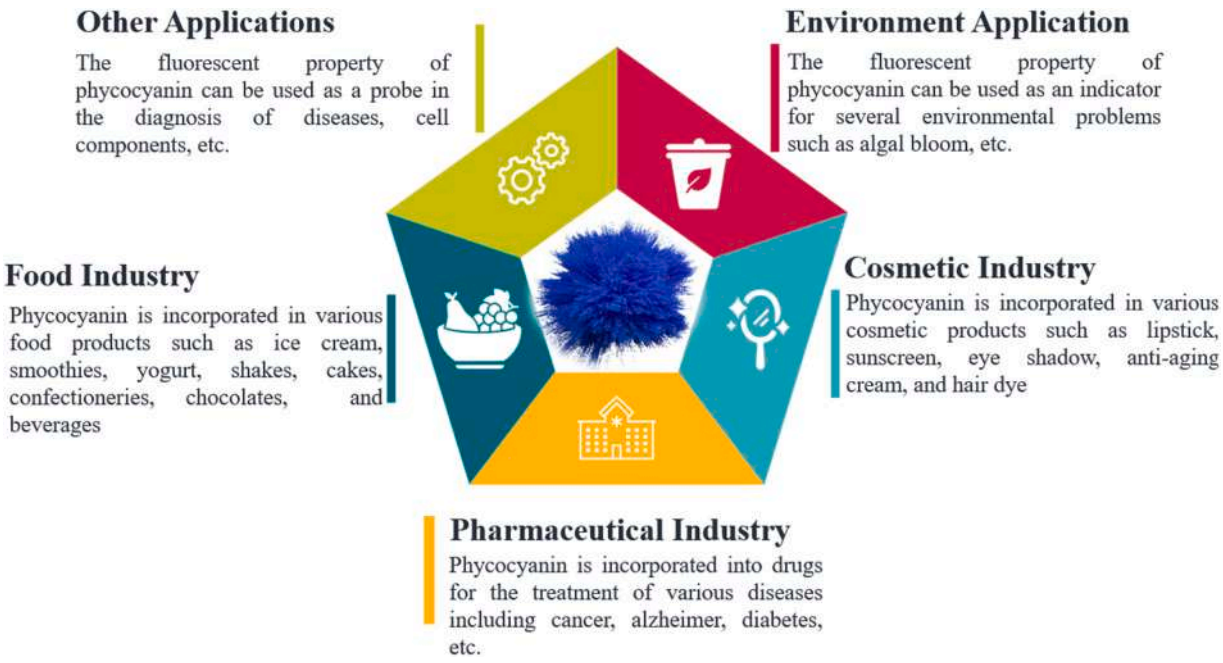

Fig. 6. Various applications of phycocyanin in food, pharmaceutical, cosmetic industries, and other industries.

Table 6
List of a few companies producing phycocyanin powder.

S.No	Name of the company	Reference
1.	Urban Platter	https://urbanplatter.in/
2.	Aspermuhle	https://www.aspermuehle.de/Algen/
3.	Organic Naturals India Private Limited	https://www.organicnaturalsindia.co.in/
4.	Parry Nutraceutical	https://parrynutraceuticals.com/
5.	Zhejiang Binmei Biotechnology Co., Ltd	https://www.binmeibio.com/
6.	Phyco-Biotech Laboratories	https://www.phytobiotech.in/
7.	AlgoSource	https://algosource.com/
8.	Merck KgaA	https://www.merckgroup.com/en
9.	Bluetec Naturals Co., Ltd	http://www.bluetecnaturals.com/
10.	Exberry	https://www.exberry.com/
11.	Givaudan SA	https://www.givaudan.com/
12.	DIC Corporation	https://www.dic-global.com/ap/

features encompass chromatin margination and condensation into dense granules or blocks, an elevated percentage of cells in the sub-G0/G1 phase, microvilli loss, cellular contraction, and membrane blebbing (Li et al., 2006). An overview of the effect of phycocyanin against various cancer cell lines has been detailed in Table 7.

Romay et al. (2018) initially reported the anti-inflammatory characteristic of phycocyanin. The study revealed that phycocyanin effectively suppressed liver microsomal lipid peroxidation, inflammation in mouse paws, and edema induced by glucose oxidase. Subsequently, the same research team detected a significant anti-inflammatory effect of PC across diverse inflammatory models. This effect was attributed to the effective reduction of histamine release, as well as the minimization of myeloperoxidase and prostaglandin E-2 levels (Romay et al., 2011). Recent research has indicated that PC exhibits anti-inflammatory properties and can target TLR (Lu et al., 2020), NF-κB (Alzokaky et al., 2020), and PI3K/Akt/mTOR pathway (Hao et al., 2018), thereby directly preventing inflammation. Furthermore, it has been observed that PC could enhance the expression of Nrf2 and subsequently inhibit the inflammatory response of tissues through its antioxidant properties (G. Liu, Li et al., 2020; Q. Liu, Li et al., 2020). More research on the inflammatory properties will aid in developing anti-inflammatory drugs made of phycocyanin.

Neurodegeneration is a condition characterized by the progressive and irreversible decline in the structure and function of neurons in both the central and peripheral nervous systems (Habib et al., 2018). Phycocyanin has been reported to promote the process of remyelination in the damaged brain tissue caused by multiple sclerosis and ischemic stroke (Pentón-Rol et al., 2018). The same research group found that the biological function of phycocyanin indicates its potential to mitigate the advancement of various neurodegenerative ailments including multiple sclerosis, Parkinson's disease, and Alzheimer's disease (Pentón-Rol et al., 2021). The compound phycocyanin exhibited scavenging properties towards peroxynitrite species and demonstrated inhibition of lipid peroxidation and oxidative DNA damage, which are recognized markers of multiple sclerosis, in the experimental autoimmune Encephalomyelitis (EAE)-induced multiple sclerosis model (Bhat & Madyastha, 2001). Research conducted on models of Parkinson's disease has demonstrated that phycocyanin provides protection against toxicity caused by α-synuclein and the formation of amyloid-β (Aβ) fibrils. Phycocyanin was observed to reduce the activity of enzymes that are linked to the development of Alzheimer's disease, including those involved in the production of Aβ (Koh et al., 2018). The potential of phycocyanin as a drug for neurodegenerative disorders is a subject of interest for future development and utilization.

Diabetes Mellitus is a chronic condition that is associated with disorders of metabolism such as hypertriglyceridemia, hyperglycemia, and hyperinsulinemia. It affects the body's ability to process sugar, which can lead to hyperglycemia. Type 1 diabetes and type 2 diabetes are the two most common kinds of diabetes. The potential of phycocyanin as a drug for neurodegenerative disorders is a subject of interest for future development and utilization (Hao et al., 2022). Researchers have been exploring phycocyanin as an anti-diabetic agent and much research has accounted that it can be used as an anti-diabetic agent and contains antiglycation activity (Husain et al., 2022). Phycocyanin as a diabetic-resistant candidate inhibited α- amylase and β-glucosidase in *in-vitro* conditions, reducing the amount of starch that is absorbed and it also promoted the uptake of glucose in the insulin-resistant cell line (Siti Halimatul Munawaroh et al., 2020). In one such research, El-Sayed et al. (2018) conducted a study wherein a rat model of type II diabetes mellitus was orally administered 50 mg/kg phycocyanin for a duration of 30

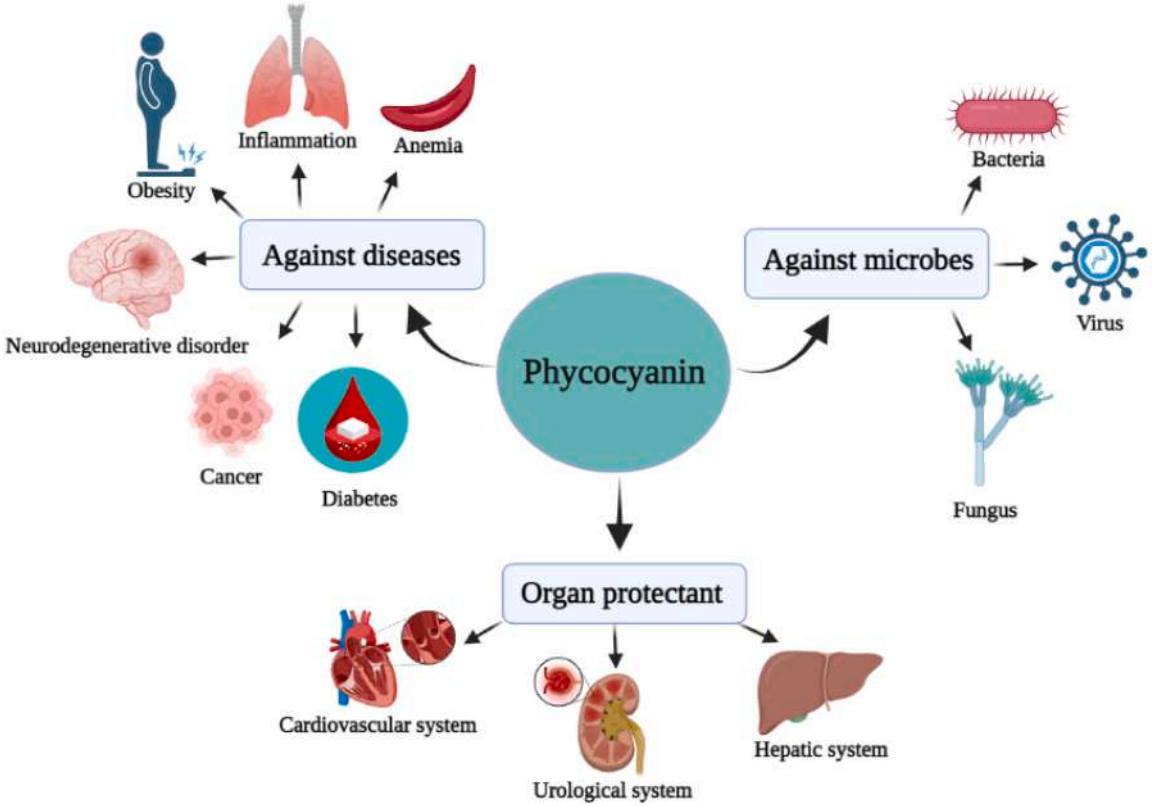

Fig. 7. Therapeutic application of phycocyanin against various diseases causing microbes,diseases and also protects various organs.

Table 7
An overview of the effect of phycocyanin in combination with other drugs or therapy in *in vivo* studies against different cancer.

S. No	Type of tumor	Cell line	Concentration of phycocyanin	Combination drug	Treatment duration (in days)	References
1.	Lung cancer	A549 cells	40 or 80 μg/L	Betaine	28	Li et al. (2015)
2.	Lung cancer	A549 cells	320 mg/mL	Retinoic acid (All-trans)	10	Bingula et al. (2016)
3.	Cervix cancer	HeLa cells/ SiHa cells	NA	The phycocyanin was functionalized using CD95sp nanoparticles	20	G. Liu, Li et al. (2020); Q. Liu, Li et al. (2020)
4.	Colon cancer	NA	Upto 200 mg/Kg body weight of the animal model	Piroxicam	42	Saini & Sanyal (2014)
5.	Breast cancer	MCF-7 cells	320 mg/mL	Photodynamic therapy	13	Li et al. (2010)
6.	Liver cancer	H22 cells	10 mg/mL	Photodynamic therapy	10	Li et al. (2015);Liu et al. (2018)

days. The results of the study indicated a decrease in insulin resistance, lipid levels, and blood glucose concentration following the treatment period. Consequently, phycocyanin has been recommended as a natural substance that alleviates insulin resistance and enhances insulin sensitivity.

7.2.3. Phycocyanin as a fluorescent marker for medical and environmental application

When the phycobiliproteins are subjected to buffer treatment during extraction, they tend to lose their excitation energy as an effect of which the molecule becomes highly fluorescent. The fluorescent property of phycocyanin has been widely explored and is also used for various medical and other applications (Bermejo, 2014). Phycocyanin has been used as a prognostic and theranostic agent for some human diseases, it has also been employed in the diagnosis of diseases in mammals and birds. Diagnosis of cell components can also be done using phycocyanin, which has been widely used in the diagnosis of cell lineage and its subsets, and other cell components (Vinothkanna & Sekar, 2020).

Phycocyanin (a natural fluorophore) has also been proposed as an alternative to ethidium bromide attributed to its higher affinity towards the genomic DNA of humans and plants (guava), and other components of blood such as red blood cells, lymphocytes, and platelets. It also possesses a high staining ability along with a very high strength of the reaction (Singh et al., 2010). It has also been found to have a wide application in fluorescence microscopy, fluorescent immunoassay, flow cytometry, histochemistry, and fluorescent-activated cell sorting (Bermejo, 2014). One such application of phycocyanin has been explored where it has been used as a fluorescent probe and used in the quantitative detection system where the phycocyanin is conjugated with a light emitting diode–charge–coupled device (LED-CCD) fluorescent density strip in the detector (Zheng et al., 2019).

Phycocyanin also finds environmental applications as it can be used as a fluorescent probe in the detection of cyanobacterial bloom in lakes, as the concentration of phycocyanin was positively correlated with the

biomass of cyanobacteria in lakes (Loisa et al., 2015). It has also been used as an *in-vivo* probe in the drinking water treatment plant to detect the cell number of the cyanobacteria present in the water sample which was considered major breakthrough research as the real-time data could be interpreted using this probe (Zamyadi et al., 2014). Thomson-Laing et al. (2020) have developed a sensor named CyanoFluor for the detection of cyanobacterial bloom in the field. This detector has embedded phycocyanin as a fluorescent sensor that was a potential alternative in terms of cost and feasibility to the existing sensors for the detection of cyanobacterial biomass.

8. Economic analysis

According to market research, the global market size (CAGR) of *Spirulina* is estimated to reach approximately 9.5% between 2023 and 2028 (www.marknteladvisors.com), with an annual production of roughly 60 to 80 thousand tonnes (see **Fig.8**). The phycocyanin market is predicted to reach $279.6 million by the year 2030 at a CAGR of 28.1% from the year 2023–2030. The phycocyanin production cost was as low as $249.70 kg^{-1} which also attributes to the large-scale production of phycocyanin and increasing market size (Chaiklahan et al., 2018). The market size of phycocyanin was $129.86 million in 2020 and $152.32 million in the year 2022 (**see Fig.9**). Also, the global phycocyanin market is anticipated to reach 3587.2 tonnes by 2030, expanding at a CAGR of 33.8% between 2023 and 2030 (www.alliedmarketresearch.com and www.marketandmarkets.com).

The research presents a competitive landscape based on an exhaustive analysis of the geographic presence, product portfolio offerings, and significant strategic moves adopted by leading market players in this market in the past three to four years. This analysis was done to provide an overview of the current state of competition in the phycocyanin market. The following companies are currently leading the pack in the global market for phycocyanin: AlgoSource (France), Bluetec Naturals Co., Ltd. (China), DIC Corporation (Japan), E.I.D. - Parry (India) Limited (India), Fuqing King Dnarmsa *Spirulina* Co., Ltd. (China), Givaudan SA (Switzerland), GNT Group B.V. (Netherlands), Hangzhou OuQi. The Asia-Pacific region is predicted to experience the most rapid growth rate from 2023 to 2030. This can be attributed to the presence of numerous local and regional stakeholders, as well as the burgeoning demand for processed food products in countries such as India, China, Indonesia, and Thailand. **Table 8** depicts the major contributing countries in the phycocyanin market till 2028. Phycoyanin can be sold in two common forms, powder and liquid and can be utilized in food and beverage, nutraceuticals, and cosmetics industries. Stakeholders in these markets are poised to benefit from the escalating demand for phycocyanin in these sectors (www.meticulousresearch.com).

9. Future prospective

The use of microalgae especially *Spirulina* biomass as a versatile source for extracting various valuable compounds, such as pigments, lipids, polyunsaturated fatty acids, vitamins, carbohydrates, and antioxidants, has increased significantly globally over the past decade. Several industries, such as cosmetics, pharmaceuticals, and nutraceuticals, are using these compounds as sustainable and renewable raw materials in the formulation of their products. Considering the harmful effects of synthetic colors and pigments, natural pigments are preferable for health applications, particularly algal pigments, which offer greater efficacy. Although micro-algal bio-pigment production technology has advanced significantly over recent years, there remain some gaps. A few bottlenecks include the development of sustainable and cost-effective cultivation methods for *Spirulina* and other microalgae to produce high-quality phycocyanin with minimal environmental impact, exploring novel extraction and purification techniques that can produce high-purity phycocyanin at lower costs and with higher yields.

Higher yield of pigments can be achieved by the following methods, bio-prospecting in unexplored micro-algal environments which might lead to the discovery of high-yielding strains, future studies must focus on the regulatory mechanisms in pigment production within the microalgae for the overproduction of pigments, in order to facilitate the application of metabolic engineering, genetic engineering, and synthetic biology-based strategies to enhance existing pathways for micro-algal pigment production or to develop new pathways for increased production. To reduce production costs, optimized technology for efficient algal biomass and pigment recovery must be used such as using standard materials when building facilities, increasing equipment size, redesigning photobioreactors so that they consume less power, and automating to reduce labor costs. Integration of phycocyanin production and utilization into circular economy models, where waste streams from other industries are used as nutrients for microalgae cultivation and phycocyanin production. Further research is needed to enhance our understanding of the wall material composition of microalgae, enabling the development of an energy-efficient method for phycocyanin extraction.

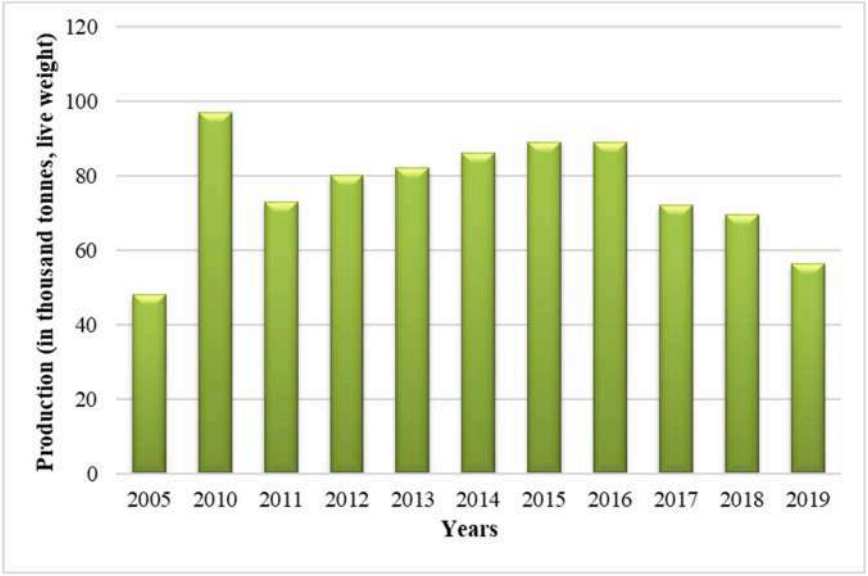

Fig. 8. Global production of *Spirulina* over years (adapted from www.fao.org),.

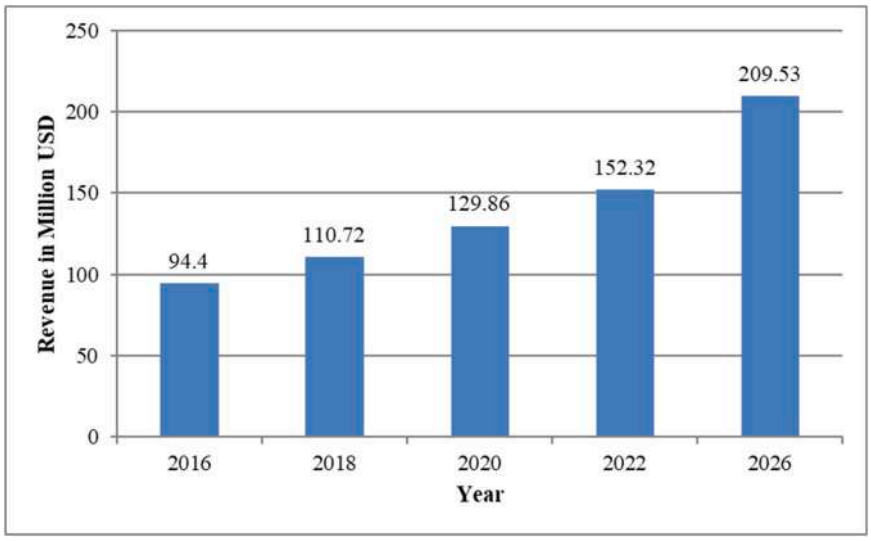

Fig. 9. Phycocyanin market trend over years (Adapted from www.profsharemarketresearch.com).

Table 8
Key Contributors in the Phycocyanin Market Across Various Regions (www.rationalstat.com).

S.No	Region	Countries
1.	North America	United States Canada
2.	Latin America	Brazil Mexico
3.	Western Europe	Germany United Kingdom France Spain Italy Benelux Nordic
4.	Eastern Europe	Russia Poland Hungary
5.	Asia Pacific	China Japan India South Korea Australia
6.	Southeast Asia	Indonesia Thailand Philippines Vietnam Malaysia
7.	Middle East and Africa	Saudi Arabia United Arab Emirates South Africa Nigeria Turkey

Further investigation of the potential health benefits of phycocyanin and its bioactive compounds, including *in vivo* studies to evaluate its therapeutic potential in various diseases, development of innovative food and nutraceutical products that incorporate phycocyanin as a natural colorant and a source of functional ingredients. In a nutshell, the ongoing research and development in the phycocyanin domain hold great promise for addressing global challenges related to food security, health, and sustainability.

10. Concluding remarks

The purpose of this article is to comprehensively review the cultivation of *Spirulina* and the extraction, purification, and application of phycocyanin in various industries. To cultivate *Spirulina*, optimal growth parameters include Zarrouk's media, a pH of 8.8–11, a temperature between 30 ± 4 °C, a light intensity of about 60 μmol photons m^{-2} s^{-1}, and a 12-hr photoperiod. Phycocyanin can be extracted from *Spirulina* using various methods, including physical methods of cell disruption such as bead milling and ultrasound, which are scalable but typically yield low-purity extracts due to the vigorous cell disruption process. Therefore, a purification step is usually required after extraction. While there are alternative techniques such as blending and uniformity, these methods require significant time investment and result in lower-purity extracts. The freeze-thaw method and pulse electric field produce a high yield of phycocyanin with comparatively higher purity, but the cost of establishing these techniques is questionable. Ammonium sulfate precipitation combined with column chromatography is the widely used purification technique, while activated charcoal is a promising alternative for enhancing phycocyanin purity. The bioactivity of phycocyanin has been widely discussed, which presents opportunities for developing numerous products with phycocyanin in various industrial sectors such as food, pharmaceutical, and nutraceutical. Further research is needed to determine the structural characterization of these compounds to discover their complete potential. Nevertheless, increasing their commercial prospects will require cost-effective production, extraction, and purification methods.

CRediT authorship contribution statement

Athiyappan Kerthika Devi: Writing – review & editing, Writing – original draft, Conceptualization. **Paramasivan Balasubramanian:** Writing – review & editing, Supervision, Formal analysis. **Routray Winny:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. On behalf of all co-authors, It is hereby declared that "authors don't have any conflict of interest and no competing interests to declare.".

Data Availability

Data will be made available on request.

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