

RESEARCH ARTICLE

Separation of phycocyanin from spirulina by photosynthetic

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Today, spirulina is cultivated on land in various ways. The mass production of microalgae and their bioactive compounds has been steadily increasing in response to the global demand for natural compounds. Spirulina in particular has been used due to its high nutritional value, especially its high protein content. Spirulina extracts are mainly associated with promising biological functions related to the high-value blue pigment phycocyanin. Phycocyanin is used in several industries such as food, cosmetics and pharmaceuticals, increasing its market value. Due to the global interest and the need to replace synthetic compounds with natural compounds, efforts have been made to optimize the large-scale production process and maintain the stability of phycocyanin, a highly unstable protein. This paper describes the production, extraction and purification methods, including the main physical and chemical parameters that can affect the purity, recovery and stability of phycocyanin.

Keywords: Dried, Spirulina, Phycocyanin, Photosynthesis, Water, Seawater, Pigment.

Introduction

Arthrospira platensis, known as Spirulina, is the most widely used natural resource, especially in the food industry.

Spirulina cultivation is cost-effective, easy to harvest and can thrive in a variety of conditions, such as high pH and eutrophic or mixed trophic conditions, thus minimizing the risk of contamination by other microorganisms.

Spirulina is rich in value-added compounds, mainly polyunsaturated fatty acids and pigments, especially phycocyanins.

Phycocyanins are water-soluble, non-toxic and blue photosynthetic pigments known to be used in the food, cosmetic and pharmaceutical industries.

The rate of extraction of phycocyanins from Spirulina, the efficiency of cell disruption methods and the instability of phycocyanins in both storage conditions and product formulations limit their applicability.

Optimization of Spirulina culture conditions for producing phycocyanins, extraction and purification conditions to achieve high purity and stability of phycocyanins for mass production are therefore of great interest to the scientific community. Optimal Spirulina cultivation conditions for phycocyanin production, extraction and purification methods and physical and chemical conditions affecting the purity and stability of this protein are described.

Blue pigment in nature

Blue is a very noticeable color on Earth. But blue is very rare when it comes to nature. Less than one in ten plants has blue flowers and much fewer animals are blue. Blue flowers are rare in plants, but few have green leaves except for a few plants found at the bottom of the rainforest. The main reason for this is related to the physics of light. Pigments appear as light colors that reflect without absorbing them. The most common plant pigment is green chlorophyll, so chlorophyll does not absorb green light, but rather reflects it, so the plant looks green. However, plants like blue light because they have more energy than any other light in the visible spectrum. Animals are much more difficult to turn blue. Many pigments in animals come from the food they eat. Soflamingos

are pink because of the dye they get when they eat shrimp, their favorite food and goldfish's golden color comes out as food. However, as we heard above, animals cannot turn blue through food because plants do not have true blue pigments. Blue is obtained from many animals by creating structures that change the wavelength of light instead of mixing or changing pigments. For example, a blue morpho butterfly gains color by having its wing scales shaped like a ridge so that the only wavelength the light reflects is blue. If the scales are different, the blue color disappears. The only exception in nature is the *Glaucopsyche xizangensis* (blue morpho butterfly), the only animal known to produce true blue pigments. Today, blue flowers are still highly regarded and many people have tried to grow and reproduce perfect blue flowers. But blue roses and carnations could still produce the first true blue chrysanthemum while avoiding us in Japan. Blue will essentially continue to be rare (Posted on August 20 2019 by Sam Le Gallou).

Pigment of marine algae

Algae contain a variety of pigments. The three main classes of pigments are chlorophyll, carotenoids (carotene and xanthophyll) and phycobilins (phycocyanin and phycoerythrin). Phycocyanin and phycoerythrin belong to the main class of phycobilins photosynthetic pigments, while fucoxanthin and peridinin belong to the carotenoid group of photosynthetic pigments. Macroalgae and microalgae (including cyanobacteria) provide a variety of metabolites, including pigments (Fig. 1).

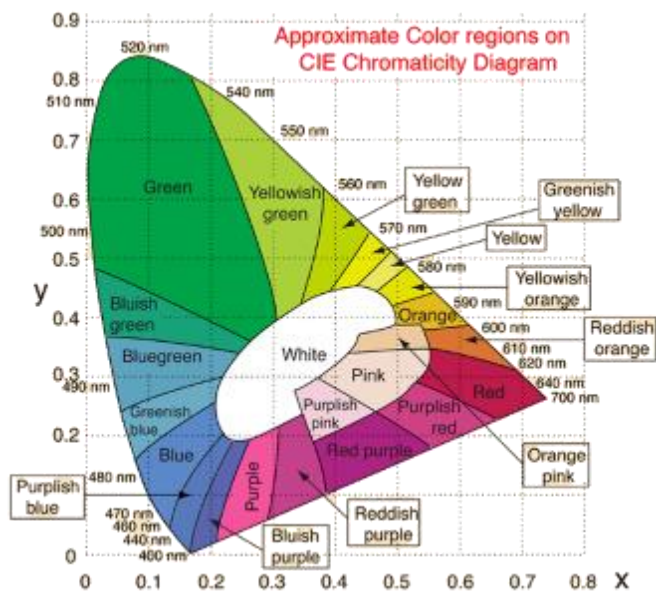


Fig. 1. Approximate color regions on CIE chromaticity diagram.

Structure of phycobiliproteins

The phycobiliproteins are antennae protein pigments found in cyanobacteria, rhodophytes, cryptomonads and cyanobacteria (Glazer, 1994). The phycobiliproteins are present as phycobilisomes anchored on the thylakoid membranes and lie adjacent to the photosynthetic reaction centre of the PS II in cyanobacteria and red algae. These chromoproteins are classified into 3 groups based on the presence of different chromophores among them (Gantt, 1994; Glazer, 1985; Zilinskas, 1986; Rowan, 1989; Sidler 1994; Mac Coll, 1998; Ducret et al., 1998). These groups are (1) Phycoerythrin (PE) λ_{\max} 480 nm-570 nm; (2) Phycocyanin (PC) λ_{\max} 590-630 nm and Phycoerythrocyanin (PEC) λ_{\max} 630-665 nm (3) Allophycocyanin (APC) λ_{\max} 620-665 nm. Core of phycobiliproteins is composed of allophycocyanin from which arise six rods of varying length consisting of phycocyanins to the proximal side of the core and phycoerythrins to the distal side of the core.

Materials and Methods

Spirulina was as a food material in the form of dried granules and powder. The drying temperature in the cultivation and production of spirulina is usually 40-60 degrees and it survives at 70-75 degrees when placed in water. The survival test of dried spirulina was

conducted outdoor and the separation experiment of phycocyanin was conducted at indoor. The experiment was conducted during the day and night and the atmosphere temperature during this period ranged from a minimum of 20 degrees to 33 degrees.

Results and Discussion

Dried spirulina is alive

The concentration of seawater and water is pH 7.0-8.2 and spirulina is in an alkaline state, so when placed in water or seawater, it photosynthesizes and lives. When nutrients are added to spirulina, the cells release spores and growing and multiply (Fig. 2).

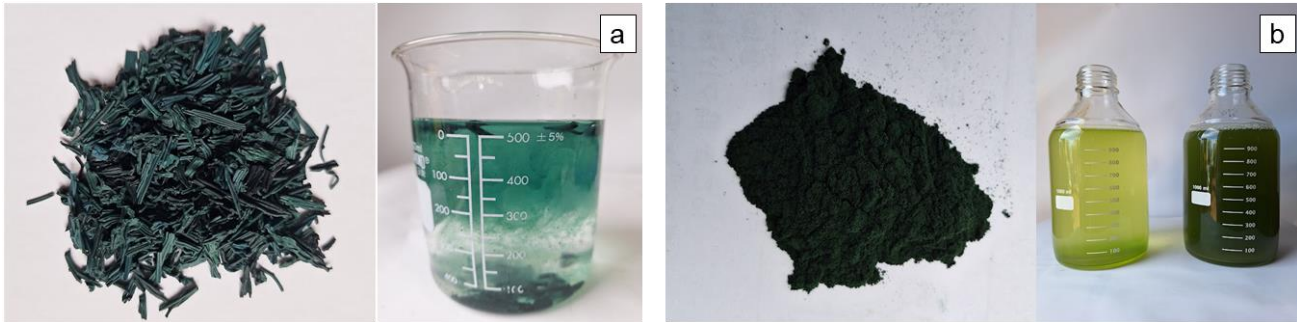


Fig. 2. Dried spirulina is sold as granules and powder for food use.

Photosynthetic spirulina reaction and separation of phycocyanin

Dried spirulina was added to 0.12-0.13 g of water and 25-28 cc of seawater and stirred. The stirred mixture was supersaturated and placed in a glass tube, where the green was separated into blue by photosynthesis. The green appeared as blue by photosynthesis and as time passed from top to bottom, the blue became saturated. The separation of blue by light reaction was fast in seawater and very slow in water. The spirulina that could not light react in a saturated state precipitated. The time required was about 9 hours in seawater and more than 15 hours in water (Fig. 3).

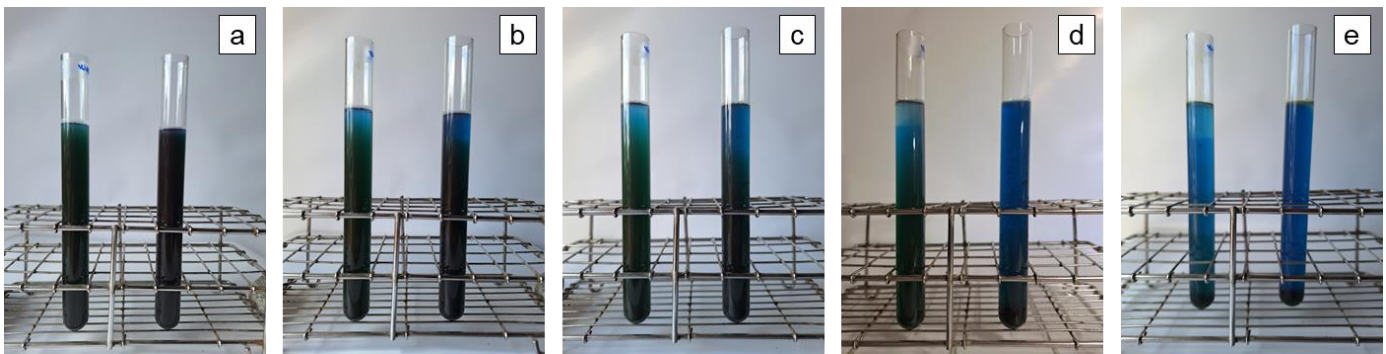


Fig. 3. The separation of phycocyanin can be discolored if exposed to excessive sunlight, so the amount of sunlight was minimized.

Spirulina in seawater

0.5 grams of spirulina was mixed with seawater and stirred, then placed in a measuring cylinder.

The seawater was divided into concentrations and the changes due to photosynthesis were confirmed. The salinity of seawater of 17.5psu reacted slowly and 27psu and 35psu were measured with the same reaction time. The sediment is the green color of spirulina. (From the left in the photo, the salinity of seawater is 17.5psu/L, 27psu/L, 35psu/L) (Fig. 4).

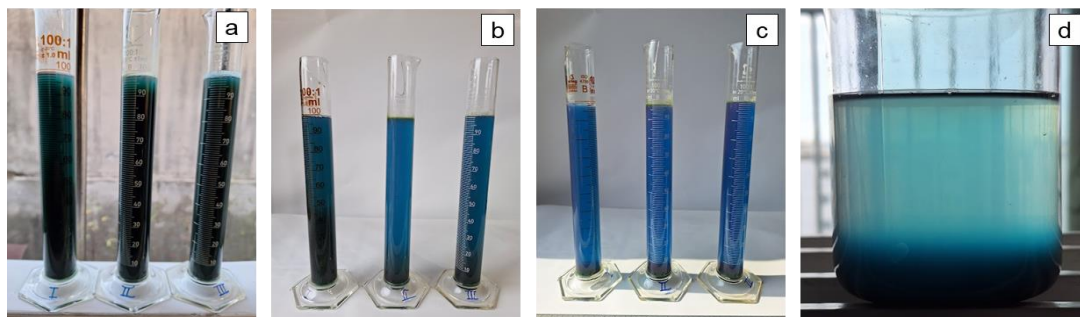


Fig. 4. Chromaticity of color and blue in phycocyanin separation.

Phycocyanin in green seaweeds of marine macro algae

Green seaweeds have nine structural colors. Photosynthetic green seaweeds produce pigments as they change color through signaling of intracellular chromosomes (Fig. 5).

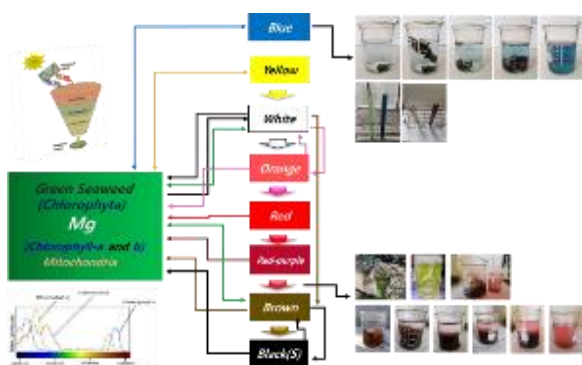


Fig. 5. Colors and pigments of photosynthetic green seaweed.

Conclusion

Phycocyanin is separated from spirulina in various ways. The process of separating pigments can cause environmental problems by consuming a lot of energy and water. Reducing the amount of energy and water consumed in the production and separation of pigments, especially by using seawater, can reduce environmental problems.

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None.

Conflict of Interest

The authors declare no conflict of interest.

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