EXTRACTION, ISOLATION AND PURIFICATION OF PHYCOCYANIN FROM BLUE GREEN ALGA *SPIRULINA PLATENSIS*

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Abstract

In this study, the blue green alga *Spirulina platensis* was obtained from Aquaculture Section, Research and Development Station, Central Arava in 2016. Phycocyanin, one of the phycobiliproteins, was extracted from blue green algae *Spirulina platensis*, followed by the isolation, and characterization of its purity grade. The phycocyanin was extracted by using SCIENTZ-10N Benchtop Freeze - Drying Lyophilizer (-20 ^{o}C) and characterized by SDS PAGE analysis methods. The results showed that the purity level of crude extract is 0.94, 50% ammonium sulphate resulted in purity of 2.10, whereas dialysis tubing was 2.41. In this condition, the phycocyanin with a purity of 3.24 was obtained based on SDS PAGE analysis. It would be concluded that using low temperature extraction method with PBS buffer (pH-6.5, 0.1M) can help to get the better purity with degraded less phycocyanin molecule, because freezing temperature disrupted completely cell membrane of *Spirulina platensis.*

Keywords: phycobiliprotein, Phycocyanin, dialysis, buffer solution, SDS PAGE, freeze -drying

Introduction

Since previous time, *Spirulina platensis* was useful natural resources mainly served as a food or food supplementary for human beings. Nowadays, it becoming popular trend to use algal products both in functional food items and also raw materials for cosmetic products. An active component of *S platensis*, Phycocyanin, is a colorant, which mainly found in cyanobacteria and red algae. In nature, it exists in the form of monomer, trimers or hexamers; small quantities of oligomers have been found as well (MacColl, 1998). Having special bioactivities, they are increasingly recognized as potential raw materials for making food products that are beneficial to health.

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Many studies have indicated that phycocyanin have bioactivities such as: antitumor, antioxidant, and anti-inflammatory effect (Romay et al., 1998; Eriksen, 2008). Since these plants grow in limiting environment, the pigments are rather expensive, and availability as pure compounds is tend to be quite hard endeavor (Silveria, 2007).

Getting phycocyanin extract from wet biomass have been attempted by various methods. The reported method of freezing and thawing (Soni et al., 2006; Stewart and Farmer, 1984) were done. It would have been suggested that the ratio of absorbance at 620 nm and 280 nm can be employed to indicate the purity of phycocyanin, while the ratio of absorbance at 650 and 280 nm can indicate the purity of allophycocyanin.

The purity of phycocyanin is generally evaluated using the absorbance ratio of A_{620}/A_{280} and a purity of 0.7 is determined as food grade 3.9 as relative grade and greater than 4.0 as analytical grade. Purity is directly related to process costs and in general, the more purified a extracted product is, the more expensive to obtain it. Many obstacles exist in the purification process of Phycocyanin (e.g.- if high purity Phycocyanin is desired, the experimental procedure is rather complicated and need a high production costs). So far, there were some studies have been carried out on extraction and purification of phycocyanin, but there still need to improve in procedure that care about the costs, uses of chemicals and efficiency. In this research work, freeze-drying (-20°C) method has been used to extract phycocyanin to avoid some unnecessary complicated procedures, while it could not be damaging the pigment intensity. Thus, the main focus of the present study was to produce the phycocyanin from *S platensis* using the freeze–drying extraction method, which followed by the dialysis by extracted buffer solution to obtain food grade dye for food industry use. (Soni et al., 2006; Sarada et al.,1999)

Materials and Methods

Materials

Sephaadex G-25, dialysis tubing, markers of known molecular weight were purchased from Sigma (St Lowis,MO, USA). All chemicals were of analytical grade.

Organism and Growth condition

Spirulina platensis was grown in a modified Zarouk medium (1966). Firstly,The algae were grown in a batch culture at 35°C, then illuminated by cool white lamp and finally, stirring was provided by bubbling with a mixture of air with 1.5% CO_2.

Extraction of Phycocyanin from *Spirulina platensis*

The concentrated *Spirulina* cells (50 g) was washed with double distilled water to remove adhered salts. Then, biomass was introduced in Bench - Top Freeze Dryer (at - 20 C for 6 hours) to degrade the cell walls of *Spirulina platensis*. After that, the slurry was put in PBS buffer (pH 6.0, 0.1M). The cell debris was removed by centrifugation (4000 rpm for 20 min) . Finally, the supernatant was obtained as crude phycocyanin.

Isolation and purification of Phycocyanin from *Spirulina platensis*

The isolated crude phycocyanin was precipitated in 50% ammonium sulfate and then recovered by centrifugation (8000rpm, for 20 min). The colourless supernatant was discarded and the blue precipitate was dissolved in the extracted buffer solution to dialyze. Dialyzed was served for thrice against 1000 ml of extraction buffer at room temperature .

The dialyzed solution after centrifugation (13000 rpm , for 10min) was eluted through Sephadex- 25 column (3cm*2cm) pre equilibrated and diluted with same buffer. The column was developed at a flow rate of 0.25ml/min and elution were collected in 2.0 ml fraction tubes .

Spectroscopic Measurements

The isolated phycocyanin pigment was evaluated by the procedures below by the ratio of 280nm for the absorbance of total proteins in phycocyanin to 620 nm of desired protein in the complex .(Liu et al ., 2005)

$$\text{Phycocyanin Purity} = A_{620}/A_{280}$$

SDS – PAGE pattern of phycocyanin

Moreover, the measurement of purity, the molecular weight of isolated phycocyanin was determined by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Mini-Protean, Bio-red Ltd., Hercules, CA USA). SDS-PAGE was confirmed using a 14% polyacrylamide slab gel and a 4.5 % stacking gel and was confirmed after staining with Coomassie blue R25 (Sigma, St. Louia, MO, USA) and distaining. The molecular weights of the Phycocyanin subunits were confirmed by comparison with a standard ladder (pre-stained SDS-PAGE standard ; Bio-Rad , USA)

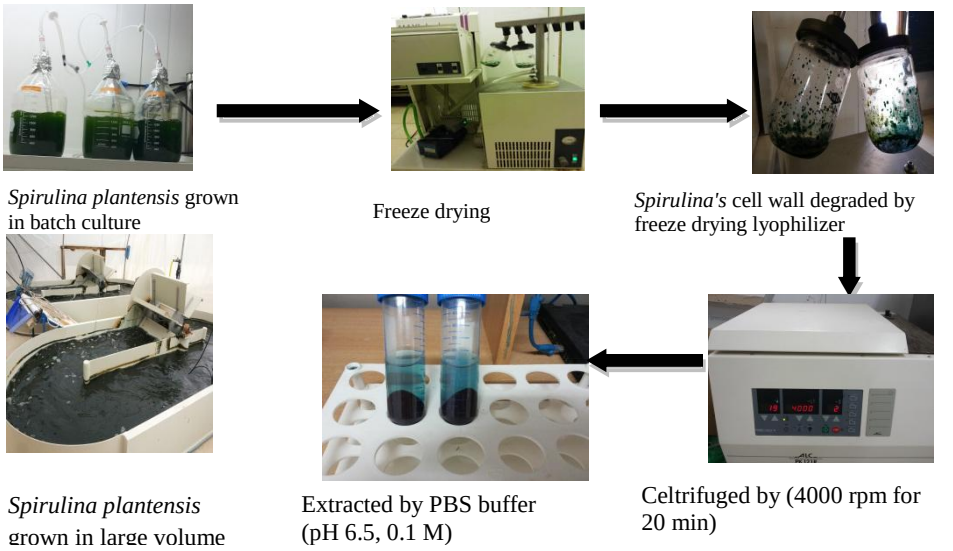

Figure 1. Extraction of crude phycocyanin from *Spirulina plantensis*

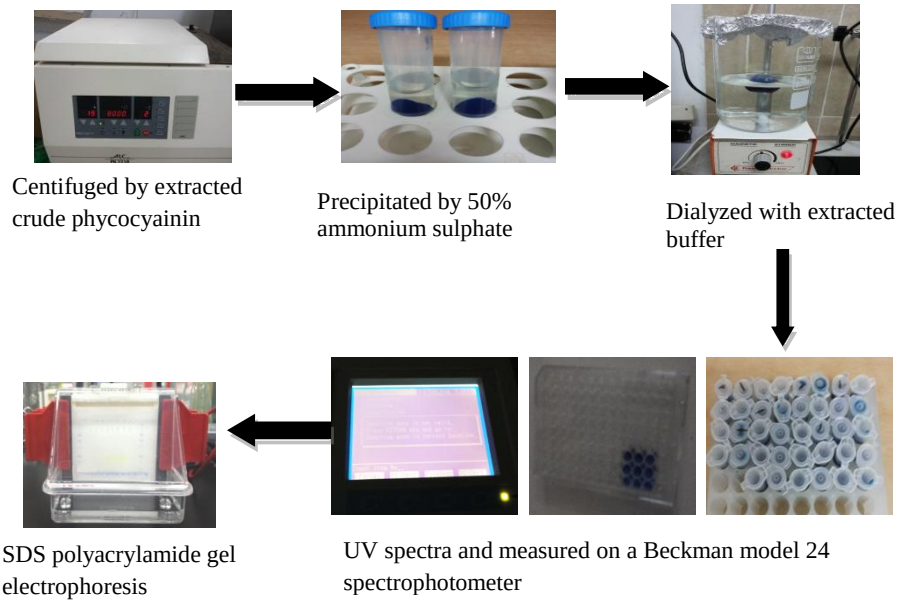

Figure 2. Isolation and purification of phycocyanin from *Spirulina plantensis*

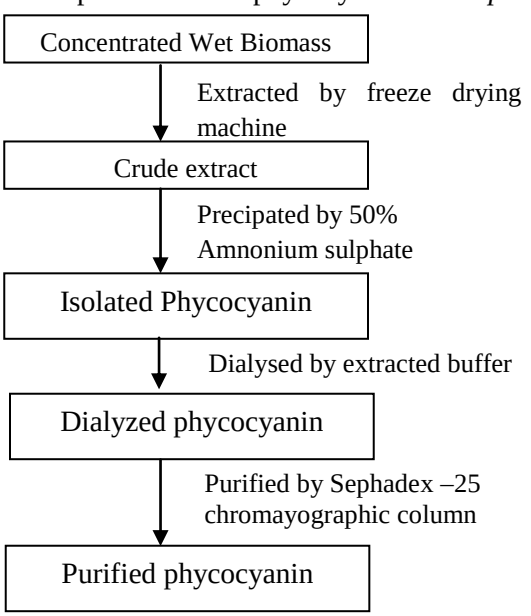

Figure 3. Process flowchart for phycocyanin from *Spirulina plantensis*

Results and Discussion

For obtaining the crude blie pigment, phycocyanin from *Spirulina platensis* is the selection of extraction procedures. In the present study, the Freeze-Drying method (using o.1M PBS buffer, pH 6.0) has been used for extraction of phycocyanin and its purity, which was assessed in Table 1. From this experiment, the purity value for crude extract has been found 0.94, which falls in the food grading. The purity of phycocyanin plays a major role in commercial application and is generally evaluated using Ultra Violet spectrometry. A purity value of isolated pigment up to 0.877 is considered as food grade (Rito – Palmores et al., 2001).

In purification of crude extract, it involves functional precipitation with 50% ammonium sulfate, which is useful in salting out unwanted proteins and at the same time to concentrate Phycocyanin (Boussiba and Richmon, 1979). Experimentally, phycocyanin becomes precipitated in this medium and improves the purity ratio increased to 2.10. After further purification, it was compulsory to be done dialysis for the removal of phycocyanin to improve the purity, which got value up to 2.41. Then, after passing through sephadex G-25 column, this also increased purity ratio to 3.24. Finally, the successive purified fractions from each steps, run on SDS PAGE, the contaminating protein band disappeared and only one band (18 kDa. subunit) was observed, which is consistence with the research found by Patel et al. (2005) Fig (4).

Table1. Purity Ratio of Phycocyanin extracted from *S.platensis* by spectrophotometric method

Steps of purification	280nm	620nm	Purity Ratio (A620/A280)
Crude extract	3.56	3.34	0.94
50%Ammonium sulfate	1.10	2.42	2.10
Dialysis	0.87	2.10	2.41
Sephadex G-25	0.364	1.18	3.24

Isolation & Purification of Phycocyanin from *Spirulina platensis*

The purity of phycocyanin plays a significant role in commercial application and is generally evaluated using the absorbance ratio of A_{620}/A_{280} where A_{620} represents maximum peak height for phycocyanin and A_{280} indicates contamination of aromatic amino acid rich proteins. A purity value up to 0.877 is considered as food grade (Rito – Palmores, 2001) .In this result, the phycocyanin purity value for crude extract was 0.94 . The purification of crude extract involves functional precipitation with 50% ammonium sulfate which is particularly useful in salting out unwanted proteins and at the same time to concentrate phycocyanin (Boussiba and Richmon, 1979). At this step phycocyanin get precipitated and improves the purity ratio is increased to 2.10 . For further purification removal of phycocyanin is compulsory and for this dialysis was carried out and improved the purity value up to 2.41. It was further purified by passing through sephadex G-25 column .This increased purity ratio to 3.24. The purity of phycocyanin was further confirmed by absorption spectral scanning. The successive purified fractions from each step were run on SDS PAGE and thecontaminating protein band disappeared. . Only one band (18 kDa. subunit) was observed. Fig (4).The molecular weight α subunit were being reported from 18 kDa in Spirulina (Patel et al., 2005).

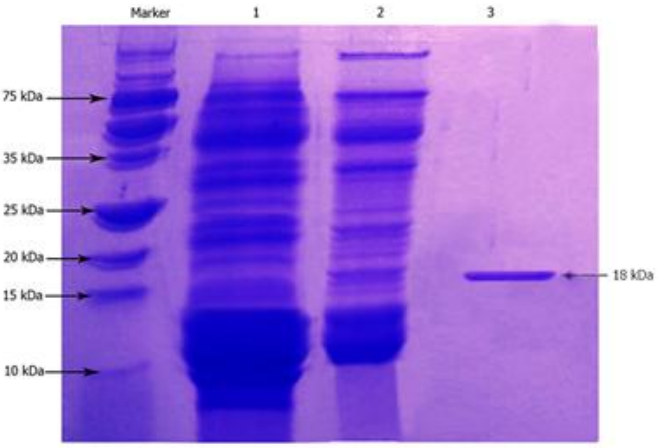

Figure 4. 14% SDS-PAGE at each stage of purification at phycocyanin from *Spirulina plantensis*
1. 50% ammonium sulphate precipitation
2. Dialyzed phycocyanin
3. Sephadex G-25

Conclusion

The present study has been extracted phycocyanin from the natural blue green algae by using freeze - drying method, whereby the resulting compound got better purity with few phycocyanin molecule degradation. It would be described as an effective method of extraction and purification of phycocyanin from *Spirulina* so far. The purity level of phycocyanin at the end of the process achieved at the food grade of 3.24.

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