Production of antioxidant C-phycocyanin using extraction process of *Spirulina platensis* in large scale industry

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**Abstract.** *Spirulina platensis* is one of the popular species that containing C-Phycocyanin. C-Phycocyanin has been used in many applications such as antioxidant, anti-inflammatory, and natural blue colorant. The increase of phycocyanin production from *S. platensis* is influenced by extraction method and solvent ratio. The phycocyanin pigment can be massive extracted using mechanical cell rupture like freezing thawing method and distillated water as solvent. This research aimed to produce high concentration of antioxidant phycocyanin from *S. platensis* using simple and efficient method for large scale industry and develop a method for primary extraction from dry biomass of *S. platensis*. There were 3 treatments and each treatment was conducted in 3 replications. Standardization of process treatments such as biomass : solvent ratio (1:6, 1:50 and 1:100) and processing time were carried out at thawing time (1 hour), freezing time (2 hours) and stirring time in water bath (0.5 hour). The phycocyanin concentration and yield were determined by using spectrophotometer. The result showed that at biomass : solvent ratio of 1:50, resulted C-PC concentration of 0.179 mg/ml and its yield of 17.944 mg/g.

1. **Introduction**

*Spirulina platensis* is a rich and inexpensive source of natural blue pigment [1]. The phycobiliprotein in cyanobacteria *S. platensis* produces allophycocyanin (APC) and C-phycocyanin (C-PC), both blue colored. C-PC as the major is a photosynthetic pigment of the phycobiliprotein family and is the major light-harvesting protein pigment present in the cells of *S. platensis*, whereas APC is a minor component [2].

C-PC has many applications in pharmaceutical (fluorescent marker [3], the neuroprotective [3], hepatoprotective [3], anti-inflammatory [4] and antioxidant [5]), cosmetic and also in food industry such as (as natural blue colorant). In general, the extraction method is the key for maximum recovery of phycobiliproteins in the natural state from algae [6]. The extraction of phycobiliproteins involves the cell rupture and release of these proteins from within the cell, and thus, the use of variations in the osmotic pressure, abrasive conditions, chemical treatment, freezing-thawing and sonication, among others, are necessary. Its extraction is difficult as the cell wall of the cyanobacteria is quite resistant [7], which is made of four layers i.e. fibril, peptidoglycan, proteins, and analogous to gram negative bacteria [8].

The extraction process should be effective in terms of high extraction yield and must be environmental friendly. However, all of these cell disruption methods lack specificity as cell debris and...
other unwanted impurities are also released. Nevertheless, phycocyanin is sensitive to light, oxygen, moisture, and temperature. Therefore, it is able to readily degrade under certain physicochemical conditions [9]. The most important are the cell disruption method, type of solvent, solvent-to-biomass ratio and extraction time [10, 11, 20].

Phycobiliprotein is a polar compound that dissolves in polar solvents. The use of water solvents in extracting phycocyanin in *S. platensis* is considered safe and can attract the active substances. The use of water as a solvent can more easily dissolve the biomass of *Spirulina* cells. Unlike methanol which includes organic solvents and is toxic, water is not an organic solvent and is not toxic. Because water does not have toxic power, no preliminary test for solvent toxicity is necessary. In addition, the economic value of water is cheaper when compared to other types of solvents [2].

This study was primarily aimed to determine the most effective solvent concentration to obtain optimize the process, minimizing costs and maximizing yield. Several factors can influence C-PC extraction. However, it is important to study the factors affecting extraction as a group in order to optimize the process, minimizing costs and without the use of chemical agents, in order to obtain this natural blue dye from *S. platensis* with a high phycocyanin yield and concentration.

2. Materials and Methods

2.1. Materials

Dry biomass of *S. platensis* was procured from PT. Neoalgae Indonesia Makmur, Sukoharjo, Indonesia which stored in aluminum phthalate pouches at room temperature (20 ± 5°C) and distillated water as solvent.

2.2. Extraction procedures

The dry biomass underwent the drying step that was carried out at 40°C for 1 hour and the freezing step at -18°C for 2 hours. Mechanical rupture was carried out by stirring in a water bath for 0.5 hour then settled for 24 hours. The extraction of C-PC was carried out according to Silveira [11], which used water as solvent under the following conditions: a biomass-to-solvent ratio of 1:6, 1:50, 1:100. Each analysis was conducted with 3 replications. The crude extract was separated from debris using a set of sieves.

2.3. Analytical procedures

2.3.1 C-PC Concentration. The C-PC concentration in mg/mL was calculated and analyzed by the optical densities of spectrophotometry at 652 nm and 620 nm, using Eq. (1) [12]:

\[
C\text{-PC} = \frac{(OD_{620} - 0.474 \times (OD_{652}))}{5.34}
\] (1)

2.3.2 Extraction Yield. The extraction yield was calculated using Eq. (2) [12]. Yield is the extraction yield of phycocyanin in mg of C-PC/ dry biomass (g), V is the solvent volume (mL) and DB is the dry biomass (g).

\[
\text{Yield} = \frac{(C\text{-PC} \times V)}{DB}
\] (2)

C-Phycocyanin concentration and yield were presented in table form then also analyzed for significance level using SPSS software based on one way Analysis of Variance (ANOVA) and Duncan's Multiple Range (DMRT) tests at a significance level of 5%.

3. Result and Discussion

The effects of the different treatments on the biomass of *S. platensis* were investigated individually (Table 1):
Table 1. Concentration and Yield of C-Phycocyanin

| Treatments | OD 620 (nm) | OD 652 (nm) | C-PC (mg/ml) | C-PC average (mg/ml) | V/DB (1000 ml/10 gr) | Yield (mg/g) | Yield average (mg/g) |
|------------|-------------|-------------|--------------|----------------------|----------------------|--------------|----------------------|
| A (1:6)    | 1           | 1.849       | 1.948        | 0.173                | 0.172                | 100          | 17.334               |
|            | 2           | 1.843       | 1.948        | 0.172                | 0.172                | 100          | 17.222               |
|            | 3           | 1.838       | 1.943        | 0.171                |                      | 100          | 17.173               |
| B (1:50)   | 1           | 1.888       | 1.958        | 0.179                | 0.179                | 100          | 17.976               |
|            | 2           | 1.888       | 1.958        | 0.179                |                      | 100          | 17.976               |
|            | 3           | 1.883       | 1.958        | 0.178                |                      | 100          | 17.882               |
| C (1:100)  | 1           | 1.878       | 1.943        | 0.179                |                      | 100          | 17.922               |
|            | 2           | 1.878       | 1.943        | 0.179                |                      | 100          | 17.922               |
|            | 3           | 1.872       | 1.943        | 0.178                |                      | 100          | 17.809               |

The table of C-Phycocyanin concentration and yield showed that ratio 1:50 was the highest among the other treatments. It resulted the concentration of of 0.179 mg/ml and yield of 17.944 mg/g. On the otherhand ratio 1:6 achieved the lowest concentration of 0.172 mg/ml and yield of 17.243 mg/g. Phycocyanin is a water-soluble protein that can be released simply by the destruction [13]. The analysis of variance (ANOVA) test results showed that the use of water solvent with different concentrations had no significant effect (P <0.05) on the C-Phycocyanin concentration and yield.

According to the literature, freeze-thawing method is widely used for the extraction of phycocyanin from fresh biomass that improves the efficiency of extraction [1, 10, 14, 15]. The main purpose of the freeze-thaw process is for stressing cells to accelerate the release of pigments from cells after the collision process. The cell breakage that is caused by freezing can improve the phycocyanin extraction yield [16]. The impact of the freezing process is the occurrence of cell swelling and cell damage caused by the formation of sharp crystals that form during clotting. The next step with the thawing process will cause cellular contraction due to the piercing of the cell due to the formation of sharp crystals, so that a cell leak will occur which results in cellular pigment discharge.

The solvent used in the extraction process must be able to pull the active component from the mixture in the sample [17, 21]. Extraction of phycocyanin with water does not differ much from that of sodium phosphate buffer (pH 7.4), it is found twofold higher extraction yield of phycocyanin in frozen biomass using distilled water as compared to phosphate buffer [11]. Through the sample immersion process, there is a breakdown of cell walls and membranes due to differences in pressure inside and outside the cell. The bioactive compounds in the cytoplasm will dissolve in the solvent and extract the compound will be perfect. Solvent volume affects extraction yield. This is in accordance previous report who extracted dyes on suji leaves with ethanol solvent, stating that extraction with a stirring speed of 300 rpm with a ratio of 5 grams of suji in 200 ml of solvent resulted in the greatest extract yield [18]. Water is not an organic solvent and is not toxic. Because water does not have toxic power, no preliminary test for solvent toxicity is necessary. In addition, the economic value of water is cheaper when compared to other types of solvents. Increasing the biomass / solvent ratio can increase effectiveness solvent into the cell and give more extract. However, an advantage solvents have been reported to absorb cavitation energy from extraction system, much of the other contaminant proteins from cytoplasm and other organelles resulting in lower extraction results, In the case of a smaller amount of solvent, biomass cannot find sufficient blood solvent, resulting in insufficient duplicate phycocyanin from the cell [19, 11].

4. Conclusion
The biomass to solvent ratio showed effect on the C-PC concentration and extraction yield. Moreover, this methodology does not employ chemical products, using only water to make the extraction, and is a simple procedure. Using dried biomass from S. platensis, in a 1:50 biomass to solvent ratio, a C-PC
concentration of 0.179 mg/mL was obtained with yield of 17.944 mg/g.

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