Advance Sustainable Science, Engineering and Technology (ASSET)
Vol. 6, No.3, July 2024, pp. 02403023-01 ~ 02403023-08
ISSN: 2715-4211 DOI: https://doi.org/10.26877/asset.v6i3.907

Comparing Conventional and Modern Methods for The Phycocyanin Extraction from *Spirullina sp*

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Abstract. *Spirulina platensis*, a blue-green algae abundant in tropical regions, is rich in minerals, vitamins, fibers, and pigments, with low nucleic acid content. It has unique chromoproteins called phycobiliproteins, notably phycocyanin, used in various applications. This study aims to optimize phycocyanin extraction using different solvents (distilled water and sodium phosphate buffer pH 6.7) and methods (freeze-thaw and sonication). Spirulina platensis biomass was extracted in both solvents, then some of them was freeze for 24 and 48 hours followed by thawing overnight. The other was sonicated for 2.5 minutes, 50 Hz then soaked for 1, 2, and 3 hours. All of the samples were centrifuged at 6000 rpm for 10 minutes and the absorbance was measured using a UV-Vis spectrophotometer at wavelengths of 280, 620, and 650 nm. with freeze-thawing for 48 hours yielded the highest phycocyanin concentration (0.55%), with a yield of 11.07 and purity of 0.21. Sonication improved phycocyanin concentration, yield, and purity significantly, yielding 1.108, 25.85, and 0.26, respectively.

Keywords: Phycoyanin, *Spirulina platensis*, sonication, freeze-thawed, sodium phosphate buffer

(Received 2024-06-15, Accepted 2024-07-17, Available Online by 2024-07-31)

1. Introduction

Blue-green algae (cyanobacteria) are among the most primitive life forms on Earth. Their cellular structure is a simple prokaryote. They share features with plants, as they have the ability to perform photosynthesis [1]. These algae use solar energy, carbon dioxide, and minerals within the water to grow, and their growth rate is very high. Additionally, microalgae can produce various products by photosynthesis during their growth. *Spirulina platensis* contains a relatively low nucleic acid content; is composed of 55%–70% protein, 6%–9% fat, and 15%–20% carbohydrate, and is rich in minerals, vitamins, fibers, and pigments. It has many unique chromoproteins known as phycobiliproteins. In some countries, phycobiliprotein, a fluorescent chromoprotein, has been used as an additive in foods, cosmetic products, and medical diagnostic reagents [1].

Phycocyanin converted into chemical energy that can be used directly by living cells. Different from higher plants and green algae, major light-harvesting antennae in cyanobacteria and red algae are a large multisubunit protein complex called phycobilisome (PBS). PBPs can be

classified into four types according to their spectral properties: phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC), and phycoerythrocyanin (PEC) [2]. C-phycocyanin (C-PC) and allophycocyanin (APC) approximately at a ratio of 10:1[3].

The application of phycocyanin by utilizing its biological activity, especially as an antioxidant compound, has been widely applied, both in the fields of nutraceutics, cosmeceutic, and pharmaceuticals. Phycocyanin has a wide range of biological activities including anticancer, antidiabetic, antimelanogenic, anti-inflammatory, and antioxidant [4] as fluorescent markers [5].

Although several methods have been developed for the separation and purification of these phycobiliproteins, they are either more tedious or time consuming. The major drawback of almost all these methods is the large number of steps involved, and it is known that higher the number of steps higher is the loss of product yield. Furthermore, the scale-up of these methods is very difficult and also expensive. Freeze thawing and maceration is one of the potential purification techniques of downstream processing. It is a better alternative to existing methods especially in the early processing stages, with regard to scale of operation, low processing time, enrichment of the product and continuous operation for the separation and purification of desired enzymes/proteins from a complex mixture.

The primary objective of this study is to develop a simple and efficient method for extracting phycocyanin from *Spirulina platensis*. This study explores the optimization of phycocyanin extraction using different solvents (distilled water and sodium phosphate buffer pH 6.7) and methods (freeze-thaw and sonication). The novel approach presented in this study aims to improve extraction efficiency and product quality, which is crucial for expanding the applications of phycocyanin in various industries. By employing innovative techniques and solvents, this research seeks to enhance the extraction process, making it more feasible and effective for large-scale applications.

2. Methods

The dry mass of *Spirulina platensis* used as the sample was procured from PT. ALBITEC, Semarang, Central Java, Indonesia. Extraction processes were performed using three different methods: sonication, freeze-thaw, and maceration. For all methods, a sample-to-solvent ratio of 1:20 was applied with two types of solvents: distilled water (aquadest) and 0.1 M sodium phosphate buffer (pH 6.7).

The sonication process was optimized with minor modifications. Ultrasonication was conducted at an amplitude of 50%, with 10 seconds on and 10 seconds off cycles for a total of 2.5 minutes. The samples were then soaked for 1 hour, 2 hours, and 3 hours, respectively, and subsequently centrifuged at 4000 rpm for 10 minutes.

For the freeze-thaw method, Spirulina was initially mixed with water and sodium phosphate buffer solution at 1000 rpm for 10 minutes. The mixture was then placed in a deep freezer overnight. The frozen samples were allowed to thaw at room temperature for 24 hours and 48 hours before being centrifuged at 4000 rpm for 10 minutes.

The maceration process was carried out similarly to the freeze-thaw method, except that the prepared samples were kept at room temperature for 24 hours and 48 hours instead of being frozen.

The absorbance of the prepared solutions was measured using UV-Vis spectrophotometry at wavelengths of 280 nm, 620 nm, and 650 nm. Yield and purity were calculated using the following equations:

$$Phycocyanin\ concentration\ \left(\frac{\text{mg}}{\text{mL}}\right) = \frac{A620 - 0{,}474 \times A650}{5.34}, \tag{1}$$

$$Phycocyanin\ yield = \frac{Phycocyanin\ concentration\ \left(\frac{mg}{ml}\right) \times volume\ of\ extracts\ (ml)}{weight\ of\ feedstoct\ (mg)} \times 100\%, \tag{2}$$

$$\text{Phycocyanin purity} = \frac{A620}{A280} \quad (3)$$

Notation:
0.474 = Chlorophyll's Reduction Factor (Photosynthetic Pigment of Spirulina)
5.34 = Molar Absorption Coefficient Conversion Factor for Phycocyanin
A280 = Absorbance at 280 nm
A620 = Absorbance at 620 nm
A650 = Absorbance at 650 nm

3. Results and Discussion

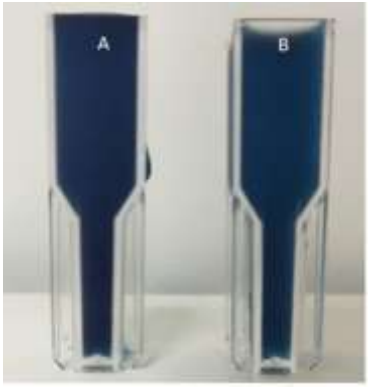

Figure 1. Phycocyanin obtained from maceration using (A) sodium phosphate buffer, (B) distilled-water.

In this research, dry mass spirulina was obtained from PT. ALBITECH. There are enormous benefits from spirulina that has been continuously explored. Therefore, it is meaningful to develop the most effective and efficient method for the spirulina extraction, mainly phycocyanin, one of phycobiliproteins in spirulina. Based on that, this study has been focused to find the best polar solvent that can be used for obtaining the optimum phycocyanin. Distilled water and sodium phosphate buffer pH 6.7 were used in this study. Figure 1 shows the Phycocyanin extracted from *Spirulina platensis* by maceration using two different solvents; sodium phosphate buffer and distilled-water. Visually, both sodium phosphate buffer and distilled-water have the similar blue pigment color density and viscosity. However, it is not the case for the yield and purity.

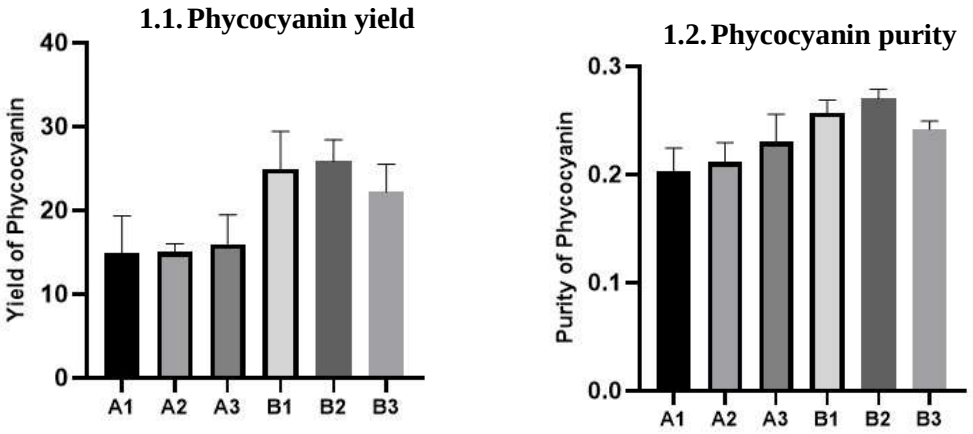

Figure 2. (2.1) Phycocyanin yield, (2.2) Phycocyanin purity, obtained from maceration method with (A1) destilled-water for 1 h, (A2) destilled-water for 2 h, (A3) destilled-water for 3 h, (B1) sodium phosphate buffer for 1 h, (B2) sodium phosphate buffer for 2 h, (B3) sodium phosphate buffer for 3 h.

Figure 2 shows the result of solvent optimation. The maceration method using distilled-water shows the highest phycocyanin yield of 15.95 (A3), while sodium phosphate buffer has the highest phycocyanin yield of 25.85 (B2). The highest phycocyanin purity from distilled-water maceration is 0.23 (A3), while sodium phosphate buffer gives the highest purity of 0.26 (B2). These results show higher concentrations compared to those reported by Wulandari et al. (2016), who extracted *Spirulina* using phosphate buffer with a freeze-thaw method for 72 hours and obtained a concentration of 8 mg/mL, and Gustiningtyas (2020), who used ultrasonic extraction with phosphate buffer and achieved a concentration of 1.25 mg/mL. Compared to previous studies, the phycocyanin purity index in this research is lower than the values reported by Ridlo et al. (2015), who achieved a purity index of 1.61 using ultrasonic extraction with phosphate buffer, and Gustiningtyas (2020), who reported a purity index of 1.33 with the same method and solvent. Additionally, Margiati et al. (2019) obtained a purity index of 1.03 using distilled water as the solvent.

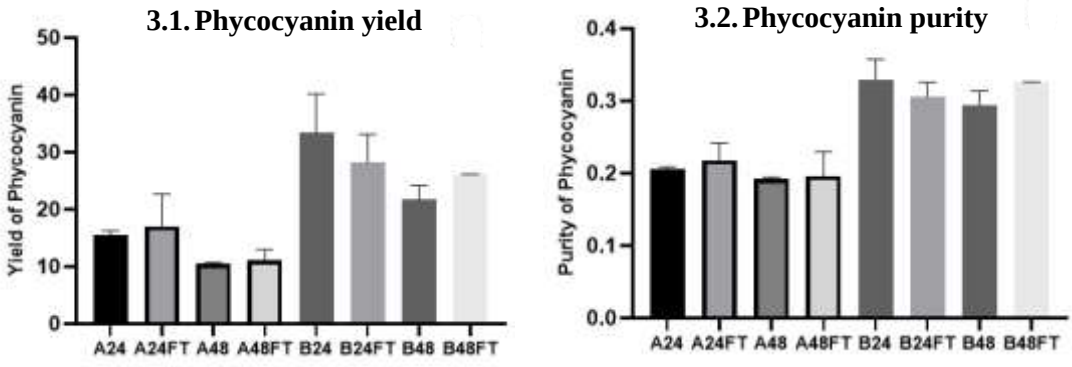

Figure 3. Phycocyanin (3.1) yield and (3.2) purity obtained with variation extraction method of freeze-thawed and maceration. (A24) distilled water-maceration for 24 h, (A24FT) distilled water-freeze thawed 24 h, (A48) distilled water-maceration for 48 h, (A48FT) distilled water-freeze thawed 48 h, (B24) buffer-maceration 24 h, (B24FT) buffer-freeze thawed 24 h, (B48) buffer-maceration 48 h, (B48FT) buffer-freeze thawed 48 h.

Figure 3. effectively illustrates the comparative yields of phycocyanin under different experimental conditions, highlighting the influence of these conditions on production efficiency. Generally, the use of sodium phosphate buffer in the extraction process yields higher amounts of phycocyanin compared to the use of distilled water. The highest yield is observed in the buffer extraction process using the maceration method for 24 hours (B24), which produces the greatest yield.

The freezing and thawing (FT) method does not consistently increase the yield, as evidenced by the minimal differences between samples treated with and without FT. For example, there is only a slight difference between A24 and A24FT, as well as between B24 and B24FT.

Further analysis of the yield and purity of phycocyanin under various conditions (Figure 3) reveals that the use of sodium phosphate buffer (B24, B24FT, B48, B48FT) consistently outperforms the use of distilled water (A24, A24FT, A48, A48FT). Among the tested conditions, the buffer extraction process combined with the maceration method for 24 hours (B24) achieves the highest yield and purity, making it the most effective method. The freezing and thawing treatments have minimal and inconsistent effects on both yield and purity.

In conclusion, the buffer extraction process, particularly B24, is optimal for maximizing the production and quality of phycocyanin. This method demonstrates superior efficiency and effectiveness compared to the other tested conditions.

Significant test between the different method and the same solvent shows no significant different, while significant test between the same method and different solvent describes the significant different. The use of distilled water together with sonication method results in all variable has p value <0.05. It describes that sonication method in distilled-water no significant different with the highest score of of phycocyanin purity 0.23 using sonication method. The use of sodium phosphate buffer together with sonication method has significant different between method and the highest phycocyanin purity of 0.33.

Phycocyanin is a natural pigment from *Spirulina platensis* which is polar, so that in its extraction a polar solvent is used [9]. The polar solvents used in this study were destilled water and sodium phosphate buffer pH 6.7. Both type of solvents can dissolve phycocyanin. The purity and yield of the phycocyanin with solvent aquadest and time of maceration 3h was not significantly higher ($P < 0.05$) than the solutions buffer pH 6.7 and time of maceration 3h. This is because the pH of the destilled water and buffer is not much different so that the ability to extract phycocyanin is not much different.

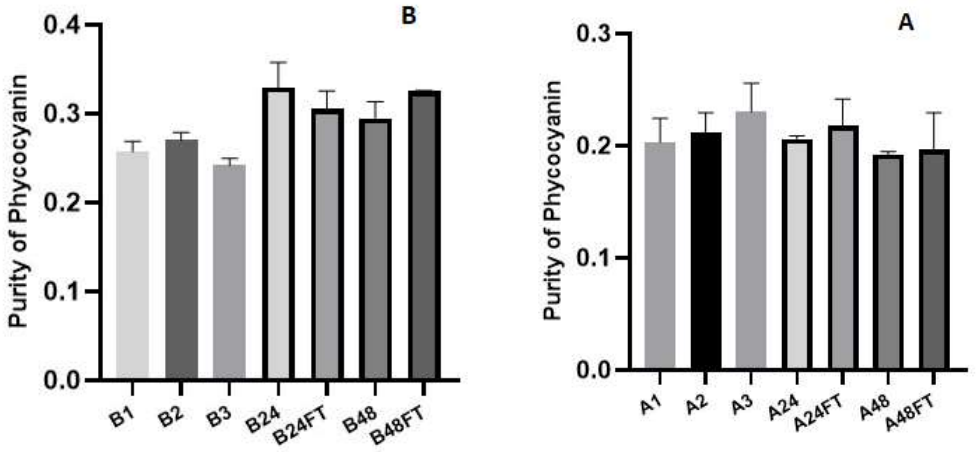

Figure 4. The effect of sonication process for cell damage to phycocyanin concentration: (A1) distilled water-maceration 1 h, (A2) distilled water-maceration 2 h, (A3) distilled water-maceration 3 h, (B1) buffer-maceration 1 h, (B2) buffer-maceration 2 h, (B3) buffer-maceration 3 h, and without sonication: (A24) distilled water-maceration 24 h, (A48) distilled water-maceration 48 h, (A24FT) distilled water-freeze thawed 24 h, (A48FT) distilled water-freeze thawed 48 h, (B24FT) buffer- freeze thawed 24 h, (B48FT) buffer-freeze thawed 48 h.

Sonication plays a crucial role in the extraction of the pigment phycocyanin from *Spirulina platensis* due to its ability to enhance extraction efficiency significantly. The process utilizes ultrasonic waves to create cavitation in the solution, leading to the formation and subsequent explosion of microbubbles. This results in the effective disruption of cell walls, allowing for the release of phycocyanin more efficiently than conventional methods. By employing sonication, the extraction process can achieve higher concentrations and purity of phycocyanin, which is essential for various applications in nutraceuticals, cosmeceuticals, and pharmaceuticals. Moreover, sonication reduces the extraction time dramatically, making it a more efficient method compared to traditional techniques like soaking or freeze-thawing [10].

Figure 4 describes the impact of sonication on phycocyanin purity affected by extraction method and solvents. Sonication significantly influences the purity of phycocyanin extracted from

Spirulina platensis. When combined with sonication, the purity of phycocyanin tends to improve due to the enhanced efficiency of cell disruption and pigment release. This method uses ultrasonic waves to create cavitation effects that break open cell walls more effectively than maceration or freeze-thaw techniques. Consequently, sonication leads to a more complete release of phycocyanin into the extraction solvent, thereby increasing its concentration and reducing the presence of contaminants.

The choice of solvent also plays a crucial role in the purity of the extracted phycocyanin. Phycocyanin extracted using sodium phosphate buffer generally exhibits higher purity compared to those extracted with distilled water. The buffer solution helps maintain an optimal pH for preserving the stability and integrity of phycocyanin, whereas distilled water may lead to less controlled conditions that can affect the protein's stability. When sonication is employed, the combination of the buffer with sonication enhances the extraction efficiency and results in a more refined product with fewer impurities. Conversely, while sonication improves extraction outcomes, the traditional freeze-thaw method combined with either solvent shows inconsistent effects on purity, indicating that sonication in conjunction with buffer solutions is more effective in achieving high-purity phycocyanin.

Phycocyanin is water-soluble and can be easily extracted from *Spirulina sp*. as a protein–pigment complex. Appropriate control of the pH and ionic strength during extraction, separation and purification processes are crucial for the stability of phycocyanin molecules. The degradation of phycocyanin depends on the aggregation state of the protein, which is influenced by parameters such as light, temperature, pH and protein concentration[10][11]. Phosphate buffer solvents can attract pigments more strongly from inside the cells because they are able to maintain both acidic and basic conditions. The function of the buffer solution is to stabilize the pH, so that after the extraction process, the phycocyanin solution is in a stable condition and is able to minimize the pigment degradation process. Phycocyanin degradation will occur if the degree of acidity decreases or the alkalinity increases (stable pH 4-9), a buffer with a pH of 6.7 is a suitable solution for phycocyanin extraction, because from the results of research by comparing pH 5, 6 and 7, the optimum pH for phycocyanin extraction is 6-7 [12]. Therefore, buffer concentration at pH 6-7 was used as a suitable pH level for C-phycocyanin extraction. Selection of a suitable buffer for phycobiliprotein extraction is also a crucial factor for obtaining high yield and quality of phycobiliproteins. Moreover, among the different buffers evaluated to maximize phycobiliproteins extraction, sodium phosphate was found to be the best [10].

When compared to alternative methods, freeze-thaw cycles have been the most efficient way to extract C-PC from wet cyanobacterial biomass[8], CPC has also been extracted after mechanical cell disruption[13]. C-phycocyanin can be extracted from cyanobacteria by different procedures which combine breakage of the cell walls and extraction of water-soluble phycobiliproteins into aqueous media [14]. Several factors influence C-phycocyanin extraction; the most important being the cellular disruption method, type of solvent, biomass-solvent ratio, and type of biomass [15]. Physical methods include sonication, cavitation, osmotic shock, and repeated freeze-thawing, whereas chemical methods use acids, alkalis, detergents, and enzymes. Combinations of a variety of physical and chemical methods are exploited for cell breakage[16]. Ultrasound is a novel technology to improve the extraction process of hydrophobic compounds from microorganisms by disrupting the cell wall of the different bio-tissues to facilitate the release of extractable compounds and enhance mass transport of solvent from a continuous phase into the cells [17].For C-phycocyanin extraction by ultrasonic methods, the biomass solution is treated ultrasonically to accelerate cell wall breakage through direct osmosis which effectively shortens the treatment time[18]. After cell breakage, clarification by centrifugation was performed, and the product is primarily isolated from the supernatant[16]. The research result method with sonication have significantly higher ($P > 0.05$) than method without sonication. Wet Spirulina and solvent buffer and time of maceration 3h have higger purity 0.27 and yield 22.18 in sonication method[19][17]. Buffer with freeze thawing method have higger purity and yield than all method.

In freeze thaw method we use 24 H and 48 H for freezing and overnight for thawing. Differences in time and methods used cause differences in yield and purity values.

4. Conclusion

The freeze-thawed using sodium phosphate buffer was better than distilled water with a freezing time of 48 hours. It shows phycocyanin concentration of 0.55, yield of 11.07, and purity of 0.21. The use of sonication process could increase the value of phycocyanin concentration, yield, and purity with the best results of 1.108, 25.85, and 0.26 respectively. Future research should focus on optimizing sonication parameters and exploring alternative solvents to enhance phycocyanin extraction efficiency and purity. Additionally, investigating the scalability of these methods and their impact on phycocyanin's bioactivity will be crucial for advancing industrial applications and ensuring high-quality production.

5. Acknowledgment

Thank you to the Ministry of Education, Culture, Research and Technology through the KEDAIREKA Matching fund program in 2022, which has funded this research. Thank you to Universitas Setia Budi, especially the Faculty of Pharmacy, Faculty of Health Sciences, and Faculty of Engineering, as well as the Faculty of Medicine, Pembangunan Nastional "Veteran" University, Jakarta and Faculty of Agricultural Science which supported this research.

References

[1] P. Saranraj and S. Sivasakthi, "Spirulina platensis - FOOD FOR FUTURE: A REVIEW," *Asian J. Pharm. Sci. Technol. www.ajpst.com*, vol. 4, no. 1, pp. 26–33, 2014, [Online]. Available: www.ajpst.com

[2] Y. C. Seo, W. S. Choi, J. H. Park, J. O. Park, K. H. Jung, and H. Y. Lee, "Stable isolation of phycocyanin from Spirulina platensis associated with high-pressure extraction process," *Int. J. Mol. Sci.*, vol. 14, no. 1, pp. 1778–1787, 2013, doi: 10.3390/ijms14011778.

[3] G. Patil, S. Chethana, M. C. Madhusudhan, and K. S. M. S. Raghavarao, "Fractionation and purification of the phycobiliproteins from Spirulina platensis," *Bioresour. Technol.*, vol. 99, no. 15, pp. 7393–7396, 2008, doi: 10.1016/j.biortech.2008.01.028.

[4] N. E. A. El-Naggar, M. H. Hussein, and A. A. El-Sawah, "Bio-fabrication of silver nanoparticles by phycocyanin, characterization, in vitro anticancer activity against breast cancer cell line and in vivo cytotxicity," *Sci. Rep.*, vol. 7, no. 1, pp. 1–20, 2017, doi: 10.1038/s41598-017-11121-3.

[5] D. Kumar, D. W. Dhar, S. Pabbi, N. Kumar, and S. Walia, "Extraction and purification of C-phycocyanin from Spirulina platensis (CCC540)," *Indian J. Plant Physiol.*, vol. 19, no. 2, pp. 184–188, 2014, doi: 10.1007/s40502-014-0094-7.

[6] L. N. Liu, "Distribution and dynamics of electron transport complexes in cyanobacterial thylakoid membranes," *Biochim. Biophys. Acta - Bioenerg.*, vol. 1857, no. 3, pp. 256–265, 2016, doi: 10.1016/j.bbabio.2015.11.010.

[7] S. Sedjati, A. Ridlo, and E. Supriyantini, "Efek Penambahan Gula Terhadap Kestabilan Warna Ekstrak Fikosianin Spirulina sp.," *J. Kelaut. Trop.*, vol. 18, no. 1, pp. 1–6, 2016, doi: 10.14710/jkt.v18i1.505.

[8] J. M. Doke, "An Improved and Efficient Method for the Extraction of Phycocyanin from Spirulina sp," *Int. J. Food Eng.*, vol. 1, no. 5, 2005, doi: 10.2202/1556-3758.1037.

[9] A. Ridlo, S. Sedjati, and E. Supriyantini, "Aktivitas Anti Oksidan Fikosianin Dari Spirulina Sp. Menggunakan Metode Transfer Elektron Dengan DPPH (1,1-difenil-2-pikrilhidrazil)," *J. Kelaut. Trop.*, vol. 18, no. 2, pp. 58–63, 2016, doi:

10.14710/jkt.v18i2.515.
[10] E. Manirafasha, T. Ndikubwimana, X. Zeng, Y. Lu, and K. Jing, "Phycobiliprotein: Potential microalgae derived pharmaceutical and biological reagent," *Biochem. Eng. J.*, vol. 109, pp. 282–296, 2016, doi: 10.1016/j.bej.2016.01.025.
[11] L. Jespersen, L. D. Strømdahl, K. Olsen, and L. H. Skibsted, "Heat and light stability of three natural blue colorants for use in confectionery and beverages," *Eur. Food Res. Technol.*, vol. 220, no. 3–4, pp. 261–266, 2005, doi: 10.1007/s00217-004-1062-7.
[12] R. Chaiklahan, N. Chirasuwan, and B. Bunnag, "Stability of phycocyanin extracted from Spirulina sp.: Influence of temperature, pH and preservatives," *Process Biochem.*, vol. 47, no. 4, pp. 659–664, 2012, doi: 10.1016/j.procbio.2012.01.010.
[13] R. A. Schmidt, M. G. Wiebe, and N. T. Eriksen, "Heterotrophic high cell-density fed-batch cultures of the phycocyanin-producing red alga Galdieria sulphuraria," *Biotechnol. Bioeng.*, vol. 90, no. 1, pp. 77–84, 2005, doi: 10.1002/bit.20417.
[14] N. T. Eriksen, "Production of phycocyanin - A pigment with applications in biology, biotechnology, foods and medicine," *Appl. Microbiol. Biotechnol.*, vol. 80, no. 1, pp. 1–14, 2008, doi: 10.1007/s00253-008-1542-y.
[15] S. T. Silveira, J. F. M. Burkert, J. A. V. Costa, C. A. V. Burkert, and S. J. Kalil, "Optimization of phycocyanin extraction from Spirulina platensis using factorial design," *Bioresour. Technol.*, vol. 98, no. 8, pp. 1629–1634, 2007, doi: 10.1016/j.biortech.2006.05.050.
[16] M. Kuddus, P. Singh, G. Thomas, and A. Al-Hazimi, "Recent developments in production and biotechnological applications of c-phycocyanin," *Biomed Res. Int.*, vol. 2013, 2013, doi: 10.1155/2013/742859.
[17] W. Pan-utai and S. Iamtham, "Physical extraction and extrusion entrapment of C-phycocyanin from Arthrospira platensis," *J. King Saud Univ. - Sci.*, vol. 31, no. 4, pp. 1535–1542, 2019, doi: 10.1016/j.jksus.2018.05.026.
[18] T. Furuki *et al.*, "Rapid and selective extraction of phycocyanin from," *J. Appl. Phycol.*, vol. 15, no. 1979, pp. 319–324, 2003.
[19] J. da C. Ores, M. C. A. de Amarante, and S. J. Kalil, "Co-production of carbonic anhydrase and phycobiliproteins by Spirulina sp. and Synechococcus nidulans," *Bioresour. Technol.*, vol. 219, pp. 219–227, 2016, doi: 10.1016/j.biortech.2016.07.133.