GSC Biological and Pharmaceutical Sciences, 2020, 11(03), 006–013

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Taguchi's design for optimization of phycocyanin extraction from *Arthrospira (Spirulina) platensis*

Şahin Oya Irmak *

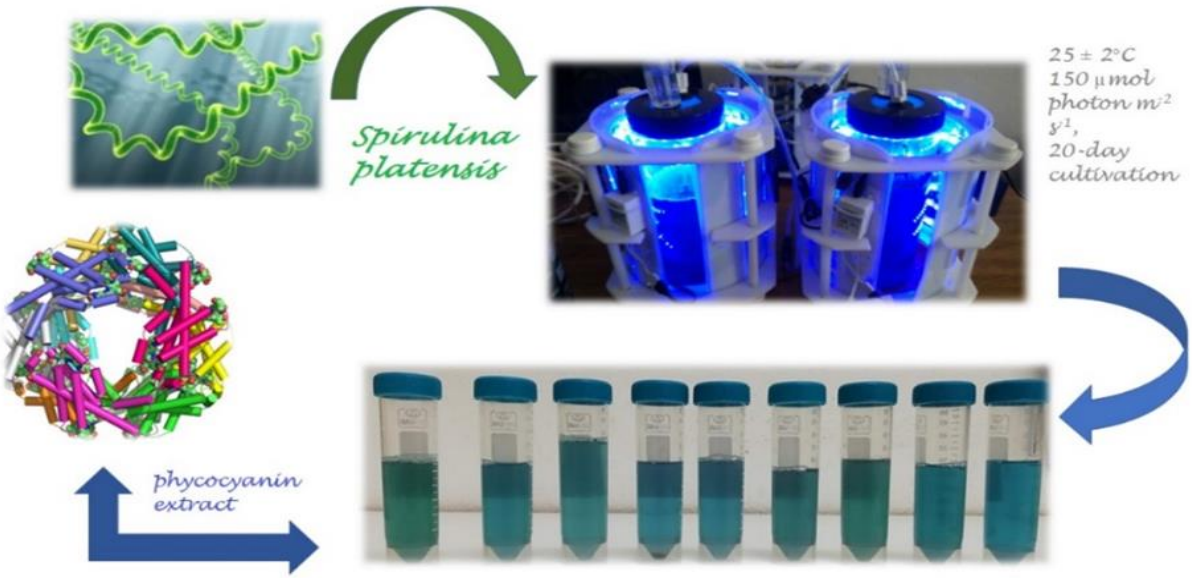
Chemical Engineering Department, Engineering Faculty, Yalova University, Yalova, Turkey

Publication history**:** Received on 28 May 2020; revised on 08 June 2020; accepted on 09 June 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.11.3.0158

Abstract

Blue-green microalgae are the source of many commercially important products such as protein, amino acids, fatty acids, carotenoids and phycobiliproteins, and biotechnological studies on this issue has been profoundly increasing. *Arthrospira (Spirulina) platensis* is the main source of cyanobacterial phycocyanin, used as a natural colorant that is called as "Lina blue" in the commercial area. The purity ratio and quantity of phycocyanin may vary depending on the processing method, also the purity of the phycocyanin is an important factor that determines its application area. Food-grade phycocyanin is utilized safely as a natural dye in the food formulation without offering health risk. Many conventional methods are used in several to obtain crude extracts of C-PC, such as freezing and thawing, sonication and mechanical disruption. Also, for the purification stage high-speed centrifugation, precipitation with ammonium sulphate, and ion-exchange chromatography methods are used. The aim of this study is to investigate the effects of a modified and combined extraction and purification method, including precipitation, chilling and centrifugation, at different centrifuge speeds, temperature and time on phycocyanin concentration to obtain a food grade phycocyanin from *Spirulina platensis*. As a result, centrifugation at 25 °C was found the higher purity ratio, above 0.7.



Keywords: Phycocyanin; Spirulina; Taguchi; Extraction; Food Grade

* Corresponding author: Şahin Oya Irmak

1. Introduction

Microalgae has recently attracted worldwide attention with its potential use in the food, feed, pharmaceutical, fertilizer, fuel, pigment and aquaculture industries and the production of secondary metabolites such as vitamins, toxins, enzymes. Blue-green microalgae are the source of many commercially important products such as protein, amino acids, fatty acids, carotenoids and phycobiliproteins, and biotechnological studies on this issue has been profoundly increasing.

The most important type of phycobiliprotein, which is found in high amounts in blue-green microalgae, is blue-coloured phycocyanin. Phycocyanin can be found in protoplasm up to about 20 % of cell dry weight. Due to the predominance of the green chlorophyll-a and blue-coloured phycocyanin pigment in the cell, the cells appear in characteristic blue green colour.

Phycocyanin got this name because of its phycocyanobilin (FSB) chromophore. Considered as the prosthetic group of the protein, phycocyanobilin exhibits a linear tetrapyrrole structure formed by four pyrrole rings. This structure forms the phycocyanin structure (Fig.1) by binding to the cysteine amino acid from different regions. The chemical structure of phycocyanobilin is similar to bilirubin, which is a bile pigment and has a strong radical removing effect in the cell, and this finding supports that phycocyanobilin's also have radical removing effects such as bilirubin [1,2].

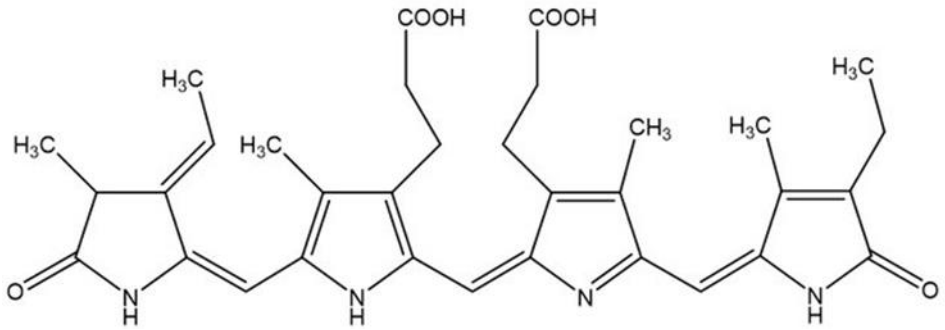


Figure 1 Chemical structure of phycocyanobilin

The main blue green microalgae responsible for the production of phycobiliprotein are *Spirulina (Arthrospira)* [3] and *Anabaena* [4] species**.** The characteristics of the development conditions applied, especially nitrogen and carbon sources, determine the phycobiliprotein content [5].

Bousibba and Richmond [6] stated that the phycocyanin serves as a storage nutrient in Spirulina. It is used as a biochemical isotope in immune-related trials. Due to the fluorescent property of the pigment, pure phycocyanin is profoundly utilised as fluorescent labelling reagents in microscopy and cytometry studies [7,8]. It has been also stated that the phycocyanin generally supports the immune system and provides protection against various diseases due to their antioxidant and anti-inflammatory properties [9,10]. Mainly in food industry, phycocyanin is preferred as a natural food colorant because of its colour of bright blue to indigo blue [11] despite its un-stability to heat.

Today, the main problem in the extraction and purification of phycocyanin is seen as low yield and purity. Many conventional methods are used in several to obtain crude extracts of C-PC, such as freezing and thawing, sonication and mechanical disruption [6,12–14]. Also, methods for the extraction stage are include nitrogen cavitation for algal-cell disruption [15,16], acidic extraction [17] and high-pressure extraction [18]. For the purification stage high-speed centrifugation, precipitation with ammonium sulphate, and ion-exchange chromatography methods are used. Each method has its advantages and disadvantages. Despite all these studies, the problems of extraction and purification processes such as the long-time, expensive, not being able to work in large volumes and low purity rate keeps remain. From this point of view, it is of great scientific and commercial importance to obtain effective and efficient phycocyanin from blue-green microalgae.

Hence, the aim of this study is to investigate the effects of a modified and combined extraction and purification method, including precipitation, chilling and centrifugation, at different centrifuge speeds, temperature and time on phycocyanin concentration and purity from *Arthrospira (Spirulina) platensis*.

2. Material and methods

2.1. Materials

All analytical grade chemicals were purchased from Merck. *Arthrospira (Spirulina) platensis*, in Schössler medium, were cultivated in a 2 L photobioreactor under conditions of 25 ± 2 °C, 150 µmol photon m^{-2} s^{-1}, for 20-day cultivation, at the Food Biochemistry and Biotechnology Laboratory, Yalova University.

The culture in the growing logarithm phase was taken from the photobioreactor and treated with ammonium citrate to obtain pH 7. Biomass yield of the culture was determined as 0.58 g L^{-1} and the added buffer ammonium citrate ratio was maintained as 1:1 (solid: liquid, g: L). Then the homogenized mixture was kept in refrigerator conditions (5 °C) overnight. Chilled mixture was separated in 50 mL centrifugate tubes for a 40 mL volume and then treated at different centrifugation speed and time.

2.2. Analysis

Phycobiliproteins (phycocyanin and allophycocyanin) were determined using Eqs. (1) and (2) [19], phycocyanin purity was evaluated using Eq. (3) and phycocyanin / allophycocyanin separation factor using Eq. (4). Absorbance values were corrected by subtracting the absorbance at 750 nm [20].

$$[PC] = \frac{A_{615} - 0.474\ x\ A_{652}}{5.34} \quad (1)$$

$$[APC] = \frac{A_{652} - 0.208\ x\ A_{615}}{5.09} \quad (2)$$

$$Purity = \frac{A_{615}}{A_{280}} \quad (3)$$

$$Separation\ Factor\ = \frac{A_{615}}{A_{652}} \quad (4)$$

A_{615}, A_{652} and A_{280} are the absorbance for phycocyanin absorption at 615 nm, allophycocyanin absorption for 652 nm and for whole protein content absorbance at 280 nm. All spectrophotometric measurements were performed with a Schimadzu 1280 (Kyoto, Japan) UV-VIS spectrophotometer (±0.001 units of absorbance, 10 mm light path)

2.3. Design of experiment

According to the Taguchi method, the L9 standard orthogonal array (Table 1) of Taguchi for three different parameters used and three different levels of each parameter (Table 2) and this design requires 9 experimental studies.

Table 1 Experimental design matrix according to the Taguchi's design approach

Factor	Name	Minimum	Maximum	Coded Low	Coded High
A	Temperature (°C)	5	25	-1 ↔ 5	+1 ↔ 15
B	Centr. Time (min)	5	25	-1 ↔ 5	+1 ↔ 25
C	Centr. Speed (rpm)	3000	8000	-1 ↔ 3000	+1 ↔ 8000

The objective in this study was to find the optimum extraction conditions for phycocyanin concentration (C-PC) and phycocyanin purity ratio, the experimental data (y_i) were converted to a signal–to– noise (S/N) ratio, so the "larger is the better" function was used to calculate S/N ratio using the following Eq. 5;

$$\frac{S}{N} = -\log_{10}\left(\frac{\Sigma\left(\frac{1}{yi^2}\right)}{n}\right) \quad (5)$$

where yi is the "i"th quality parameter and "n" is the number of trials. The expected values of observations (Y_{opt}), i.e., the phycocyanin concentration and the purity ratio, were calculated using the following Eq. 6;

$$Y_{opt} = \bar{T} + \sum(\bar{F}_i - \bar{T}) \quad (6)$$

where T and F_i are the grand averages of the S/N ratios and the factor averages at each factor level, respectively. The main effect was the difference between the maximum and minimum values of the factor averages at each factor level, while the percent main effect of each factor was calculated as the percentage of its main effect divided by the sum of the main effects of all the factors.

Table 2 Experimental design matrix with actual and coded values

Actual values				**Coded values**		
Run Order	*Temperature (°C)*	*Centrifugation Time (min)*	*Centrifugation Speed (rpm)*	*A*	*B*	*C*
1	25	15	3000	3	2	1
2	25	5	8000	3	1	3
3	5	25	8000	1	3	3
4	15	15	8000	2	2	3
5	5	15	5000	1	2	2
6	25	25	5000	3	3	2
7	15	25	3000	2	3	1
8	15	5	5000	2	1	2
9	5	5	3000	1	1	1

3. Results and discussion

3.1. Taguchi's approach results

One of the most important process to obtain phycocyanin from microalgae is optimizing the extraction and purification steps. Many techniques can be used to extract and purify the phycobiliproteins, but unfortunately, the techniques or the procedures may be varied from one organism to another organism. The release of C-phycocyanin is directly related to cell rupture, but small algae such as *Spirulina* have resistant multi-layered cell walls, making the extraction procedure difficult.

Table 3 Results of experiments according to Taguchi orthogonal design and S/N ratios

St Order	**Run Order**	**A**	**B**	**C**	**Mean value of Phycocyanin Purity**	**S/N ratio of Phycocyanin Purity**	**Mean value of C-PC (g/L)**	**S/N ratio of C-PC (g/L)**
1	9	5	5	3000	0.7007	3.08936	0.2064	19.7517
2	5	5	15	5000	0.6446	3.81419	0.1922	20.3818
3	3	5	25	8000	0.4521	6.89531	0.1625	21.7662
4	8	15	5	5000	0.7795	2.16368	0.2041	19.8365
5	4	15	15	8000	0.5701	4.88098	0.1878	20.5467
6	7	15	25	3000	0.5586	5.05798	0.1991	20.0698
7	2	25	5	8000	0.7236	2.81003	0.2131	19.7096
8	1	25	15	3000	0.6929	3.18659	0.2222	19.0001
9	6	25	25	5000	0.7209	2.84250	0.2181	19.3155

In the presented study, *Arthrospira (Spirulina) platensis* was cultivated for 20 days with an initial OD at 680 nm as 0.30 and the final OD as 1.073. Then the culture was taken from the bioreactor at the stationary phase, which biomass yield was calculated as 0.58 $g.L^{-1}$. Phycocyanin yield was also obtained between 38.31 $mg.g^{-1}$ (run 1) and 28.02 $mg.g^{-1}$ (run 3).

The results obtained from the parameters designed according to the Taguchi method are converted to the signal / noise (S / N) ratio and are expressed in decibels (dB). The signal value represents the actual value given by the system and desired to be measured, and the noise factor represents the share of undesired factors within the measured value. S / N ratio; smaller is better, larger is better and nominal is better, calculated with different formulas according to target value. Whichever S / N ratio formula is used, the value with the largest S / N ratio among the factor levels in the experimental design is the value that provides the best performance. According to Taguchi experiment design, the experiment configuration determined using the L9 orthogonal index, the mean value for each experiment and the S / N ratio calculated according to "larger is better" were given in Table 3. As shown in table, the higher PP was obtained at (run 8) 15 °C, 5 min and at 5000 rpm as 0.7795 and also, the higher C-PC was obtained as 0.2222 $mg.L^{1}$culture at (run 1) 25 °C, 12 min. and 3000 rpm.

The level of the greatest values of the parameters (Table 4) indicates the test result corresponding to the best level of that parameter. These calculated values were shown graphically in Figure 2 for each parameter. As a result, the values with the highest S / N ratios are shown in the circle, giving the optimum design. These optimum parameters were designated as $A_3B_1C_1$ for C-PC and $A_3B_1C_2$ for PP.

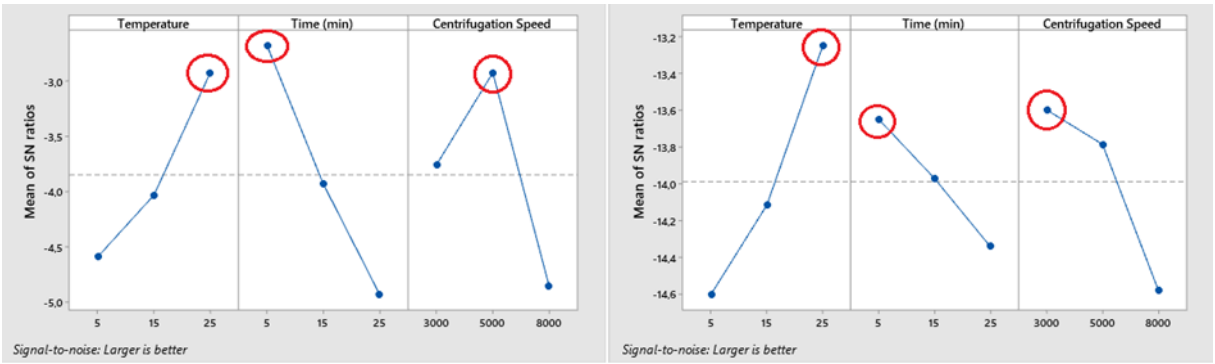


Figure 2 Main effects graphics for S/N ratio a) of PP, b) of C-PC

Table 4 Response Table for Signal to Noise Ratios

Level	Temperature	Centrifugation Time (min)	Centrifugation Speed (rpm)
Purity			
1	4.600	2.688	3.778
2	4.034	3.961	2.940
3	2.946	4.932	4.862
Delta	1.653	2.244	1.922
Rank	3	1	2
C-PC			
1	-14.60	-13.65	-13.60
2	-14.12	-13.97	-13.79
3	-13.25	-14.34	-14.58
Delta	1.36	0.69	0.99
Rank	1	3	2
Larger is better			

3.2. ANOVA Analysis

In order to test the statistical reliability of the results obtained and to determine how much variation was contributed by each factor, variance analysis (ANOVA) was made using the Minitab 19 program using the S/N ratios responses. As seen in Table 5 C-PC is highly affected by temperature and centrifugation speed (Contribution ratio is 0.5440) while the purity ratio was contributed with all parameters. Temperature and centrifugation speed parameters were found statistically significant for both PP and C-PC.

Table 5 ANOVA of factors affecting PP and C-PC

Source	DF	Contribution	F-Value	P-Value
Phycocyanin Purity				
Temperature	2	23.81%	125.27	0.000*
Time (min)	2	44.14%	232.27	0.000*
Centrifugation Speed	2	31.00%	163.12	0.000*
Error	11	1.05%		
Lack-of-Fit	2	1.01%	150.14	0.000
Pure Error	9	0.03%		
Total	17	100.00%		
Model Summary			R^2	R^2 (adj)
			98.95%	98.38%
Phycocyanin Concentration				
Temperature	2	54.40 %	54.20	0.001*
Time (min)	2	11.82 %	11.78	0.002*
Centrifugation Speed	2	28.26 %	28.16	0.001*
Error	11	5.52 %		
Lack-of-Fit	2	3.51 %	7.84	0.011
Pure Error	9	2.01 %		
Total	17	100.00 %		
Model Summary			R^2	R^2 (adj)
			94.48 %	91.47 %

3.3. Process Validation

Confirmation testing is a necessary requirement of the Taguchi method. Confirmation tests were both conducted for C-PC and PP using the optimum settings of the process parameters, as $A_3B_1C_1$ and $A_3B_1C_2$. The confirmation test results for the set of optimization conditions (Table 6) revealed a comparable predicted value with confirmation of the phycocyanin concentration (C-PC) and the phycocyanin purity (PP). As seen from the results (Table 6) for the prediction, C-PC and PP results were found closely to the observations.

Table 6 Predicted and experimental values of optimum parameters by Taguchi's design

Optimum Designs	Predicted Values		Experimental Values	
	C-PC	PP	C-PC	PP
A3B1C1	0.2372	0.8157	0.2305	0.7872
A3B1C2	0.2327	0.8798	0.2284	0.8294

Many researches have been reported that the phycocyanin extraction from biomass showed freezing and thawing was considered to be best when compare with other methods studied by Sarada et al. [17] and Acker & McGann [21]. Walter et al. [22] studied different light intensity with different filters (red-blue-yellow).After sodium phosphate buffer treatment with several freeze-thaw steps, 0.237 $mg.mL^{-1}$ and 0.8 purity ratio was observed. In recent years, some papers reporting the C-phycocyanin extraction from wet biomass were published [3,23–25]. Moraes et al [26] extracted phycocyanin from wet Spirulina biomass and used different extraction methods and obtained an extraction yield of 43.75 $mg.g^{-1}$ and a C-phycocyanin concentration of 0.21 $mg.mL^{-1}$. These results were similar to presented work findings, also in this study Spirulina biomass was only treated with sodium citrate buffer and then centrifugation step was performed.

Compared to previous studies, it was observed that the centrifugation procedure after citrate buffer treatment is sufficient for the food grade phycocyanin. Other purification techniques based on chromatography, for example, can provide a higher degree of purity, if needed, but for the presented extraction procedure there is no need for an extra purification step for the usage in food industry.

4. Conclusion

The presented work hypothesised optimum cultivation of *Arthrospira platensis*, in a 2 L photobioreactor under conditions of 25 ± 2 °C, 150 µmol photon m^{-2} s^{-1} for 20-day cultivation, and a minimal extraction treatment of the un-harvested culture could be reached a food grade phycocyanin with an acceptable phycocyanin concentration. To prove the hypothesis the Taguchi's approach was used. Taguchi method is a high-quality tool for the optimization of biotechnological processes involving microorganism. In this study, the influence of the extraction parameters on phycocyanin from *Arthrospira platensis* could be tested with only 9 runs with the consequent saving time and cost. The optimal conditions for phycocyanin purity (PP) and phycocyanin concentration (C-PC) were obtained as 25 °C, 5 minutes and 5000 rpm.

Compliance with ethical standards

Acknowledgments

No funding has been received to support this work. No funds have been received, to cover the costs to publish in open access.

Disclosure of conflict of interest

The author declares no conflict of interest.

References

[1] Benedetti S, Rinalducci S, Benvenuti F, Francogli S, Pagliarani S, Giorgi L, Micheloni M, D'Amici G M, Zolla L and Canestrari F. (2006). Purification and characterization of phycocyanin from the blue-green alga Aphanizomenon flos-aquae. Journal of Chromatography B, 833, 12–18.

[2] Fernández-Rojas B, Hernández-Juárez J and Pedraza-Chaverri J. (2014). Nutraceutical properties of phycocyanin. Journal of Functional Foods, 11, 375–392.

[3] Bermejo Román R, Alvárez-Pez J M, Acién Fernández F G and Molina Grima E. (2002). Recovery of pure B-phycoerythrin from the microalga Porphyridium cruentum. Journal of Biotechnology. 93, 73–85.

[4] Fan M, Liao Z, Wang R xin and Xu N. (2013). Isolation and antibacterial activity of anabaena phycocyanin. African Journal of Biotechnology, 12 (15), 1869-1873.

[5] Kathiravan A, Chandramohan M, Renganathan R and Sekar S. (2009). Spectroscopic studies on the interaction between phycocyanin and bovine serum albumin. Journal of Molecular Structure, 919, 210–214.

[6] Boussiba S and Richmond AE. (1980). C-phycocyanin as a storage protein in the blue-green alga Spirulina platensis, Arch. Microbiol, 125, 143–147.

[7] Glazer AN. (1994). Phycobiliproteins — a family of valuable, widely used fluorophores. Journal of Applied Phycology, 6, 105–112.

[8] Telford WG, Moss MW, Morseman JP and Allnutt FCT. (2001). Cyanobacterial stabilized phycobilisomes as fluorochromes for extracellular antigen detection by flow cytometry. Journal of Immunological Methods, 254, 13–30.

[9] Zhang Y-M and Chen F. (1999). A simple method for efficient separation and purification of c-phycocyanin and allophycocyanin from Spirulina platensis. Biotechnology Techniques, 13, 601–603.

[10] Romay C, Gonzalez R, Ledon N, Remirez D and Rimbau V. (2003). C-Phycocyanin: A Biliprotein with Antioxidant, Anti-Inflammatory and Neuroprotective Effects. Current Protein and Peptide Science, 4, 207–216.

[11] Yoshida A, Takagaki Y and Nishimune T. (1996). Enzyme immunoassay for phycocyanin as the main component of spirulina color in foods. Bioscience, Biotechnology, and Biochemistry, 60, 57–60.

[12] Minkova KM, Tchernov AA, Tchorbadjieva MI, Fournadjieva ST, Antova RE and Busheva M. (2003). Purification of C-phycocyanin from Spirulina (Arthrospira) fusiformis. Journal of Biotechnology, 102, 55–59.

[13] Silveira ST, Burkert JFM, Costa JAV, Burkert CAV and Kalil SJ. (2007). Optimization of phycocyanin extraction from Spirulina platensis using factorial design. Bioresource Technology, 98, 1629–1634.

[14] Furuki T, Maeda S, Imajo S, Hiroi T, Amaya T, Hirokawa T, Ito K and Nozawa H. (2003). Rapid and selective extraction of phycocyanin from Spirulina platensis with ultrasonic cell disruption. Journal of Applied Phycology, 15, 319–324.

[15] Gottlieb RA and Adachi S. (2000). Nitrogen Cavitation for Cell Disruption to Obtain Mitochondria from Cultured Cells. Methods in Enzymology, ed: J C Reed (Academic Press), 213–221.

[16] Viskari PJ and Colyer CL. (2003). Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. Analytical Biochemistry, 319, 263–271.

[17] Sarada R, Pillai MG and Ravishankar GA. (1999). Phycocyanin from Spirulina sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. Process Biochemistry, 34, 795–801.

[18] Herrero M, Martín-Álvarez PJ, Señoráns FJ, Cifuentes A and Ibáñez E. (2005). Optimization of accelerated solvent extraction of antioxidants from Spirulina platensis microalga. Food Chemistry, 93, 417–423.

[19] Bennett A and Bogorad L. (1973). Complementary Chromatic Adaptation in a Filamentous Blue-Green Alga. The Journal of Cell Biology, 58, 419–435.

[20] Lawrenz E, Fedewa E J and Richardson TL. (2011). Extraction protocols for the quantification of phycobilins in aqueous phytoplankton extracts. Journal of Applied Phycology, 23, 865–871.

[21] Acker J P and McGann LE. (2003). Protective effect of intracellular ice during freezing? Cryobiology, 46, 197–202.

[22] Walter A, Carvalho JC de, Soccol VT, Faria ABB de, Ghiggi V and Soccol CR. (2011). Study of phycocyanin production from Spirulina platensis under different light spectra. Brazilian Archives of Biology and Technology, 54, 675–682.

[23] Soni B, Trivedi U and Madamwar D. (2008). A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from Phormidium fragile and its characterization for antioxidant property. Bioresource Technology, 99, 188–194.

[24] Soni RA, Sudhakar K and Rana RS. (2019). Comparative study on the growth performance of Spirulina platensis on modifying culture media. Energy Reports, 5, 327–336.

[25] Bermejo P, Piñero E and Villar Á M. (2008). Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulina platensis Food Chemistry, 110, 436–445.

[26] Moraes CC, Sala L, Cerveira GP and Kalil SJ. (2011). C-phycocyanin extraction from Spirulina platensis wet biomass. Brazilian Journal of Chemical Engineering, 28, 45–49.

How to cite this article

Şahin OI. (2020). Taguchi's design for optimization of phycocyanin extraction from *Arthrospira (Spirulina) platensis*. GSC Biological and Pharmaceutical Sciences, 11(3), 06-13.