Optimization of C-phycocyanin Extraction from *Arthrospira maxima* Grown in a Raceway System

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***Abstract*— Microalgae convert light energy into chemical energy, producing high-value molecules. *Arthrospira maxima* is rich in carbohydrates, proteins, lipids, vitamins and minerals. It also produces phycocyanin, a protein with antioxidant and anti-inflammatory properties. The objective of this study was to evaluate the optimal conditions for the extraction of phycocyania produced by *A. maxima* grown in a raceway, using the Response Surface methodology.**

***Keywords*— *Arhrospira*, phycocyanin, raceway**

I. Introduction

The global demand for protein of animal origin is continuously growing, which is why meat is considered a high-value product [1]. That is why microalgae have acquired greater interest as a nutritional supplement.

Microalgae are microorganisms that convert light energy into chemical energy through photosynthesis [2].

Arthrospira, commonly known as Spirulina is one of the best known species and its biomass is rich in carbohydrates, proteins, vitamins, minerals, fatty acids and pigments such as chlorophyll, phycocyanin and carotenoids. [3]. Spirulina has been part of the human diet for more than a thousand years as it was used as food. It is currently classified as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration) [4].

It is well recognized for synthesizing phycocyanin (C-PC), a water-soluble phycobiliprotein. It is used as a pigment in food products and has gained interest because it has been shown to have antioxidant, anti-inflammatory, and anticancer effects [5].

There are various methods for phycocyanin extraction such as sonication, grinding with glass beads, and freeze/thaw cycles [6].

The objective of this study was to optimize the phycocyanin extraction in *A. maxima* grown in a raceway reactor using the Response Surface Methodology (RSM).

The experimental design Response Surface Methodology is a statistical tool that relates the input and output controllable variables to find the optimal input conditions that allow maximizing or minimizing the output response [7].

II. Methodology

A. Microoganism and Culture Conditions

The *Arthrospira maxima* strain, obtained from the Environmental Remediation Laboratory of the Faculty of Agronomy, was cultivated in Zarrouk Medium containing: $NaHCO_3$ (16.8 g L^{-1}), $NaNO_3$ (2.5 g L^{-1}), NaCl (1.0 g L^{-1}), K_2SO_4 (1.0 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), $MgSO_4.7H_2O$ (0.2 g L^{-1}), EDTA (0.08 g L^{-1}), $CaCl_2.2H_2O$ (0.04 g L^{-1}), $FeSO_4.7H_2O$ (0.01 g L^{-1}). The pH was adjusted to 9.0 with NaOH.

The raceway reactor was made of acrylic, it is 1.40 m long, 0.45 m wide and 0.25 cm deep. We worked with a total volume of 80 L with 8 h of shaking at 15 rpm and lighting of 2760 lux for 12 days, as shown in Fig. 1.

At the end of the culture time, the biomass was harvested for filtration through a 165-wire screen mesh and dried at 35°C in a food dehydrator (Septree, China).

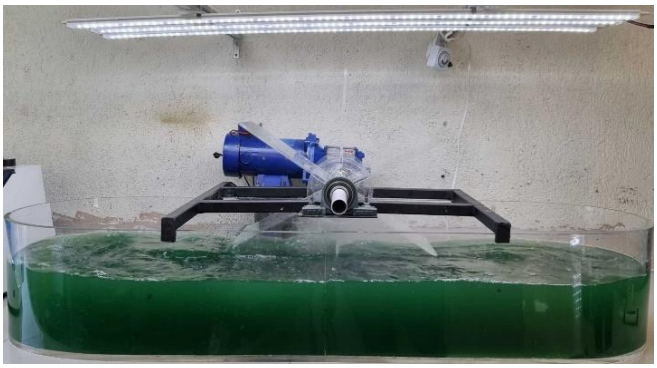

Fig. 1. Raceway reactor

B. Experimental Design

A central composite design and three central points were used. Different pH, buffer concentrations, amount of biomass and number of cycles were evaluated (Table I). Using the Design Expert software (version 12), 29 experimental runs were obtained as shown in Table II.

TABLE I
RANGE OF LEVELS OF VARIABLES USED FOR THE OPTIMIZATION OF THE EXTRACTION OF C-PC S

Independient variables	Factor	Low (-1)	Medium (0)	High (+1)
pH	A	5.5	6.75	8.0
Buffer concentration (mM)	B	100	300	500
Number of cycles	C	1	3	5
Biomass amount (mg)	D	50	100	150

C. C-PC Extraction

The extraction process is illustrated in Fig 2. The dry biomass was weighed, and 10 mL of potassium phosphate buffer was added at the corresponding concentration and pH according to the experimental design. It was mixed with a spatula for good homogenization and frozen at -20°C ± 2°C in a Torrey brand freezer. After 24 hours, they were removed to allow complete thawing.

According to the corresponding number of cycles, the thawed tubes were centrifuged, and the supernatant was quantified at 652, 615, 562, and 280 nm. Subsequently, the phycocyanin concentration (mg. mL^{-1}) was calculated using equation (1) according to [8].

$$C - PC = (A_{620} - 0.474\ x\ A_{652})/5.34 \quad (1)$$

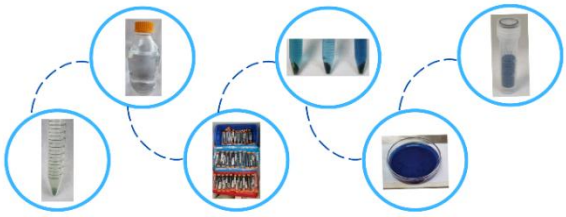

Fig. 2. Phycocyanin extraction process.

III. RESULTS AND DISCUSSION

The phycocyanin concentration obtained from each of the experimental runs is shown in conjunction with the value predicted by the design in Table II.

TABLE II
SET OF VARIABLES AND RESPONSE OF THE CENTRAL COMPOSITE DESIGN MATRIX TYPE STYLES

Run	A	B	C	D	C-PC Conc. (mg mL^{-1})	
					Exp.	*Predicted*
1	6.75 (0)	100 (-1)	3 (0)	150 (+1)	0.221	0.206
2	6.75 (0)	300 (0)	1 (-1)	150 (+1)	0.173	0.180
3	5.5 (-1)	300 (0)	5 (+1)	100 (0)	0.134	0.130
4	8 (+1)	300 (0)	1 (-1)	100 (0)	0.064	0.068
5	8 (+1)	300 (0)	3 (0)	150 (+1)	0.132	0.138
6	6.75 (0)	300 (0)	3 (0)	100 (0)	0.133	0.128
7	6.75 (0)	100 (-1)	1 (-1)	100 (0)	0.123	0.115
8	6.75 (0)	300 (0)	3 (0)	100 (0)	0.133	0.128
9	6.75 (0)	300 (0)	1 (-1)	50 (-1)	0.054	0.054
10	5.5 (-1)	300 (0)	3 (0)	50 (-1)	0.067	0.058
11	5.5 (-1)	300 (0)	3 (0)	150 (+1)	0.205	0.206
12	6.75 (0)	500 (+1)	3 (0)	150 (+1)	0.181	0.178
13	6.75 (0)	300 (0)	3 (0)	100 (0)	0.138	0.128
14	5.5 (-1)	300 (0)	1 (-1)	100 (0)	0.111	0.113
15	6.75 (0)	100 (-1)	5 (+1)	100 (0)	0.128	0.128
16	8 (+1)	500 (+1)	3 (0)	100 (0)	0.086	0.076
17	6.75 (0)	300 (0)	5 (+1)	50 (-1)	0.072	0.069
18	8 (+1)	300 (0)	5 (+1)	100 (0)	0.084	0.082
19	8 (+1)	100 (-1)	3 (0)	100 (0)	0.074	0.082
20	6.75 (0)	300 (0)	3 (0)	100 (0)	0.127	0.128
21	5.5 (-1)	100 (-1)	3 (0)	100 (0)	0.115	0.129
22	6.75 (0)	300 (0)	5 (+1)	150 (+1)	0.191	0.195
23	6.75 (0)	500 (+1)	3 (0)	50 (-1)	0.059	0.074
24	8 (+1)	300 (0)	3 (0)	50 (-1)	0.038	0.033
25	6.75 (0)	500 (+1)	5 (+1)	100 (0)	0.120	0.124
26	6.75 (0)	100 (-1)	3 (0)	50 (-1)	0.056	0.058
27	6.75 (0)	500 (+1)	1 (-1)	100 (0)	0.110	0.106
28	5.5 (-1)	500 (+1)	3 (0)	100 (0)	0.126	0.122
29	6.75 (0)	300 (0)	3 (0)	100 (0)	0.108	0.128

Fig. 3 shows the values predicted by the model versus the experimental data, showing a linear pattern indicating that it is a good model for the data set.

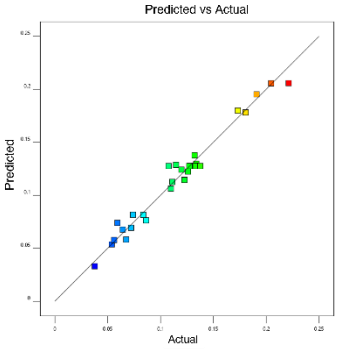

Fig. 3. Plot of prediction versus actual.

The results of the analysis of variance (ANOVA) are shown in table III. According to the analysis, the variables that are statistically significant are the pH, the number of cycles and the amount of biomass.

TABLE III
ANOVA OF THE QUADRATIC MODEL DOR THE EXTRACTION OF C-PHYCOCYANIN

ANOVA	
R^2	0.9718
p- value of model	0.0001
F- value of model	34.48
Standard deviation	0.0111
Mean	0.1159
C. V %	9.61
df	14
F- value of A	52.46
F- value of B	0.8114
F- value of C	5.82
F- value of D	384.22
p- value of A	0.0001
p- value of B	0.3829
p- value of C	0.0301
p- value of D	0.0001
Sum of squares	0.0599
Adeq. precision	21.5647

The 3D graphs shown in Fig. 4 allow us to understand the effect of the interactions of the amount of biomass with pH, buffer concentration and number of cycles. The red área is where the highest concentration of phycocyanin was obtained. Fig. 4a shows the interaction of the amount of biomass and pH. As expected, the greater the amount of biomass, the more phycocyanin is obtained, but the effect of pH is important because phycocyanin is unstable at pH values below 4.8. Under acidic conditions it is insoluble [9].

The interaction of the variables amount of biomass and buffer concentration is shown in Fig. 4b. It is observed that a lower concentration of the buffer is adequate. A similar result was obtained by [10], who after testing different concentrations of potassium phosphate buffer, determined that the best was 0.125 M.

As observed in Fig 4c, a higher concentration of phycocyanin is obtained as the number of cycles increases. This coincides with what was reported by [11] who evaluated the freeze-thaw and electric field method in *Nostoc commune* finding that the highest concentrations of C-PC were achieved with the first method, especially increasing the number of cycles.

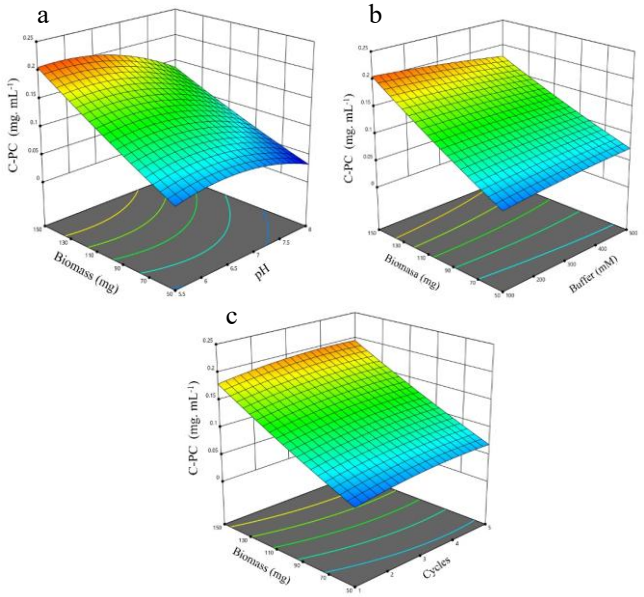

Fig. 4. 3D graphs of the RSM of the interaction of biomass with a) pH, b) buffer concentration and c) number of cycles.

According to Tavanandia et al. [12], who tested different extraction methods in *A. platensis* found that for this same method a higher yield is obtained by increasing the number of cycles up to a maximum of 5.

IV. Conclusion

Phycocyanin is a phycobiliprotein with high nutritional value that is mainly produced by cyanobacteria and some red algae. In this study, *Arthrospira maxima* was grown in a raceway reactor to extract phycocyanin using the Response Surface methodology.

It was determined that using a 100 mM potassium phosphate buffer with a pH of 5.8, 150 mg of biomass and 4 freeze-thaw cycles are optimal to obtain a higher concentration of phycocyanin.

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References

[1] B. A. Altmann and S. Rosenau. "Spirulina as animal feed: opportunities and challenges". *Foods*, vol. 11, no. 7, pp. 965, 2022. DOI: https://doi.org/10.3390/foods11070965.

[2] R. A. Soni, K. Sudhakar and R.S. Rana. "Spirulina- From growth to nutritional product: A review". *Trends in Food Science and Technology*, vol. 69, pp. 157-171, 2017. DOI: https://doi.org/10.1016/j.tifs.2017.09.010.

[3] B. B. Andrade, L. G. Cardoso, D. J Assis, J. A. V. Costa, J. I. Druzian and S. T. Lima. "Production and characterization of Spirulina sp. LEB 18 cultured in reused Zarrouk's medium in a raceway-type bioreactor". *Bioresource Technology*, vol. 284, pp. 340-348 2019. DOI: https://doi.org/10.1016/j.biortech.2019.03.144.

[4] K. Z. N. AlFadhly, N. Alhelfi, A. B. Altemimi, D. K. Verma, F. Cacciola and A. Narayanankutty. "Trends and technological advancements in the possible food applications of spirulina and their health benefits: a review". *Molecules*, vol. 25, 2022. DOI: https://doi.org/10.3390/molecules27175584.

[5] I. Chentira, M. Hamdib, S. Li, A. Doumandji, G. Markou and M. Nasri. "Stability, bio-functionality and bio-activity of crude phycocyanin from a two-phase cultured Saharian Arthrospira sp. Strain". *Algal Research*, vol. 35, pp. 395-406, 2018. DOI: https://doi.org/10.1016/j.algal.2018.09.013.

[6] D. P. Jaeschke, I. R. Teixeira, L. D. F. Marczak and G. D. Mercali. "Phycocyanin from Spirulina: A review of extraction methods and stability". *Food Research International*, vol. 143, 2021. DOI: https://doi.org/10.1016/j.foodres.2021.110314.

[7] S. M. Z. Hossain, A. Alnoaimi, S. A. Razzak, H. Ezuber, N. Al-Bastaki, M. Safdar, S. Alkaabi and Mohammad M. Hossain. "Multiobjective optimization of microalgae (Chlorella sp.) growth in a photobioreactor using Box Behnken design approach". T*he Canadian Journal of Chemical Engineering*, vol. 96, pp. 1903-1910, 2018. DOI: https://doi.org/10.1002/cjce.23168.

[8] A. Bennett and L. Bogorad. "Complementary chromatic adaptation in a filamentous blue-green algae". *Journal Cell Biology*, vol. 58, pp. 419-435, 1973. DOI: https://doi.org/10.1083/jcb.58.2.419.

[9] B. Yuan, Z. Li, H. Shan, B. Dashnyam, X. Xu, D. J. McClements, B. Zhang, M. Tan, Z. Wang and C. Cao. "A review of recent strategies to improve the physical stability of phycocyanin". *Current Research in Food Science*, vol. 5, 2022. DOI: https://doi.org/10.1016/j.crfs.2022.11.019.

[10] E. Manirafasha, T. Murwanashyaka, T. Ndikubwimana, Q. Yue, X. Zeng, Y. Lu and K. Jing. "Ammonium chloride: a novel effective and inexpensive salt solution for phycocyanin extraction from Arthrospira (Spirulina) platensis". *Journal of Applied Phycology*, vol. 29, pp. 126-1270, 2017. DOI: https://doi.org/10.1007/s10811-016-0989-y.

[11] S. Chittapun, V. Jonjaroen, K. Khumrangsee and T. Charoenrat. "C-phycocyanin extraction from two freshwater cyanobacteria by freeze thaw and pulsed electric field techniques to improve extraction efficiency and purity". *Algal Research*, vol. 46, 2020. DOI: https://doi.org/10.1016/j.algal.2020.101789.

[12] H. A. Tavanandia, R. Mittala, J. Chandrasekharb and K.S.M.S. Raghavarao. "Simple and efficient method for extraction of C-Phycocyanin from dry biomass of Arthospira platensis". *Algal Research*, vol. 21, 2018. DOI: https://doi.org/10.1016/j.algal.2018.02.008.