

Stability of C-Phycocyanin, a Blue Dye from *Spirulina Maxima*

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Abstract— *Spirulina maxima* is characterized by its high protein content, which represents up to 70% of its dry mass. Among its nutrients is C-Phycocyanin (C-PC), a phycobiliprotein responsible for the blue-green color. The FDA (Food and Drug Administration) in the United States recognized *Spirulina maxima* as GRAS (generally recognized as safe). To obtain the C-PC extract, *S. maxima* biomass was dried and suspended in 40 mL of 100 mM phosphate buffer, pH 7.8, using an orbital shaker at 150 rpm and 40°C for 25 min. To calculate the concentration of C-PC (mg mL⁻¹), the supernatant was read at 615, 620, 652, and 280 nm. The stability of the extract was analyzed for 0, 3, 6, 18, 24, 48, and 72 h, its response to temperature (4, 20, 40, 60, and 100 °C), pH (3, 5, 7, 7.8, and 9), and exposure to light (90 μmol m⁻² s⁻¹). In C-PC, greater stability was observed at pH 4-7 and 20°C, but under light. As the pigment concentration increased, the tendency for the drink to turn blue also increased. However, in the sensory evaluation, no significant differences were observed. This study demonstrated the stability of this natural pigment as a feasible alternative for the addition of various products, indicating its commercialization potential.

Keywords—Cyanobacteria, microalgae, natural pigment.

I. INTRODUCTION

Microalgae can be divided into four research areas: wastewater treatment, CO₂ removal, biofuel production, and high-value-added molecule production. The interest in health and safety in the consumption of processed foods has increased, which is why microalgae have gained popularity as natural additives [1]. The high protein content of several species has been a significant reason for considering them as a food source [2]. The richness of microalgae, thanks to their compounds, can favor the development of a food industry that seeks to produce and use them to create functional and innovative food products and thus obtain viable health benefits [3].

Spirulina maxima has a spiral shape, with cylindrical cells measuring 3-13 μm in diameter and 100-200 μm long [4]. It is made up of 65% protein, 55% lipids, 7% minerals, 20% carbohydrates, and 3% moisture. One of the crucial percentages for which it has been very notorious is the protein content, which is part of the benefits found. The vitamins provided by this cyanobacterium are: vitamin A (beta-carotene), vitamin B1 (thiamin), vitamin B2 (riboflavin),

vitamin B3 (niacin), vitamin B6 (pyridoxine), vitamin B12, and vitamin E (alpha-tocopherol) [5]. Among its other benefits are its immunological, antioxidant, antiviral, anticancer, antitoxic, and regulatory properties against hyperglycemia and hyperlipidemia [6]. Due to the interest in its research and use for human consumption, the FDA has indicated since 1981 that it is a protein source that contains vitamins and minerals [4].

Among the proteins found in *Spirulina maxima* are phycobiliproteins (PBPs), which are responsible for 50% of the light it absorbs. These are classified according to the color of the pigment they give: C-PC (blue), A-PC (red), and C-PE (pink) [7]. The main application of these compounds is as natural colorants, although research into their properties and effects on human health has increased, expanding their use into the pharmaceutical market [8]. C-PC is a water-soluble blue pigment used in the formulation of food products, although there are limited options for obtaining the blue color naturally. It has also been given increased importance and use for its antioxidant, anti-inflammatory, and anticancer activity [9].

A. Justification

Currently, the use of C-PC in food is limited by its high cost, low stability, and discoloration at high temperatures. Therefore, it is of interest to analyze its stability and potential as a better natural blue pigment for use in a wide variety of foods.

B. Objective

This research aimed to evaluate the stability of C-PC under different conditions, such as pH, temperature, and exposure to light, as well as to analyze its acceptance in food.

II. MATERIALS AND METHODS

A. Extraction

For pigment extraction, 200 mg of *Spirulina maxima* biomass were suspended in 40 mL of 100 mM phosphate buffer and stirred at 150 rpm for 25 min at 40°C. Subsequently, it was centrifuged (Civeq 80-2, Velaquin) at room temperature for 10 min, and the blue supernatant was collected, representing the crude extract of C-PC. The yield of C-PC was expressed in mg mL⁻¹, equation (1). At the same

time, the purity of the extracts was determined by the purity index, equation (2) [10].

$$C - PC = [(A_{615} - 0.474 * A_{652})]/5.34 \quad (1)$$

$$PI = A_{620}/A_{280} \quad (2)$$

B. Stability

The C-PC extract was evaluated at different pH levels (3, 5, 7, 7.8, and 9), later the extract with pH 7 was used at 4, 20, 40, 60, and 100°C, and finally with a pH 7 at 20°C with a light intensity of 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ [11]. Absorbance was measured with a UV-Vis spectrophotometer (Thermo Scientific, Evolution 201, China) at times 0, 3, 6, 18, 24, 48, and 72 h.

C. Application in food

The extract was precipitated with 5% sodium citrate and dried at 40°C for 24 h. At the end of this time, it was used to enrich a drink. A 7-point hedonic test was conducted with 25 semi-trained panelists, where 1 corresponds to 'I dislike very much' and 7 to 'I like it very much'. The objective was to determine the degree of acceptance for smell, flavor, color, sweetness, and overall acceptability across all treatments [12].

A Konica Minolta CR-410 digital colorimeter was used to measure the beverage samples on the day of their preparation; each treatment was measured in triplicate. The analysis was based on the $L^* a^* b^*$ system, where L^* is the luminosity on a scale that varies from zero to one hundred (minimum brightness is zero and maximum brightness is 100), a^* indicates the intensity of the green colors and red (negative values is a tendency to green and positive values is a tendency to red) and b^* indicates the intensity of the yellow and blue colors (negative values is a tendency to blue and positive values is a tendency to yellow), the values of Hue (tone) and Chroma were determined [13].

III. STATISTICAL ANALYSIS

The results were analyzed using Minitab 19, with a one-factor analysis of variance, and Tukey's comparison of means was performed.

IV. RESULTS AND DISCUSSION

A. Stability

At pH 7 and 7.8, no visible change was observed, but at pH 3, the extract lost almost all its color. The pH levels of 7 and 7.8 were not statistically different during the first 48 h (Table I). In other studies, they showed greater stability at pH 7, and in the first 6 h, there was no significant difference compared with PBP R-Phycocerythrin [14]. At pH 3, they exhibited a color change and high instability in R-PE [15]. However, in another investigation, it was found that C-PC was slightly more stable under acidic conditions than under neutral or alkaline conditions [16]. Table II shows that the PI was not statistically different at pH 7 and 7.8 for the first 48

h. However, pH 7 showed the highest purity, while pH 3 showed the lowest.

TABLE I
RESULTS OF C-PC AT DIFFERENT pH LEVELS

T (h)	pH 3	pH 5	pH 7	pH 7.8	pH 9
0	0.01±0.00 ^a	0.07±0.00 ^b	0.23±0.00 ^a	0.19±0.00 ^a	0.13±0.00 ^{ab}
3	0.00±0.00 ^b	0.07±0.00 ^b	0.22±0.01 ^a	0.19±0.01 ^a	0.10±0.00 ^c
6	0.00±0.00 ^b	0.07±0.00 ^b	0.23±0.00 ^a	0.19±0.00 ^a	0.11±0.01 ^{bc}
18	0.00±0.00 ^b	0.08±0.00 ^b	0.23±0.00 ^a	0.19±0.00 ^a	0.12±0.00 ^{abc}
24	0.00±0.00 ^b	0.09±0.01 ^a	0.24±0.00 ^a	0.20±0.00 ^a	0.13±0.01 ^a
48	0.00±0.00 ^b	0.09±0.00 ^a	0.21±0.03 ^a	0.18±0.02 ^a	0.08±0.01 ^d
72	0.01±0.00 ^a	0.07±0.01 ^b	0.14±0.02 ^b	0.12±0.01 ^b	0.08±0.01 ^d

* Different letters in the same parameter show a significant difference.

TABLE II
RESULTS OF PI AT DIFFERENT pH LEVELS

T (h)	pH 3	pH 5	pH 7	pH 7.8	pH 9
0	0.03±0.00 ^b	0.18±0.00 ^{cd}	0.44±0.01 ^a	0.37±0.01 ^a	0.26±0.01 ^{ab}
3	0.02±0.00 ^b	0.17±0.00 ^d	0.44±0.01 ^a	0.36±0.01 ^a	0.28±0.01 ^{cd}
6	0.02±0.00 ^b	0.17±0.00 ^d	0.43±0.01 ^a	0.37±0.01 ^a	0.23±0.02 ^{bc}
18	0.02±0.00 ^b	0.18±0.00 ^{bcd}	0.43±0.01 ^a	0.39±0.01 ^a	0.26±0.00 ^a
24	0.02±0.00 ^b	0.19±0.01 ^b	0.44±0.00 ^a	0.40±0.01 ^a	0.28±0.01 ^a
48	0.02±0.00 ^b	0.23±0.00 ^a	0.40±0.04 ^a	0.36±0.03 ^a	0.20±0.01 ^{de}
72	0.08±0.03 ^a	0.19±0.01 ^{bc}	0.28±0.01 ^b	0.26±0.02 ^b	0.18±0.01 ^e

* Different letters in the same parameter show a significant difference.

In the evaluation at different temperatures (100 and 60°C), the color was lost within the first 3 h, whereas at 4, 20, and 40°C it was maintained. Table III shows that all treatments were statistically different. When the extract was kept at 4 °C and 20°C, its concentration did not decrease. In another investigation, a decrease in the rate of color preservation of C-PC was seen at temperatures above 50°C at pH 7 [3]. However, with another PBP, they reported stable results up to 40°C; visible changes in the extract occurred at 45-50°C [11]. It was also found that C-PC degrades slowly at room temperature, whereas the degradation rate was higher at 47 and 69°C [17]. The PI was statistically different (Table IV), but the 4 and 20°C treatments did not decrease its purity.

TABLE III
RESULTS OF C-PC AT DIFFERENT TEMPERATURES

T (h)	4°C	20°C	40°C	60°C	100°C
0	0.23±0.00 ^{ab}	0.23±0.00 ^c	0.23±0.00 ^c	0.23±0.00 ^a	0.23±0.00 ^a
3	0.23±0.00 ^{ab}	0.24±0.00 ^{bc}	0.24±0.00 ^{ab}	0.07±0.01 ^b	0.07±0.00 ^b
6	0.23±0.00 ^a	0.24±0.00 ^b	0.24±0.00 ^a	0.05±0.00 ^c	0.06±0.00 ^c
18	0.23±0.00 ^a	0.26±0.01 ^a	0.24±0.00 ^{ab}	0.04±0.00 ^{cd}	0.04±0.00 ^d

24	0.23±0.00 ^{ab}	0.26±0.00 ^a	0.23±0.00 ^{bc}	0.04±0.00 ^{cd}	0.04±0.00 ^e
48	0.22±0.01 ^b	0.25±0.00 ^a	0.21±0.00 ^d	0.04±0.01 ^{cd}	0.01±0.00 ^f
72	0.23±0.00 ^{ab}	0.24±0.01 ^{bc}	0.20±0.00 ^c	0.03±0.00 ^d	0.01±0.00 ^f

* Different letters in the same parameter show a significant difference.

TABLE IV
RESULTS OF PI AT DIFFERENT TEMPERATURES

T (h)	4°C	20°C	40°C	60°C	100°C
0	0.45±0.00 ^{ab}	0.45±0.00 ^c	0.45±0.00 ^a	0.45±0.00 ^a	0.45±0.00 ^a
3	0.46±0.00 ^{ab}	0.47±0.01 ^{bc}	0.47±0.02 ^a	0.13±0.01 ^b	0.15±0.01 ^b
6	0.46±0.00 ^a	0.47±0.01 ^{bc}	0.47±0.01 ^a	0.11±0.00 ^c	0.13±0.01 ^c
18	0.47±0.00 ^a	0.48±0.01 ^{ab}	0.45±0.01 ^a	0.10±0.00 ^c	0.10±0.00 ^d
24	0.45±0.00 ^{ab}	0.50±0.01 ^a	0.44±0.01 ^a	0.09±0.00 ^{cd}	0.09±0.00 ^d
48	0.44±0.02 ^b	0.49±0.01 ^{ab}	0.41±0.01 ^b	0.09±0.01 ^{cd}	0.03±0.01 ^e
72	0.46±0.02 ^{ab}	0.47±0.02 ^{bc}	0.38±0.01 ^b	0.08±0.01 ^d	0.03±0.00 ^e

* Different letters in the same parameter show a significant difference.

When the C-PC extract was exposed to light at 20°C, it showed a statistically significant difference. Table V shows that it decreased in 3 h and then had no notable changes. The PI was statistically different. A 68% decrease in a PBP from *P. cruentum* was observed after 48 h at 79 μmol m⁻² s⁻¹ [11]. Other studies reported that the best storage conditions are in the dark, as C-PC decreases by 20% after 36 h of exposure at 100 μmol m⁻² s⁻¹ [17].

TABLE V
RESULTS OF C-PC AND IP UNDER LIGHT

T (h)	C-PC	PI
0	0.27±0.01 ^a	0.53±0.02 ^a
3	0.22±0.01 ^b	0.43±0.01 ^b
6	0.21±0.01 ^b	0.42±0.01 ^b
18	0.23±0.02 ^b	0.44±0.03 ^b
24	0.24±0.01 ^{ab}	0.45±0.03 ^b
48	0.21±0.01 ^b	0.42±0.01 ^b
72	0.21±0.01 ^b	0.43±0.03 ^b

* Different letters in the same parameter show a significant difference.

B. Application in food

Table VI shows the color parameters (L*, a*, b*, C*, and H*) for all treatments on the day of preparation. When comparing the treatments, significant differences (p < 0.05) were observed in all the study parameters. The control treatment had a higher L* value than the other treatments; that is, when adding C-PC, luminosity decreased, causing the drink to darken. On the other hand, the a* values were all negative, but the control treatment showed the most significant tendency toward green with -61.67. The value of b* in the control treatment was positive, whereas the other treatments showed a tendency toward blue when b* was negative. As the concentration of C-PC increased, the tendency to blue in the drink also increased. The differences in the color parameters were due to the natural color of the pigment derived from the blue-green microalgae. In a similar

investigation, the addition of C-PC to a soft drink was evaluated, and the color was visible when 5, 10, or 15 mL of C-PC extract was added per 10 mL. This drink remained stable during and after its preparation, thanks to the determination of optimal conditions for C-PC extraction [18].

The results of the sensory evaluation are shown in Table VII, which were not statistically different. Similar results were observed with a soft drink enriched with C-PC extract, which also showed no significant differences across the three sensory tests [18]. On the other hand, when C-PC was incorporated into yogurt, there was no significant difference, but yogurt fortified with 4% C-PC had greater overall acceptability [19].

TABLE VI
RESULTS BEVERAGE COLOR

Value	Control	0.01	0.03	0.05
L*	85.83±0.16 ^a	83.97±0.29 ^b	82.12±1.06 ^c	80.54±0.15 ^d
a*	-61.67±1.53 ^b	-2.22±0.20 ^a	-3.31±0.66 ^a	-4.40±0.16 ^a
b*	0.63±0.01 ^a	-1.31±0.22 ^b	-3.00±0.91 ^c	-4.80±0.27 ^d
C*	0.88±0.01 ^d	2.65±0.21 ^c	4.47±1.11 ^b	6.51±0.31 ^a
H*	134.51±1.17 ^d	210.37±1.90 ^c	221.74±2.77 ^b	227.41±0.56 ^a

* Different letters in the same parameter show a significant difference.

TABLE VII
RESULTS OF THE SENSORY EVALUATION OF THE DRINK

Value	Control	0.01	0.03	0.05
Smell	5.75±1.02 ^a	5.30±1.13 ^a	5.25±1.37 ^a	4.15±1.53 ^a
Flavor	5.40±1.35 ^a	5.50±1.00 ^a	5.50±1.40 ^a	4.75±1.62 ^a
Color	5.00±1.30 ^a	5.55±1.10 ^a	5.40±1.27 ^a	5.25±1.65 ^a
Sweetness	5.20±1.36 ^a	5.20±1.24 ^a	5.35±1.31 ^a	4.55±1.60 ^a
Overall acceptability	5.55±1.00 ^a	5.50±1.15 ^a	5.50±1.24 ^a	4.75±1.58 ^a

* Different letters in the same parameter show a significant difference.

V. CONCLUSIONS

The C-PC extract showed stability at pH 7, at 4 °C and 20 °C, but not when exposed to light. The sensory evaluation of the drink with C-PC had no statistically significant differences. As the concentration of C-PC increased, the blue tendency of the drink also increased.

The results showed the potential of *Spirulina maxima* biomass as a good source of natural pigments and as a natural food coloring.

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REFERENCES

- [1] W. Levasseur, P. Perré, and V. Pozzobon, "A review of high value-added molecules production by microalgae in light of the classification," *Biotechnology Advances*, vol. 41, pp. 1-26, Nov. 2020. DOI: <https://doi.org/10.1016/j.biotechadv.2020.107545>
- [2] A. Paggi, "Microalgae as a Potential Source of Proteins," in *Proteins: Sustainable Source Processing and Applications*. Ed. Elsevier, 2019. DOI: <https://doi.org/10.1016/B978-0-12-816695-6.00003-9>
- [3] G. Martelli, C. Folli, L. Visai, M. Daglia, and D. Ferrari, "Thermal stability improvement of blue colorant C-Phycocyanin from *Spirulina platensis* for food industry applications", *Process Bioch.*, vol. 49, pp. 154-159, 2014. DOI: <https://doi.org/10.1016/j.procbio.2013.10.008>
- [4] M. Caporgno and A. Mathys, "Trends in microalgae incorporation into innovative food products with potential health benefits", *Frontiers nutrition*, vol. 5, pp. 1-10, 2018. DOI: <https://doi.org/10.3389/fnut.2018.00058>
- [5] E. Ponce López, "Superalimento para un mundo en crisis: Spirulina a bajo costo" *IDESIA*, vol. 31, pp. 135-139. 2013. DOI: <http://dx.doi.org/10.4067/S0718-34292013000100016>
- [6] R. Henrikson, *Microalga Spirulina – Superalimento del futuro*, Ed. Urano S. A., Barcelona, España, 1994.
- [7] A. Belay, "The potential application of Spirulina as a nutritional and therapeutic supplement in health management", *JANA*, vol. 5, pp. 27-48, 2002.
- [8] D. Pez Jaeschke, I. Rocha Teixeira, L. D. Ferreira Marczak, and G. Domeneghini Mercali, "Phycocyanin from Spirulina: A review of extraction methods and stability", *Food Research International*, vol. 143, pp. 1-12, 2021. DOI: <https://doi.org/10.1016/j.foodres.2021.110314>
- [9] P. Spolaore, C. Joannis-Cassan, E. Duran, and A. Isambert, "Commercial Applications of Microalgae", *Journal of Bioscience and Bioengineering*, vol. 101, pp. 87-96, 2006. DOI: <https://doi.org/10.1263/jbb.101.87>
- [10] I. Chentir, M. Hamdi, 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>
- [11] W. Choi and H. Lee, "Effect of ultrasonic extraction on production and structural changes of C-Phycocyanin from marine *Spirulina maxima*" *International Journal of molecular Sciences*, vol 19, pp. 1-11, 2018. DOI: <https://doi.org/10.3390/ijms19010220>
- [12] M. Munier, S. Jubeau, A. Wijaya, M. Morançais, J. Dumay, L. Marchal, P. Jaouen, and J. Fleurence, "Physicochemical factors affecting the stability of two pigments: R-Phycocerythrin of *Grateloupia turuturu* and B-Phycocerythrin of *Porphyridium cruentum*", *Food Chemistry*, vol. 150, pp. 400-407, 2014. DOI: <https://doi.org/10.1016/j.foodchem.2013.10.113>
- [13] G. Voss, M. Monteiro, P. Jauregui, L. Valente, and M. Pintado, "Functional characterization and sensory evaluation of a novel symbiotic okara beverage" *Food Chemistry*, vol. 340, pp. 1-12, 2021. DOI: <https://doi.org/10.1016/j.foodchem.2020.127793>
- [14] Z. Uribe-Wandurraga, M. Igual, R. Rufino, C. Savall, P. García-Segovia, and J. Martínez-Monzó, "Physicochemical and rheological characterization of microalgae-enriched ketchups and their sensory acceptability" *International Journal of Gastronomy and Food Science*, vol. 26, pp. 1-10, 2021. DOI: <https://doi.org/10.1016/j.ijgfs.2021.100424>
- [15] T. Pereira, S. Barroso, S. Mendes, R. Amaral, J. Dias, T. Baptista, J. Saraiva, N. Alves, and M. Gil, "Optimization of phycobiliprotein pigments extraction from red algae *Gracilaria gracilis* for substitution of synthetic food colorants" *Food Chemistry*, vol. 321, pp. 1-9, 2020. DOI: <https://doi.org/10.1016/j.foodchem.2020.126688>
- [16] E. González-Ramírez, M. Andújar-Sánchez, E. Ortíz-Salmerón, T. Mazzuca-Sobezuk, M. Ibañez, A. Cámara-Artigas, and S. Martínez-Rodríguez, "Thermal and pH Stability of the B-Phycocerythrin from the Red Algae *P. cruentum*", *Food Biophysics*, vol. 1, pp. 1-12, 2014. DOI: <https://doi.org/10.1007/s11483-014-9331-x>
- [17] M. Wan, H. Zhao, J. Guo, L. Yan, and Y. Li, "Comparison of C-Phycocyanin from extremophilic *Galdieria sulphuraria* and *S. platensis* on stability and antioxidant capacity" *Algal Research*, vol. 58, pp. 1-15, 2021. DOI: <https://doi.org/10.1016/j.algal.2021.102391>
- [18] A. Adjali, I. Clarot, Z. Chen, E. Marchioni, and A. Boudier, "Physicochemical degradation of phycocyanin and means to improve its stability: A short review", *Journal of Pharmaceutical Analysis*, vol. 12, pp. 406-414, 2022. DOI: <https://doi.org/10.1016/j.jpha.2021.12.005>
- [19] Y. Benchikh, A. Filali, and S. Rebai, "Modeling and optimizing the phycocyanins extraction from *Arthrospira platensis* (Spirulina) algae and preliminary supplementation assays in softs beverage as a natural colorants and antioxidants", *Journal of Food Processing and Preservation*, vol. 45, pp. 0-2, 2021. DOI: <https://doi.org/10.1111/jfpp.15170>
- [20] H. Hernández, M. Nunes, C. Prista, and A. Raymundo, "Innovative and Healthier Dairy Products through the Addition of Microalgae: A Review", *Foods*, vol. 11, pp. 1-15, 2022. DOI: <https://doi.org/10.1111/ijfs.17466>