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Production of nutritionally active compounds from *Spirulina platensis* under various stress conditions

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ABSTRACT

The free-floating, filamentous cyanobacteria Spirulina platensis is highly valued and in great demand worldwide for its high-value colors and phytonutrients. These compounds find use in health foods, feed, medicines, and diagnostics. The primary objective of this work was to enhance the growth of Spirulina under both oxidative and physiological stress conditions in order to maximize the synthesis of phycocyanin and carotenoid pigments. Cultures were subjected to different concentrations of hydrogen peroxide (H_2O_2) and sodium chloride for varying temperatures and pH levels. Under lower concentrations of H_2O_2 (4mM), the maximum carotenoid content was increased, but the phycocyanin content was found to be stimulated at 10mM. Moreover, the synthesis of both pigments was shown to be highest under physiological stress circumstances (40mM and 60mM NaCl concentration) and at an optimal pH of 8-9, helped by a temperature range of 25-30°C. The experimental results demonstrate that both carotenoid and phycocyanin exhibit antioxidant properties even in the presence of oxidative stress. This suggests that the aforementioned circumstances can be applied in future investigations to extract different antioxidants from *S. platensis*.

Introduction

Over the years, spirulina has gained recognition as a nutraceutical due to its rich content of proteins, carbohydrates, essential amino acids, vitamins (such as folic acid, biotin, nicotinate, and pantothenic acid), minerals, phenolics, pigments (including chlorophyll, C-phycocyanin, carotenoids, and phycobilin), and polyunsaturated fatty acids. As a result of its value products, Spirulina is the most extensively cultivated microalga (Ghaeni *et al.*, 2011; Matos, 2017; Shao, 2019). It synthesizes antioxidants that have the ability to inhibit certain diseases, enhance the immune system, and counteract oxidative stress (Barrow & Shahidi, 2007). Spirulina has wide application in the fields of cosmetics, medicines, poultry, biofertilizers, food and feed, as well as plant "bio stimulants". The concentration of c-phycocyanin and carotenoid in *Spirulina platensis* is influenced by the nutrients in the culture media. To improve the biomass and pigment accumulation of *Spirulina platensis*, it is necessary to determine the ideal culture conditions. This may be achieved by investigating the impact of stress variables such as salinity, pH, and temperature (Sharma *et al.*, 1988). Spirulina species exhibited increased synthesis of β-carotene (a precursor for vitamin A), allophycocyanin, and total lipids in response to biotic stress (Awatif *et al.* 2013). Under low oxygen pressure, Spirulina functions as a highly effective singlet oxygen quencher and radical trapping antioxidant, reducing nuclear damage and preventing lipid peroxidation (Yang *et al.* 2018). By mixing carotenoids or establishing complexes with other antioxidants, such as vitamin E, their capacity to counteract free radicals can be enhanced. Pinero *et al.* (2001) determined that the phycocyanin present in Spirulina confers resistance to renal damage induced by various hospital-administered drug regimens. Additionally, they suggested that phycocyanin may enhance the immune system and be employed in the treatment of cancer in mice. Moreover, Ramirez *et al.* (2002) showed that it functions as an inhibitor of the allergic inflammatory reaction. Sharma *et al.* (1988) extensively studied the impact of carbon content, pH, and salinity on the accumulation of bio pigments and biomass in *Spirulina platensis*. The objective was to optimize the formation of phycocyanin in the culture.

Materials and Methods

Sample Collection

The *Spirulina platensis* strain was procured from the School of Studies in Biotechnology at Jiwaji University, Gwalior, M.P. *Spirulina* culture was maintained

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at 4°C slants in Zarrouk's agar medium (Zarrouk, 1966).

Media composition

Zarrouk's medium (Zarrouks.,1996) was considered as a specific and standard synthetic medium to be developed for the growth of *Spirulina platensis,* which was grown under alkaline aqueous medium with abundant salt concentrations.

Inoculum preparation

Spirulina was cultivated in Erlenmeyer flasks using fluorescent lights set at a temperature of 30 ± 1ºC during a 12-hour light/dark cycle. The experiment was carried out using a 10% (v/v) inoculation approach. In this study, *Spirulina platensis* was cultivated in flasks using Zarrouk's medium supplemented with different concentrations of H2O2 (2, 4, 6, 8, and 10 mM). Another experiment was carried out using various concentrations of NaCl (20, 40, 60, 80, and 100 mM). The impact of temperature was investigated at temperatures of 20, 25, 30, 35, and 40°C. The growth of *Spirulina platensis* was monitored at different pH levels (6, 7, 8, 9, 10). Each experiment was carried out in triplicate.

Harvesting and dry weight measurement

Spirulina platensis was filtered using screen printing cloth and wet biomass was weighed after complete drying at room temperature (Abd Ei-Baky *et al.*, 2003; Aly and Amber, 2011).

Total algal carotenoids

Carotenoids were isolated using acetone extraction and then analysed using spectrophotometry at 450 nm to quantify the total algal carotenoids. Next, the algal suspension was subjected to centrifugation for 5 minutes at 3000 revolutions per minute. The pellet was then washed with distilled water and supplemented with 2-3 ml of 85% acetone. The combination was then successively frozen and thawed. The concentration of the isolated carotenoid pigment in the supernatant was determined using the formula provided by Devanathan and Rmanathan (2012).

$$\textbf{Total Carotenoids} = D \times V / 2500 \times 100$$

D= Optical Density of Sample at 450 nm, V= Volume of Extract/Volume of Sample, F= 4.5 (Factor to Equal the Reduction in Absorbance), 2500 = Extinction Coefficient.

Total Algal Phycocyanin

Forty milligrammes of spirulina powder were placed into the centrifuge tube and agitated for five minutes. The sample was refrigerated overnight to inhibit the breakdown of phycocyanin. Furthermore, it was centrifuged for 5 minutes at 3500 revolutions per minute and at 10C.Using phosphate buffer as a blank, the supernatant was separated and quantified spectrophotometrically at 620 nm. The concentration of the isolated phycocyanin pigment in the supernatant was determined using the formula provided by Boussiba and Richmond 1976.

$$\textbf{Crude Phycocyanin \%} = \frac{A_{620} \times \text{Total Volume (10)} \times (100)}{3.39 \times (\text{mg sample} \times \% \text{ dry wt.})}$$

were 3.39 is extinction coefficient of phycocyanin at 620 nm.

Results and Discussion

Assessment of Carotenoid and Phycocyanin content in *Spirulina platensis* under oxidative Stress

Modulated concentrations of hydrogen peroxide (2, 4, 6, 8, and 10 millimolar) were introduced into the culture medium. Figure 1(A) demonstrates that the dry spirulina biomass exhibited a progressive decline when subjected to H2O2 stress, with the timing and concentration of exposure determining the extent of this decline. At elevated concentrations, the algal cells started to undergo collapse, resulting in a decrease or complete failure of several cellular activities. Observations revealed that the highest carotenoid content in S platensis was 0.043 mg/ml at an H2O2 concentration of 4 mM, but decreased to 0.01 mg/ml at a concentration of 10 mM. The phycocyanin content reached its maximum value of 106.3 mgDw/g at a concentration of 10mM. Nevertheless, its decrease at lower concentrations of H2O2 is indicated in Figure 1 (B).

Assessment of Carotenoid and Phycocyanin content *Spirulina platensis* under salinity stress

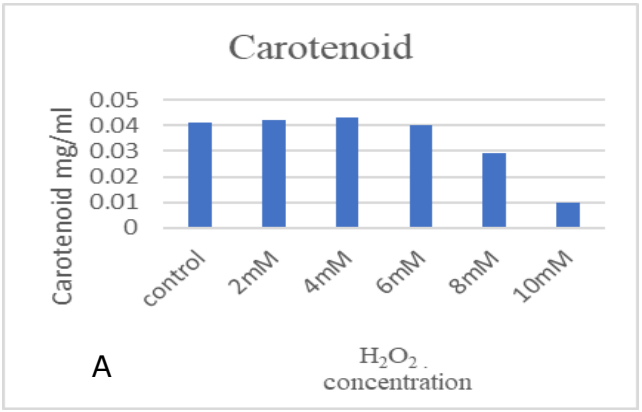
Diagram (A) illustrates the temporal variation in carotenoid content of *Spirulina platensis* under various sodium chloride concentrations (20, 40, 60, 80, and 100 mM). At a NaCl concentration of 40mM, the carotenoid content reached its maximum value of 175 mg/ml. Figure 2(B) exhibits the highest phycocyanin concentration of 137.4 mgDw/g at a NaCl concentration of 20mM. Yet, it declines when the concentration of NaCl falls.

Assessment of Carotenoids and Phycocyanin content in *Spirulina platensis* under temperature stress Figure 3(A) illustrates the distribution of carotenoid concentration in *Spirulina platensis* at different temperature exposures (20°C, 25°C, 30°C, 35°C, and 40°C). The maximum carotenoid concentration of 0.042 mg/ml was measured at 25°C, however it was shown to be insignificant at 40°C compared to the control. At 25°C, Figure 3 (B) revealed a phycocyanin concentration of 123.1 mgDw/g, which decreased to 120.2 mgDw/g at 30°C.

Assessment of carotenoids and phycocyanin content *Spirulina platensis* under different pH conditions

The development of *Spirulina platensis* was quantified to determine the quantities of carotenoid and phycocyanin at various pH values of 6, 7, 8, 9, and 10, as shown in Figure 4 (A). Variations in pH levels influenced the carotenoid content concentration in *Spirulina platensis*.At pH 8, S. platensis has the highest concentration of carotenoids, namely 0.053 mg/ml. Conversely, at pH 10, it is shown to have the lowest concentration.

Equally, the fluctuation in phycocyanin concentration was also noted, as illustrated in Figure 4(B). The phycocyanin concentration in S. platensis is 125.3 mgDw/g, with the lowest concentration seen at pH of 6. Salinity, pH, temperature, light, and nutritional circumstances exert an influence on the growth of Spirulina. The findings of this work demonstrate that the levels of carotenoid and phycocyanin depend on the specific cultivation conditions. Hanaa *et al.* (2007) investigate the feasibility of enhancing the concentrations of some bioactive chemicals in *Spirulina platensis*. The present study identified a progressive rise in the carotenoid content of S. platensis under H2O2 stress. The highest carotenoid content in S. platensis was found to be 0.043 mg/ml at an H2O2 concentration of 4 mM. The study conducted by Norun *et al.* (2014) demonstrated that the addition of H2O2 has a significant impact on the production of antioxidant compounds.



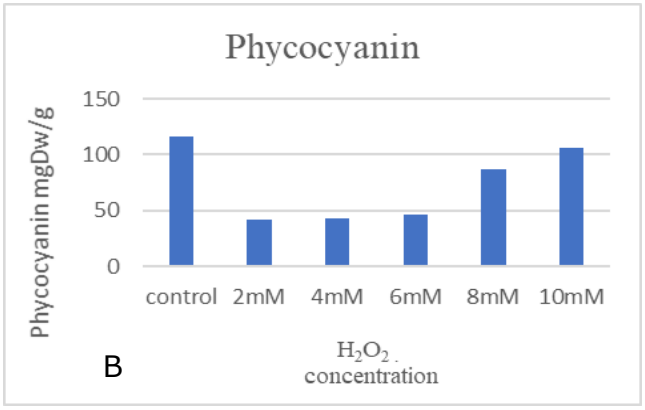
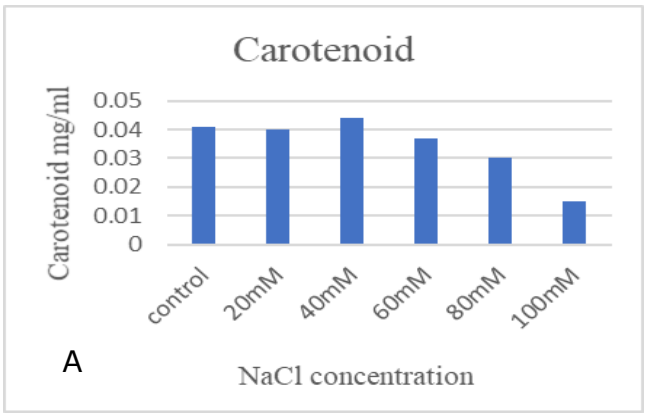


Figure.1 (A) Carotenoids content (mg/ml) (B) C-Phycocyanin content (mgDw/g) of *Spirulina platensis* with different concentration of H_2O_2



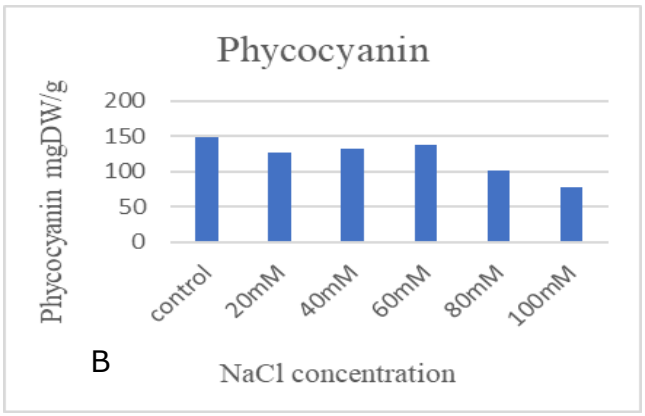
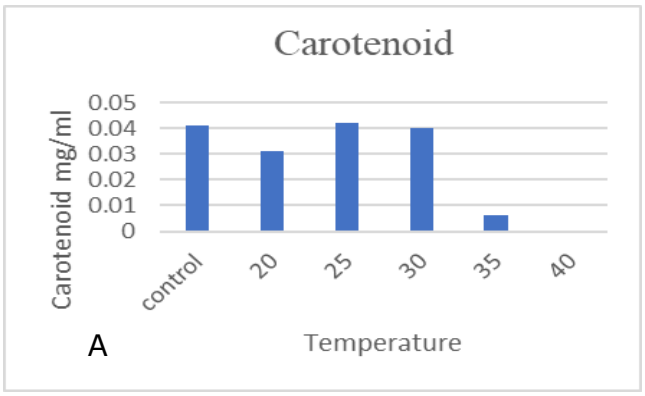


Figure 2. (A) Carotenoid content (B) C-Phycocyanin content of *Spirulina platensis* with different salinity concentration



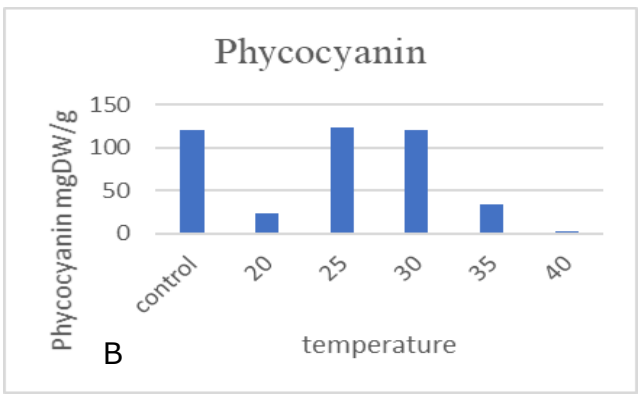


Figure 3(A). Carotenoid content (B) C-Phycocyanin content of *Spirulina platensis* with different temperatures

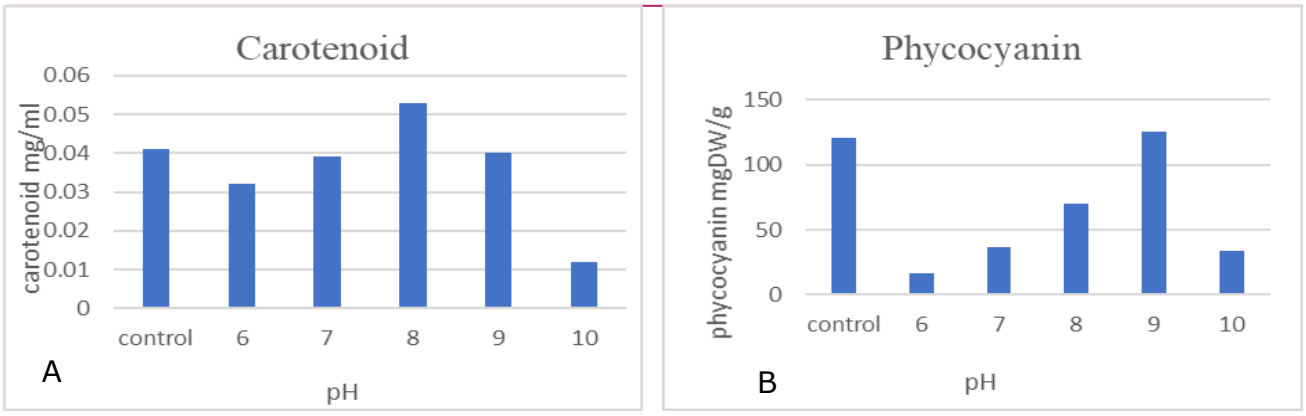


Figure 4 (A) Carotenoid content (B) C-Phycocyanin content of *Spirulina platensis* with different pH

In the current work, we examined the effect of H2O2 on the growth of Spirulina platensis. Specifically, we investigated the impact of H_2O_2 at a concentration of 10mM on the enhanced phycocyanin content. The results showed a significant effect on the nutritional value of Spirulina platensis.The aforementioned results align with the conclusions of Gaurav *et al.*, (2014) that higher salt stress leads to reduced levels of carotenoid and phycocyanin content. The greatest carotenoid content of 175 mg/ml was seen at 40mM, which is much higher than the control. Phycocyanin reached its maximum value at 60 mM (137.4 mgDw/g), indicating that the growth of Spirulina was greatly suppressed with increasing salinity stress concentration. Principally, this is attributed to the decline in respiratory and photosynthetic systems, resulting in a rapid decrease in both growth and biomass. The present results indicate that elevated temperatures lead to reduced levels of carotenoids and phycocyanin, possibly caused by the denaturation of protein macromolecules. These findings are consistent with the results reported by Ghiselli *et al*. (2000) Parwani and Singh (2019).The maximum carotenoid concentration of 0.042 mg/ml was measured at a temperature of 25°C, while a phycocyanin content of 123.1 mgDw/g was observed at the same temperature. Muthu *et al.* (2020) examined the impact of pH on the growth of Spirulina platensis. The findings reported in this study align with the current experiment, indicating that the highest phycocyanin content was observed at the optimal pH of 9 (125.3 mgDw/g), whereas the highest carotenoid content was identified at pH 8.Therefore, it can be inferred that augmenting the pH level leads to a significant decrease in the growth of Spirulina. This is attributed to the degradation of non-enzymatic functions, which ultimately weakens the activity of antioxidant enzymes.

Conclusion

The synthesis of nutritionally active substances, such as carotenoid and phycocyanin content, in *S. platensis* was significantly influenced by exposure to culture conditions including both oxidative stress and physiological stress. Chronic oxidative stress can advance to oxidative damage that affects cellular proteins. Sufficient nutrition supply and tolerance to fluctuations in H2O2 are necessary for Spirulina to generate carotenoid and phycocyanin content, which act as antioxidant stress sensors. Inhibition of growth and productivity of pigments by temperature and salt stress is commonly linked to reduced photosynthesis. It is evident that both carotenoid and phycocyanin exhibit antioxidant properties even in stressful situations. This suggests that the aforementioned conditions can be applied in future research to extract different antioxidants from Spirulina platensis.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

Abd El-Baky, H. H, (2003). Overproduction of phycocyanin pigment in "blue green" alga *Spirulina* and its inhibitory effect on growth of Ehrlich ascites carcinoma cells. "*Journal of Medical Sciences*", *2*, 222-240.

Aly, M.S., & Ambers., (2011). Production and application of *Spirulina platensis* rich in fatty acids and vitamins. "*Journal of American Science*",*7(2),*36-42.

Awatif, M.A., Sahar, A.M., & Hayfaa, A.S., (2013). Evaluation of analgesic activity and toxicity of alkaloids in *Myristica fragrans* seeds in mice. "*Journal of Pain Research*", *6* ,611 -615.

Barrow, C., Shahidi, F., (2007). *Marine nutraceuticals and functional foods*" *book*, "Food Science & Technology".

Boussiba, S., & Richmond, A., (1979). Isolation and purification of phycocyanin from *Spirulina platensis* (CCC540). *Indian Journal of Plant Physiology,19(2),*184-188.

Devanathan, J., & Ramanathan, N., (2012). Pigment production from *Spirulina platensis* using seawater supplemented with

dry poultry manure. *Journal of Algal Biomass,* 3 (4), 66-73.

Gaurav, S., Manoj, K., Mohammad, I.A., & Nakuleshwar, D.J., (2014). Effect of carbon content, salinity and pH on *Spirulina platensis* for phycocyanin, allophycocyanin and phycoerythrin accumulation. *Journal of Microbial and Biochemical Technology,* 6(4),202-206.

Ghaeni, F.A., Hosseinzadeh, H., Sadeghnia, H.R., & Motamedshariaty, V.S., (2011). Effects of saffron (*Crocus sativus* L.) and its active constituent, crocin, on recognition and spatial memory after Chronic Cerebral Hypoperfusion in Rats. *Journal of Phytotherapy Research*, *26 (3),*381-386.

Ghiselli, A., Serafini, M., Natella, F., & Scaccini, C., (2000). Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free radical biology and Medicine*, *29(11)*,1106-1114.

Matos, A.P.,(2018). Microalgae as a potential source of proteins. *Sustainable Source, Processing and Applications*.63-96.

Muthu, B., Kamalanathan, A., Janarthanan, S., Kalaiyarasu T. (2020). Effect of pH on *Arthrospira Platensis* production. *Alok Chandra Journal.9(5),*2297-2305.

Parwani, L., & Singh, J., (2019). Effect of temperature on nutritional values of *Spirulina*: useful for nutrient sustainable food preparations to combat malnutrition. *proceedings of the national academy of india, section B: Biological Science*,*89 (4),*1259-1265.

Pinero-Estrada, J.E., Bermejo-Bescos, P., &Villar Del Fresno, A.M., (2001). Antioxidant activity of different fractions of *Spirulina Platensis* protean extract II. *Farmaco*, *56 (5-7),* 497-500.

Ramírez, D., Gonzalez, R., Merino, N., RodríguezS., & Ancheta, O., (2002). Inhibitory effects of *Spirulina* in zymosan-induced arthritis in mice.*Mediators of Inflammation*. *11(2),* 75-79.

Shao, W .,Ebaid R., El-Sheekh, M., Abomohra A., & Eladel, H., (2019). Pharmaceutical applications and consequent environmental impacts of *Spirulina* (*Arthrospira*):An overview. *Grasas Y Aceites*,*70(1),*292-304.

Sharma, R.M. & Azeez, P.A. (1988). Accumulation of copper and cobalt by blue-green algae at different temperature.*International Journal of Environment Analytical Chemistry*, *32*,87–95.

Yang, Y.K., Lu, H.K., Hsieh, C.C., & Hsu, J.J., (2018). Preventive effects of *Spirulina platensis* on skeletal muscle damage under exercise-induced oxidative stress. *European journal of Applied Physiology* ,*98*, 220-226.

Zarrouk, C., (1966). Influence de Contribution a L'etudeD'une Cyanobacterie: Influence de Divers Facteurs Physiques et Chimiques sur la Croissance et la Photosynthese de Spirulina maxima (Setchell et Gardner) Geitler. PhD Thesis, University of Paris, Paris.divers facteurs physiques et chimiques sur la croissance et la photosynthèse de Spirulina maxima (Setch et Gardner) Geitler", University of Paris. PhD Thesis, *University of Paris*, France, *4*