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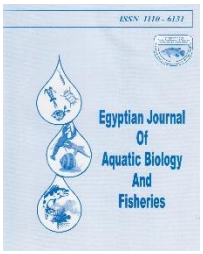

Monosodium Glutamate (MSG) as Metabolic Stressors Stimulate the Production of Valuable Compounds in *Spirulina platensis*

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ABSTRACT

Spirulina platensis is a blue-green alga with columnar cells forming a spiral-like twisted (helix). This research aimed to boost cell growth and the simultaneous production of high-value compounds in response to the varying concentration of MSG as metabolic stressors in *Spirulina platensis*. The methods used in this study included biomass growth as measured by the cell density method with a wavelength of 680nm, while phycocyanin concentration and phycocyanin purity were measured by wavelengths of 620 and 280nm. Carotenoid content was measured using a spectrophotometer, while total lipids were extracted by Bligh and Dyer method, and then fatty acids profiling was evaluated using GC-MS. In the total lipid data, a drastic increase occurred at a concentration of 7.5 mM which was about 3 times higher than the control and 5 mM MSG. The results showed that the addition of monosodium glutamate had a significant effect on the biomass growth rate of microalgae, but it could increase the phycocyanin content. The greatest value of phycocyanin concentration was obtained with the addition of 7.5 mM monosodium glutamate which was 0.116 mg/mL, compared to the control which was 0.060 mg/mL. While the purity at the best concentration of 7.5 mM monosodium glutamate was 2.049 ± 0.159 and 1.518 ± 0.266 for the control. In this study, it was shown that sodium glutamate is a good metabolic stress in the growth of phycocyanin production from the microalgae *Spirulina platensis.*

INTRODUCTION

Spirulina platensis is one of the microalgae often cultivated and developed commercially **(Madkour *et al*., 2012)**. The superiority of *Spirulina platensis* protein lies in the complete composition of essential amino acids, protein and carbohydrate **(El-Chaghaby *et al*., 2019)**. Besides its high protein production (50-60% of dry weight biomass), *Spirulina platensis* contains high carotenoid bio-pigment essential compounds (beta-carotene, lutein, and phycocyanin) which can function as human health nutrients **(Al-Zayat, 2019)**. The largest essential bio pigment compound in *Spirulina platensis* is Phycocyanin, containing 1500 to 19000 mg in 100 grams of *Spirulina platensis* dry

biomass. Phycocyanin can function as an antioxidant that can counteract free radicals, and cancer cell growth and increase immunity and stamina **(Capelli & Cysewski, 2010)**. Phycocyanin has been reported to exhibit strong cancer chemo-preventive activity and exert anti-cancer effects on various types of cancer cells such as liver cancer and lung cancer **(Roy *et al*., 2007)** as well as breast cancer **(Chen & Wong, 2008)**. In general, the environmental variables and suitable medium are two main factors for the production of high value-added products by microalgae of *Spirulina platensis*, especially to achieve microalgae with the high amount of biomass and phycocyanin **(Manirafasha *et al*., 2017)**. The manipulation of nutrient conditions can affect the biochemical composition of algal cells including lipid, carotenoid and phycocyanin production by microalgae *Spirulina platensis.* One of the efforts to achieve higher biomass and phycocyanin content can be done by regulating cellular mechanisms through the manipulation of nutrients by adding compounds that act as metabolic stressors **(Paliwal *et al.*, 2017)**.

Metabolic stress is currently a promising approach for manipulating the cell pathway and enhancing the production of metabolites from microalgae. Recently, several investigations have been accomplished by supplementation of monosodium glutamate (MSG) and aspartate (ASP) as a metabolic stressor to increase phycocyanin productivity and carotenoid content by 83.36 % and 64.77% in *Arthrospira platensis* **(Fekrat *et al*., 2021)**. This substance can act as a substrate in manipulating the biosynthetic process of chemical compounds in microalgae cells. One example that has been done is monosodium glutamate (MSG), which is able to increase the pigment and protein content of the microalga *Spirulina platensis.* The addition of this substrate as stress is a strategy to increase the accumulation of the desired metabolite target **(Li *et al.*, 2016)**. Several reports presented that the addition of substrates such as glutamate could increase the biomass production of *Spirulina platensis* from 3.19 mg/L to 3.97 mg/L and increase the production of protein from 55% to 66% **(Prabha *et al.*, 2016)**. In line with previous research conducted by Manirafasa reported that the addition of metabolic stress could improve the production of phycocyanin around 51.85 mg by adding 7.5 mM of the succinic acid into the medium **(Manirafasha *et al*., 2017)**. A thorough evaluation of the relevant literature reviews some data about applying MSG supplements in *Spirulina platensis* cultivation. Nevertheless, only few reports have been conducted on the use of MSG supplement in *Spirulina platensis* cultivation and their effect on biochemical metabolite.

This research aimed to boost cell growth and the simultaneous production of high-value compounds in response to the varying concentration of MSG as metabolic stressors in *Spirulina platensis* under suitable culture medium conditions. Furthermore, a network correlation analysis was applied to biomass growth and phycocyanin production in microalgal cells. Additionally, the content of lipid, chlorophyll and carotenoid was determined in the microalgal biomass.

MATERIALS AND METHODS

1. Inoculation of *Spirulina platensis*

The microalgae *Spirulina platensis* used in this study were isolates of microalgae *Spirulina platensis* obtained from the Biochemistry Laboratory of Andalas University. *Spirulina platensis* was previously tested using an Olympus BX40 binocular microscope

to see the morphology of *Spirulina platensis*. The microalgae isolates were then cultured in Zarrouk's medium consisting of (per Liter): 4.2 g $NaHCO_3$ (Merck®), 0.5 g K_2HPO_4 (Sigma Alderich®), 3.75 g $NaNO_3$ (Merck®), 1 g K_2SO_4 (Merck®), 1 g NaCl (Merck®) , 0.2 g $MgSO_4.2H_2O$ (Merck®), 0.04 g $CaCl_2.2H_2O$ (technical), 0.01 g $FeSO_4.7H_2O$ (Sigma Alderich®), 0.08 g EDTA and 1 ml of the top-stuffed trace metal solution (per Liter): 2.86 g H_3BO_3 (Merck®), 1.81 g $MnCl_4.4H_2O$ (Merck®), 0.222 g $ZnSO_4.4H_2O$ (Merck®), 0.10177 g Na_2MoO_4 (Sigma Alderich®), 0.079 g $CuSO_4.5H_2O$ (Merck®). The medium was then grown for 4 days with initial cell density reaching 0.6 at OD_{680} **(Manirafasha *et al.*, 2017)**.

2. Microalgae cultivation conditions and growth optimization

Microalgae were cultivated at a temperature of 27°C (relative laboratory room temperature), which was carried out with a 24 hour cycle, using fluorescent lamps for lighting. The zarrouk medium was prepared at this stage and treated by adding monosodium glutamate in medium to detect the optimum concentration limits for each substance. The variation of sodium glutamate concentrations in the culture medium was 0 (control), 5 mM, 7,5 mM, and 10 mM. The addition of monoodium glutamate was carried out at the beginning of cultivation **(Manirafasha *et al.*, 2017)**. Each treatment measured Phycocyanin content and microalgae growth curve based on optical density (OD_{680}) and dry biomass weight, and then the optimum conditions were obtained for monosodium glutamate treatment. This was conducted 3 times.

3. Determining the growth rate of *Spirulina platensis*

Microalgae dry biomass was determined by weight based on a standard calibration curve that relates the absorbance (optical density) and dry weight. Microalgae isolate cultures at the highest optical density were filled into five vials that had been weighed beforehand with volumes of 5, 10, 15, 20, 25 and 30 mL, respectively. The volume of all cultures was made up to 30 ml, with the addition of distilled water to form five variations of isolate concentration and the optical density was measured. The culture was dried in an oven at 50°C and weighed until a constant weight was obtained. The standard curve was substituted for the optical density value at optimal growth into the obtained regression equation, with x as dry biomass weight and y as optical density so that the dry biomass weight of each isolate can be determined **(Lee & Shen, 2004)**. The dry weight of microalgae was calculated using the following regression equation.

$$Y = 0.5587x + 0.115 \quad \text{.....(1)}$$

4. Assessment of total carotenoids

Dried biomass of 50mg was suspended to methanol (5mL) in term to calculate concentrations of total carotenoids and kept in the dark/incubation in water bath at 40±5 ℃ for 30 minutes, followed by cooling in room temperature. The mixture was centrifuged at 3500 rpm for 10 minutes. Aliquots of each extract were measured by recording the optical densities at 470, 652 and 665 nm for optical density using a spectrophotometer UV VIS (Thermo Scientific Genesys 20) using the following equations as described by **Lichtenthaler (1987)**.

$$Chlo\ a\ (\mu g/g) = (16.72 \text{ x A.665 nm}) - (9.16 \text{ x A.652 nm})$$

$$Chlo\ b\ (\mu g/g) = (34.09 \text{ x A.652 nm}) - (15.2 \text{ x A.665 nm})$$

$$Carotenoid\ Total\ (\mu g/g) = \frac{(1000 \times A.470\ nm) - (1.63 \times Chlo\ a) - (104.96 \times Chlo\ b)}{221}$$

5. Determination of lipid content

Lipids were extracted based on the modified Bligh and Dyer method with modification according to **(Perdana *et al.*, 2021)**. Dried biomass 50 was added 100 µL dionisation water then added with 300 µL a mixture of chloroform and methanol at the 1:2 ratio (v/v) and vortex for 2 min. A 100 µL chloroform was added to mixture and then vortex for 1 min. The lipid-containing chloroform layer at the bottom of the separating funnel was removed by centrifuged at 2500 x g for 6 min. The chloroform phase was recovered, the residue was reextracted with 100 µL of chloroform three times. Then, the extracts were air-dried and total lipid (TL) percentage was determined by following formula:

$$\%\ Total\ Lipid = \frac{Total\ lipid\ weight\ (g)}{Total\ biomass\ weight\ (g)}\ x\ 100\%$$

Lipid extracted from microalgae biomass was methylated by modified methanolic HCl-transesterification method. Microalgae lipid was incubated at 90°C for 2 hours with methanol: HCl: chloroform (10: 1: 1). Thereafter, lipid was added to 1 mL distilled water and fatty acid methyl ester extracted by adding 2 mL hexane-chloroform (4: 1); vortexed and top layer was recovered. The organic layer was injected to GC-MS with specification where column oven temperature 50°C, injection temperature 250°C, pressure 85 kPa, total flow 60 mL/min, start time 2.00 min, end time 60.54 min, start m/z 40.00, and end m/z 500.00 **(Cavonius *et al.*, 2014)**.

6. Determining phycocyanin production of *Spirulina platensis*

60 mg of air-dried biomass was suspended in 10mL of 0, 1 M phosphate buffer (pH=7), and the solution was stored in a refrigerator at 4°C. After 20 hours, the solution was then centrifuged at 3.500 rpm for 15 minutes for removing contamination, and the supernatant (blue color) was collected **(Horváth *et al.*, 2013)**. The absorbance of the pure extract was determined by UV/Vis at wavelengths of 620 and 652 nm **(Simis & Kauko, 2012)**. For the last step, the Phycocyanin concentration of the extract was calculated using the following formulas **(Bennett & Bogobad, 1973)**.

$$\text{Phycocyanin consentration} = \frac{(OD_{620} - 0.474 \text{ x } OD_{652})}{5.34} \quad \text{.....(2)}$$

The absorbance of the pure extract was determined by UV/Vis at wavelengths of 620 and 652 nm. Afterward, the Phycocyanin concentration of the extract could be calculated.

$$\text{Purity of Phycocyanin} = \frac{A_{620}}{A_{280}} \qquad (3)$$

Phycocyanin content per cell weight using equation (4) **(Sharma *et al*., 2014)**

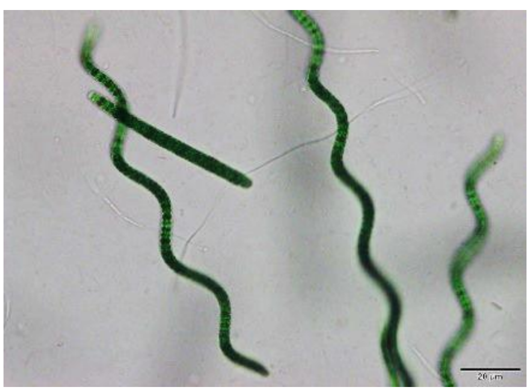
$$\text{Phycocyanin content} = \frac{PC_C\, V_e}{M_E} \qquad (4)$$

Where, PCc is the concentration of Phycocyanin obtained in equation (2) (mg mL^{-1}); V_e is the volume of the extract (mL), and M_E is the biomass used in the extraction (g)

RESULTS AND DISCUSSION

1. Morphopogy of *Spirulina platensis*

The study of morphological features of microalgae *Spirulina platensis* and their suspensions is displayed in Fig. (1). This microalgae has a blue-green color with columnar cells forming spiral-like twisted filaments (helix) so it is also called blue-green filamentous algae (*cyanobacterium*). *Spirulina platensis* filaments live independently and can move freely **(Koru, 2012)**. One of the most prominent morphological features of the genus *Spirulina* a is the spiral coiling of multicellular trichomes with easily visible cross-walls **(Nowicka-Krawczyk *et al*., 2019)**.



(a)

(b)

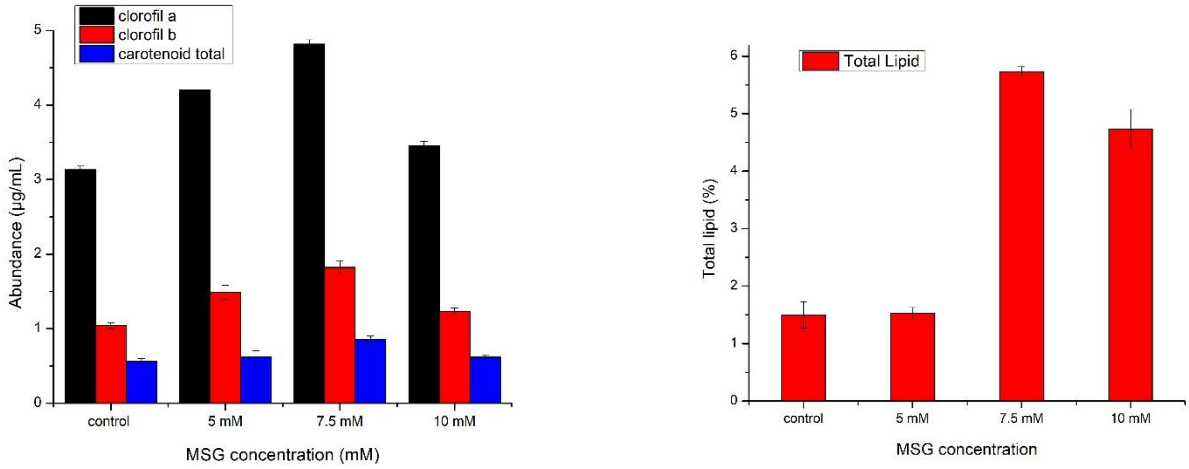
Fig. 1. The results of studying the morphological features of the *microalgae Spirulina platensis* and their suspensions: **(a)** Cells under a microscope; **(b)** The suspension's appearance

From Fig. (1), it can be seen that, not all *Spirulina platensis* microalgae cells resemble spirals. In this study, some *Spirulina platensis* microalgae cells are like linear filaments and have different cell lengths. According to research conducted by **Wijayanti *et al*., (2020)**, *Spirulina platensis* cultured with different media had several linear/straight and spiral morphologies. Furthermore, *Spirulina platensis* cells were spiral and linear, indicating that there were differences in genes **(Yadav *et al*., 2020)**. This is due to

differences in the light used, radiation and temperature, as well as environmental influences, both physical and chemical. *Spirulina platensis* is an organism that is susceptible to mutations and sequence changes in its genomic **(Vonshak & Tomaselli, 2006)**.

2. Carotenoid and lipid content accumulation

The chlorophyll a and total carotenoid concentrations were simultaneously increased in the *Spirulina platensis* under MSG treatment (Fig. 2). The highest chlorophyll a and b content were observed in the 7.5 mM MSG treatment, with amounts of 4.18 ± 0.58µg/ mg and 1.82 ± 0.83µg/ mg, respectively. The treatment condition with MSG 7.5 mM, recorded the highest carotenoid level 0.856 ± 0.04 µg/mg and also the highest lipid content 5.57 %. The results showed that there was an increase in the total carotenoid content, accompanied with an increase in the total content of chlorophyll a and b. The optimal increase in total carotenoid content was obtained at a concentration of 7.5 mM. However, the addition of monosodium glutamate which exceeds the optimal conditions causes a decrease in total carotenoid content. In terms of carotenoids, at higher stressor concentrations the growing cells contained higher amounts of total carotenoids and higher carotene production (Fig. 2). This is similar to previous studies in *Dunalina salina* microalgae. In this context, **Pisal and Lele (2005)** reported that, carotene is a secondary metabolite, and these molecules are produced by cells under stress conditions as a cell protective mechanism. Therefore, there was an increase in total carotenoids and carotene content at higher stress conditions.



(a) **(b)**

Fig. 2. Effect of MSG concentration on (a) Carotenoid content (b) Total lipid

After testing the carotenoid levels then continued to calculate the lipid levels from the different concentrations of monosodium glutamate. In the total lipid data above, a drastic increase occurred at the concentration of 7.5 mM, which was about 3 times higher than the previous concentration, control and 5 mM MSG. These data are in line with the findings of **Kandasamy and Nagarajan (2014)** who reported that, chlorophyll, phycocyanin and lipid content in *Spirulina platensis* microalgae increased when stressors

were added in the form of salt with concentrations of 0.1 and 0.2 M. Lipid accumulation in algae usually occurs during environmental stress. Cells adapt to stress by undergoing changes in morphology and developmental patterns and physiological as well as biochemical processes. Other research shows that lipid productions of *A. Platensis* were significantly higher grown in 35 psu (14.635±0.087% dry weight) after modifying the growth medium **(Almahrouqi *et al.*, 2015)**. The highest lipid content, namely the addition of 7.5 mM MSG, was then esterified and identified using GC-MS. The results of the GC analysis were obtained in the form of a chromatogram using an MS detector. Certain fatty acid compounds will come out at a certain retention time and then by the MS detector, compounds with small molecular weights will be identified. In this study, the effect of 7.5 mM monosodium glutamate on the fatty acid profile in *Spirulina platensis* microalgae is shown in Table (1).

Table 1. Fatty acid composition of microalgae biomass *Spirulina platensis*

RT	Compound name	Molecule Formula	Molecule weight (g/mol)	Abundance (%)	
				Control	7.5 mM MSG
10.929	napthalene	$C_{10}H_8$	128.174	4.35	4.48
12.721	6-Octen-1-ol, 3,7 dimethyl, (R)	$C_{13}H_{24}O_2$	212.33	1.59	-
12.883	azulene	$C_{10}H_8$	128.174	2.66	-
13.423	2,6 Octadin-1-ol, 3,7 dimethyl (E)	$C_{10}H_8O$	154.2493	10.5	3.33
13.689	dianhydromannitol	$C_6H_{10}O_4$	146.14	2.55	2.83
16.587	2,6-Octadien-1-ol,3,7 dimethyl-acetate (E)	$C_{13}H_{22}O_2$	210.31	29.55	5.35
17.335	caryophyllen	$C_{15}H_{24}$	204.367	6.3	-
18.939	napthalene, 1,2,3,4,4a,5,6,8,8a-octahydro-7n	$C_{15}H_{24}$	204.350	3.63	-
21.422	heptadecane	$C_{17}H_{36}$	240.475	17.59	68.47
22.080	8,11-octadecadioic acid, metyl ester	$C_{19}H_{34}O_2$	294.5	11.14	-
22.700	phytol	$C_{20}H_{40}O$	296.539	10.4	-
11.009	benzaldehyde, 3-methyl	C_8H_8O	120.148	-	4.68
14.059	hexanedionic acid, dimetyl ester	$C_8H_{14}O_4$	174.194	-	4.11
20.115	hexadecane	$C_{16}H_{34}$	226.448	-	7.76

Fourteen compounds in the total polar lipid of *Spirulina platensis* were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula

(MF) and concentration (%) are presented in Table (1). The prevailing compounds in control were 2,6 Octadin-1-ol, 3,7 dimethyl (E) (10.05%), 2,6-Octadien-1-ol,3,7 dimethyl-acetate (E) (29.55%), caryophyllen (6.3%), napthalene, 1,2,3,4,4a,5,6,8,8a-octahydro-7n (3.63%), 8,11-octadecadioic acid, metyl ester (11.4%), and phytol (10.4%). Interestingly, at the concentration of MSG 7.5, there were several compounds that experience an drastic increase, such as napthalene (4.48%), heptadecane (68.47%) and dianhydromannitol (2.83%). Several compounds also appeared such as benzaldehyde, 3-methyl (4.68%), hexanedionic acid, dimetyl ester (4.11%) and hexadecane (7.76%). At this concentration, fatty acid production has decreased compared to control.

2. Phycocyanin production

Microalgae can accumulate desired biochemical products such as Phycocyanin by changing certain environmental factors, one of which is the provision of nutrients into the growth medium. Glutamate, pyruvate and succinic-coenzyme A are precursors in the tetrapyrole biosynthetic metabolite pathway such as porphyrin, pycobilin and chlorophyll that can act as nutrients **(Fujisawa *et al.*, 2010)**. Microalgae that had been cultivated for 4 days in Zarrouk medium were then re-cultivated with variations of monosodium glutamate (MSG) concentrations to determine the growth rate and concentration of phycocyanin. The following is a growth curve and graph of Phycocyanin production obtained for MSG as metabolic stress in *Spirulina platensis* (Fig. 4). Based on the results in (Fig. 4a) shows that there had been a negative effect of monosodium glutamate on dry biomass on the growth of *Spirulina platensis*. This trend is in line with the findings of previous studies on the impact of increasing salt concentration on biomass production in *Spirulina platensis*. When cells were exposed to high salinity as a stressor, special processes are activated such as, a) pressure recovery, b) regulation of ion uptake and export through cell membranes, and c) induction of protective solute accumulation as well as protein stress. This mechanism in turn led to decrease the growth conditions **(Shalaby *et al.*, 2010)**.

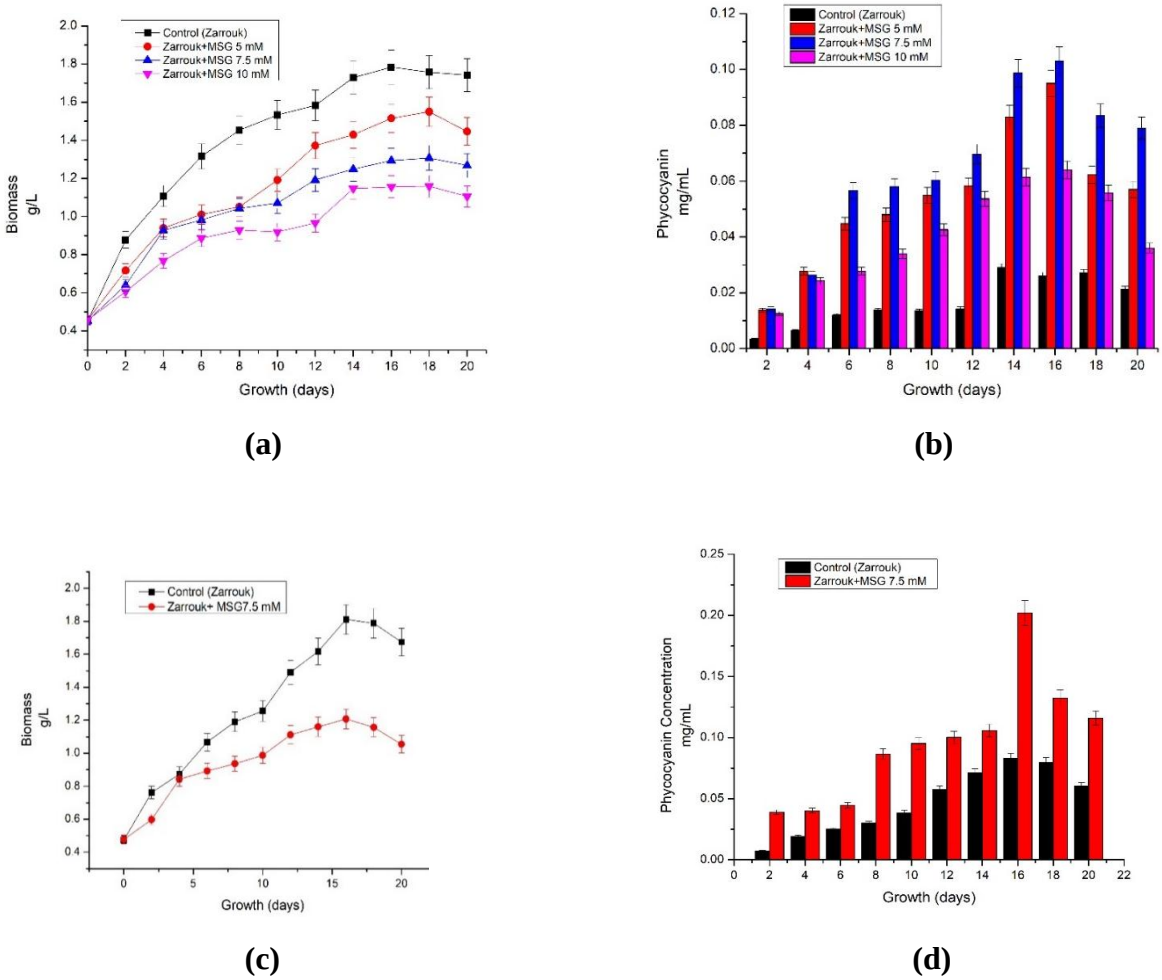


Fig. 4. (a) Biomass growth curve; and **(b)** Phycocyanin production graph in *Spirulina platensis* with the addition of monosodium glutamate (MSG); **(c)** *Spirulina platensis* biomass growth curve, and **(d)** Phycocyanin production on 7.5 mM MSG addition.

After obtaining the best concentration of 7.5 mM monosodium glutamate substrate, microalgae cultivation was carried out again with a concentration to detect the effect on biomass growth rate and phycocyanin production in *Spirulina platensis* microalgae. From Fig. (4c) it can be seen that, the biomass growth curve of *Spirulina platensis* with zarrouk medium (control) was in the highest position by producing dry cell weight or dry biomass weight of microalgae of 1.811 g/L at the beginning of the stationary phase, followed by the addition of MSG. 7.5 mM, with an absorbance value of 1.208 g/L. For the stationary phase, the two variations were on days 16 to 18. Then, the dry biomass decreased on the death phase on day 20, with a value of 1.674g/ L for control and 1.054g/ L for the addition of MSG. With a decrease in the value of biomass, it could be concluded that the more substrate additions to the growth medium, the smaller the absorption value of *Spirulina platensis* growth. Fig. (4d) shows the effect of MSG on the growth of

Phycocyanin production. From the overall graph given, it can be seen that the increase during the cultivation process reached the peak of Phycocyanin production on day 16, which was 0.083mg/ mL for the control and 0.0202mg/ mL for the highest peak at a concentration of 7.5 mM monosodium glutamate (MSG). Nevertheless, it decreased on day 20 with the concentration of phycocyanin in the final stationary phase of 0.060 mg/mL for the control, 0.116 mg/mL for the best variation of MSG concentration. The following is a graph of phycocyanin production obtained for 7.5 mM MSG addition in *Spirulina platensis* culture (Fig. 5).

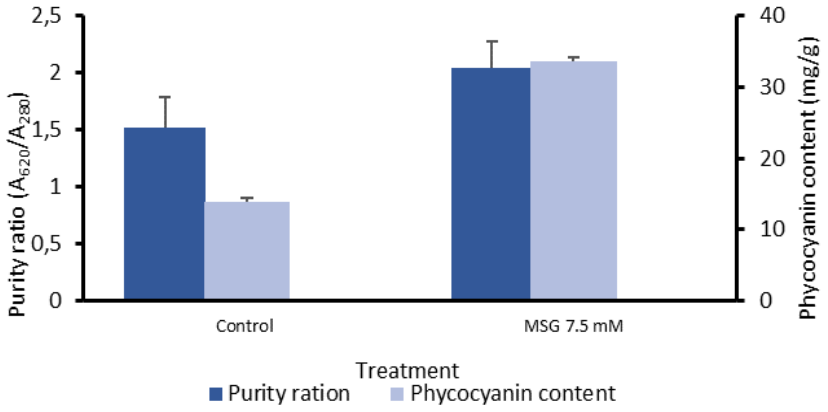


Fig. 5. Ratio of purity and Phycocyanin content in *Spirulina platensis*

The concentration of phycocyanin produced in the control and MSG can be seen in Fig. (5), which is 0.083 ± 0.003 mg/mL for the control and 0.202 ± 0.004 for MSG. The total Phycocyanin in MSG increased almost 3 times, namely 33.717 ± 0.670 when compared to the control (13.856 ± 0.507). The ratio of phycocyanin purity was determined by the ratio between the absorbance of the sample of the pigment at 620 nm (A_{620}) and 280 nm (A_{280}). Samples of Phycocyanin with A_{620}/A_{280} ratio greater than 0.7 are considered as food grade, while those with A_{620}/A_{280} ratio of 3.9 are considered reactive values, while values above 4.0 are considered as from analytical grade **(Eriksen, 2008)**. In this study, the obtained phycocyanin acted as a food grade, namely 2.049 ± 0.159 and 1.518 ± 0.266 in MSG and controls. The interaction between trace metals and other nutrients added to the growth medium will increase phycocyanin by 2.3 times in *P. ceylanium.*

CONCLUSION

In conclusion, it was found that, *Spirulina platensis* responded differentially to varaying range of metabolic stress for high value compound productivity. Monosodium glutamate (MSG) has an impact on biomass growth of *Spirulina platensis* microalgae the more MSG was given, the slower and smaller the biomass produced. However, the

consentration of 7.5 mM of MSG was found as substrate for stimulating carotenoid, lipid and the ratio of purity of Phycocyanin as well as Phycocyanin content. These findings provide important data for future metabolic studies to improve valuable compounds accumulation using metabolic stressor in *Spirulina platensis*.

REFERENCES

Al-Zayat, Ahmed M. 2019. "Effect of Various Levels of Spirulina (*Arthrospira platensis)* as Feed Supplement on Growth Performance, Feed Utilization, Immune Response and Hematology of the Nile Tilapia (Oreochromis Niloticus) Fingerlings." Egyptian Journal of Aquatic Biology and Fisheries., 23(3): 361–70.

Almahrouqi, H.A.; Sukumaran, P.; Naqqiuddin, M.A. and Al-sabahi, J. 2015. "The Effect of Salinity on Growth , Biochemical Composition and Fatty Acid Profile of Spirulina (*Arthrospira Platensis*) Grown in Sheltered Outdoor Conditions in Oman. Journal of Algal Biomass Utilization., 6(2):61-67.

Bennett, A. and Bogobad, L. 1973. "Complementary Chromatic Adaptation in a Filamentous Blue-Green Alga." Journal of Cell Biology.,58(2): 419–35.

Capelli, B. and Cysewski, G.R. 2010. "Potential Health Benefits of Spirulina Microalgae * A Review of the Existing Literature." Nutra Foods., 9(2): 19–26.

Cavonius, L.R.; Carlsson, N.G. and Undeland, I. 2014. "Quantification of Total Fatty Acids in Microalgae: Comparison of Extraction and Transesterification Methods." Analytical and Bioanalytical Chemistry., 406(28): 7313–22.

Chen, T. and Wong, Y.S. 2008. "In Vitro Antioxidant and Antiproliferative Activities of Selenium-Containing Phycocyanin from Selenium-Enriched *Spirulina Platensis*." Journal of Agricultural and Food Chemistry., 56(12): 4352–58.

El-Chaghaby, G.A.; Rashad, S.; Abdel-Kader, S.F.; Rawash, E.A. and Moneem, M.A. 2019. "Assessment of Phytochemical Components, Proximate Composition and Antioxidant Properties of Scenedesmus Obliquus, *Chlorella Vulgaris* and *Spirulina platensis* Algae Extracts." Egyptian Journal of Aquatic Biology and Fisheries., 23(4): 521–26.

Eriksen, Niels T. 2008. "Production of Phycocyanin - A Pigment with Applications in Biology, Biotechnology, Foods and Medicine." Applied Microbiology and Biotechnology., 80(1): 1–14.

Fekrat, F.; Shahbazi, M.; Nami, B.; Hejazi, M,A. and Ghaffari, M.R. 2021. "Nitrogen-Containing Metabolic Stressors Stimulate High-Value Compounds Accumulation in *Arthrospira Platensis*." Authorea., - ; 1-13.

Fujisawa, T.; Narikawa, R.; Okamoto, S.; Ehira, S.; Yoshimura, H.; Suzuki, I.; Masuda, T.; Mochimaru, M.; Takaichi, S.; Awai, K.; Sekine, M.; Horikawa, H.; Yashiro, I.; Omata, S.; Takarad, H.; Katano, Y.; Kosugi.; Tanikawa., S.; Ohmori, K.; Sato.; Ikeuchi, M.; Fujita, N. and Ohmori, M. 2010. "Genomic Structure of an Economically Important Cyanobacterium , Genomic Structure of an Economically Important Cyanobacterium, *Arthrospira (Spirulina) platensis* NIES-39." DNA Research., 17(2): 85–103.

Horváth, H.; Attila, W. K.; Riddick, C. and Présing, M. 2013. "Extraction Methods for Phycocyanin Determination in Freshwater Filamentous Cyanobacteria and Their Application in a Shallow Lake." European Journal of Phycology., 48(3): 278–86.

Kandasamy, S. and Nagarajan. P. 2014. "Effect of Salinity on Biomass and

Biochemical Constituents of *Spirulina Platensis* (Geitler).” International Journal of Plant Protection., 7(1); 71-73

Lee, Y. K. and Shen, H. (2004). "Basic Culturing Techniques. Handbook of Microalgal Culture: Applied Phycology and Biotechnology." Richmon, A. (Ed). Blackwell Publishing Ltd., Israel, pp. 40–56.

Li, Y.; Wan, M.; Wang, Z.; Zhang, Z.; Wang, J.; Li, S. and Yu, A. 2016. “A Novel Paradigm for the High-Efficient Production of Phycocyanin from Galdieria Sulphuraria.” Bioresource Technology., 218: 271-278.

Lichtenthaler, H.K. 1987. “Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes.” Methods in Enzymology., 148(C): 350–82.

Madkour, F.F.; Kamil, A.E.W. and Nasr, H. S. 2012. “Production and Nutritive Value of *Spirulina Platensis* in Reduced Cost Media.” Egyptian Journal of Aquatic Research., 38(1): 51–57.

Manirafasha, E.; Murwanashyaka, T.; Ndikubwimana, T.; Rashid, A.N.; Liu, J.; Lu, Y.; Zeng, X.; Ling, X. and Jing, K. 2017. “Enhancement of Cell Growth and Phycocyanin Production in *Arthrospira (Spirulina) Platensis* by Metabolic Stress and Nitrate Fed-Batch.” Bioresource Technology., 255: 293–301.

Nowicka-Krawczyk, P.; Mühlsteinová, R. and Hauer, T. 2019. “Detailed Characterization of the Arthrospira Type Species Separating Commercially Grown Taxa into the New Genus Limnospira (Cyanobacteria).” Scientific Reports., 9(1): 1–11.

Paliwal, Chetan.; Mitra, M.; Bhayani, K.; Vamsi Bharadwaj, S.V.; Ghosh, T.; Dubey, S. and Mishra, S.. 2017. “Abiotic Stresses as Tools for Metabolites in Microalgae.”Bioresource Technology., 244(2): 1216–26.

Perdana, B. A.; Dharma, A.; Zakaria, I.J. and Syafrizayanti. 2021. “Freshwater Pond Microalgae for Biofuel: Strain Isolation, Identification, Cultivation and Fatty Acid Content.” Biodiversitas., 22(2): 505–11.

Pisal, Dipak S., and S.S. Lele. 2005. “Carotenoid Production from Microalga, *Dunaliella Salina*.” Indian Journal of Biotechnology., 4(4): 476–83.

Prabha, Y.; Tyagi, A. and Soni.; S.K. 2016. “Impact of Monosodium Glutamate on the Production and Chemical Constituents of *Spirulina Maxima*.” International Journal of Pure & Applied Bioscience., 4(1): 180–84.

Roy, K.R.; Arunasree, K.M.; Reddy, N.P.; Dheeraj, B., Reddy, G.V. and Reddanna, P. 2007. “Alteration of Mitochondrial Membrane Potential by Spirulina *Platensis* C-Phycocyanin Induces Apoptosis in the Doxorubicinresistant Human Hepatocellular-Carcinoma Cell Line HepG2.” Biotechnology and Applied Biochemistry., 47(3): 159.

Shalaby, E.A.; Sana M.M. Shanab. and Singh, V. 2010. “Salt Stress Enhancement of Antioxidant and Antiviral Efficiency of *Spirulina Platensis*.” Journal of Medicinal Plants Research., 4(24): 2622–32.

Sharma, G.A.; Manoj K.; Ali, M.I. and Jasuja, N.D. 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): 202–6.

Simis, Stefan G.H. and Hanna M.K. 2012. “In Vivo Mass-Specific Absorption Spectra of Phycobilipigments through Selective Bleaching.” Limnology and Oceanography Methods., 10: 214–26.

Vonshak, A. and Tomaselli, L. 2006. “Arthrospira (Spirulina): Systematics and

EcophysioIogy." The Ecology of Cyanobacteria., 505–22.
Wijayanti, M.; Syaifudin, M.; Yulisman.; Nurianti, Y.; Hidayani, A. and Gofar, N. 2020. "Characterization of Arthrospira *Platensis* Cultured in Wastewater of Clarias Catfish Farming Media: Dna Barcode, Helical Form, Growth, and Phycocyanin." Biodiversitas., 21(12): 5872–83.
Yadav, A., Monsieurs, P.; Misztak, A.; Waleron, K.; Leys, N.; Cuypers, A. and Janssen, P.J. 2020. "Helical and Linear Morphotypes of Arthrospira Sp. PCC 8005 Display Genomic Differences and Respond Differently to 60 C Gamma Irradiation." European Journal of Phycology., 55(2): 129–46.