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PRODUCTION ENHANCEMENT of SOME VALUABLE COMPOUNDS of *ARTHROSPIRA PLATENSIS*

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Abstract

Arthrospira platensis is a non toxic edible cyanobacterium which contains high amounts of various valuable nutrients such as essential amino acids, essential fatty acids and carotenoids. It also contains compounds having medicinal effects, such as phycocyanin and different polysaccharides. The present study examined the effect of nitrogen, phosphorus, sulfur and salinity on biomass, lipid, protein, carbohydrate and phycocyanin productivities of Arthrospira platensis. Decreasing nitrogen to 50 % than the concentration mentioned in the modified Zarrouk medium resulted in a reduction of biomass, lipid, protein, carotenoids and phycocyanin productivities by 20, 12, 20, 2 and 32 %, respectively. While 100 % phosphorus deficiency enhanced lipids and carotenoids productivities by 128 % and 64 %, respectively, with 25 % reduction in biomass. In addition, sulfur limitation led to 280 % increase in carbohydrate productivity with insignificant changes in biomass productivity. Finally, increasing salinity caused significant increase of lipid, carbohydrate and carotenoids productivities up to 93, 84 and 64 %, respectively. Whereas, increasing salt concentration, increased lipid and carotenoid productivities. The present study showed that phosphorus and sulfur deficiencies and high salinity increased the productivity of some valuable products of Arthrospira platensis. Therefore alteration of growth conditions changes its nutritional compounds. This means that we could obtain Arthrospira with different nutritional composition by altering the composition of cultivation medium.

INTRODUCTION

Arthrospira sp is a filamentous cyanobacterium that has a long history of in being used as food supplement. *Arthrospira* is rich in important nutrients, such as proteins (50-70 %), vitamins, minerals, carbohydrates and essential fatty acids such as gamma linolenic acid **(Vonshak *et al.*, 1988)**. It is gaining more and more attention, not only for its nutritional value, but also for its pharmaceutical aspects (**Quoc and Pascaud, 1996**). In addition to its effectiveness in reducing hyperlipidemia, diabetes, high blood pressure, it has been proved to have anti-viral and anti-cancer effects. It has also been reported to enhance immune functions (**Belay, 2002**). *Arthrospira* sp. is being commercially cultivated and sold in the market as food supplement due to its medicinal and nutritional importance. *Arthrospira* sp. are grown in many liquid and solid media such as; Bold Basal Medium (**Bischoff and Bold, 1963**), Zarrouk (**Zarrouk, 1966**), BG11 (**Stanier *et al.*, 1971**) and modified Zarrouk (**Aiba and Ogawa, 1977**).

Arthrospira platensis is influenced by cultivation conditions such as; nitrogen concentration (**Vonshak *et al.*, 1982**), light intensity and the presence of contaminants (**Vonshak *et al.*, 1982; Walach *et al.*, 1987**), temperature (**Vonshak *et al.*, 1988**), presence of bicarbonate ions (**Vonshak *et al.*, 1982; Costa *et al.*, 2002**), salt stress (**Shalaby *et al.*, 2010**), phosphate concentration (**Vonshak, 1997**) and initial biomass concentration (**Pelizer *et al.*, 2003**). So, many trials were applied to optimize the growth and production of specific important compounds of *Arthrospira.* Many of these compounds showed effective biological activities, e.g. carotenoids have powerful antioxidant activity (**Abd El-Baky *et al.*, 2003**), phycocyanin was reported to be a free radical scavenger and microsomal lipid inhibitor (**Estrada *et al.*, 2001**) having anticancer, anti-inflammatory and antioxidant activity (**Abd El-Baky *et al.*, 2003**). Moreover, polysaccharides extracted from *A. platensis* exhibited antiviral activity against both human immunodeficiency virus type 1 (HIV-1), herpes simplex virus type 1 (HSV-1) and cytomegalovirus (CMV) (**Hayashi *et al.*, 1996; Ayehunie *et al.*, 1998; Hernández-Corona *et al.*, .2002; Lee *et al.*, 2004**). In addition, many fatty acids extracts from *Arthrospira* showed antibacterial and antifungal activity (**Ozdemir *et al.*, 2004; Kumar *et al.*, 2011; Abd El- Baky and El-Barouty, 2012**). Many studies were established to optimize these important compounds production (**Vonshak *et al.*, 1982**); however, those studies depended on the measurement of "content" of the desired product as mg g^{-1} of dry weight or as a percent of the dry weight. Although, production of certain compounds is stimulated under stresses, the growth usually decreases; therefore, the term "content" doesn't refer to the real production efficiency of a certain compound. The present study aimed to enhance the production of these compounds, which were calculated as "productivity" in mg L^{-1} d^{-1}, by modification of media composition. The present study aims to optimize the growth conditions of *Arthrospira platensis* for the highest biomass production and to control the productivity of carotenoids, phycocyanin, lipids, proteins and carbohydrates as nutritional valuable compounds.

MATERIALS AND METHODS

Cyanobacterium and growth conditions

Arthrospira platensis was obtained from Phycology Research Lab at Botany Department, Faculty of Science, Tanta University. It was cultivated axenically as batch culture using modified Zarrouk medium described by **Aiba and Ogawa (1977)**. Cultures were illuminated by tubular fluorescent lamps (PHILIPS Master TL-D 85 W/840). The light intensity at the surface of the culturing vessels was 2500 Lux at 31 ± 1 °C. Cultures were shacked once a day to keep them homogenous.

Alteration of medium composition

The effect of different nutrients namely nitrogen [control (2.5 g L^{-1}), - 50 % , - 100 % and + 100 %], sulfur [control (1g L^{-1}) , - 50 % , - 100 % and + 100 %], phosphorus [control (0.5 g L^{-1}) , - 50 % , - 100 % and + 100 %] and sodium chloride [control (1 g L^{-1}) , - 50 % , - 100 %, + 100 %, + 200 % and + 300 %] on growth, carotenoids, phycocyanin, lipids, proteins and carbohydrates was studied. An equal volume of exponentially growing *A. platensis* cells, which has been precultured in 300 ml modified Zarrouk medium, was used as inoculum in 750 ml of modified Zarrouk medium in 1 L Erlenmeyer flasks at an initial OD_{750} of 0.06.

Biomass assay

Cyanobacterial growth was monitored using the optical density of the culture according to **Bhattacharya and Shivaprakash (2005)** at 750 nm (OD_{750}) and by determination of cyanobacterial cellular dry weight (CDW). Biomass productivity was calculated according to the modified method of **Abomohra *et al.* (2013)**.

$$\text{Biomass productivity (g CDW } L^{-1} d^{-1}) = (CDW_L\text{-}CDW_0)/t$$

Where; CDW_0 and CDW_L represent the CDW (g L^{-1}) at the start of the culture and late exponential phase, respectively during time (t).
Estimation of carotenoids

Estimation of carotenoids

A known volume of *A. platensis* culture was centrifuged at 3000 rpm for 10 min. The supernatant was decanted and an equal volume of methanol added to the pellet, then it was incubated in a water bath at 55°C for 15 min. and centrifuged. The absorbance of the extract (A) was measured against blank of free methanol at 650, 665 and 452 nm. Carotenoids were estimated as mg ml^{-1} of culture suspension using the following equation **MacKinney (1941)**

$$\text{Carotenoids (mg ml}^{-1}) = 4.2\ A_{452} - (0.0246\ ((10.3\ A_{665} - (0.918\ A_{650}))$$

Estimation of total soluble proteins

After carotenoids extraction, residual cells were extracted using 1 N NaOH in a boiling water bath for 2 h as described by **Payne and Stewart (1988)**. Protein concentration as mg ml^{-1} was determined according to Bradford method (**Bradford, 1976**) using bovine serum albumin as a standard reference.

Estimation of total carbohydrates

Total carbohydrates were quantitatively determined by the phenol sulphuric acid method described by **Kochert (1978)** using glucose as a standard reference.

Estimation of C-phycocyanin

Phycobiliproteins were determined according to the method described by **Bennett and Bogorad (1973)**. Briefly, 50 ml of cyanobacterial suspension were centrifuged at 3000 rpm for 10 min. The obtained cells were resuspended in 20 ml of sterile distilled water. The quantitative extraction of phycobiliproteins was achieved by combination of prolonged freezing and sonication, followed by centrifugation at 4000 rpm for 20 min. The crude extract was completed to 50 ml and the concentration of c-phycocyanin (CPC) was calculated by measuring the absorbance (A) at 615 and 652 nm using the following equation:

$$CPC\ (mg\ ml^{-1}) = (A_{615} - 0.474\ A_{652})/\ 5.34$$

Estimation of total lipid

Extraction of lipids was done using chloroform: methanol (2:1). The lipid extracts were dried under a stream of argon. The pre-weighted glass vials containing the lipid extracts were dried at 80 °C for 30 min, cooled in a desiccator and weighed (**Folch *et al.*, 1957**).

Productivity calculation

Productivities of different measured parameters (lipids, protein, carbohydrates, c-phycocyanin and carotenoids) were calculated according to the modified method of **Abomohra *et al.* (2013)**

$$Productivity\ (mg\ L^{-1}\ d^{-1}) = (P_L - P_0)/t$$

Where; P_0 and P_L represent the concentration of the desired product (mg. ml^{-1}) at the start of the culture and at the late exponential phase, respectively during time (t).

Statistical analysis

Results are presented as mean ± standard deviation (SD) from three replicates. The statistical analyses were carried out using SAS (v 6.12, 1997). Data obtained were analyzed statistically to determine the degree of significance using one way analysis of variance (ANOVA, $p \leq 0.05$).

RESULTS

Nitrogen supply is essential for amino acids synthesis and is often added to the culture in the form of nitrate. The effect of different nitrogen concentrations (in the form of sodium nitrate) on the growth of *A. platensis* was recorded as OD_{750} at 2 days interval for 20 days of incubation (Figure 1). The obtained results revealed that a decrease in sodium nitrate concentration led to growth reduction. The most pronounced inhibition reached a 98 % reduction after 20 days in a nitrogen free medium. However, a 100 % increase of nitrogen did not affect the growth pattern of *A. platensis*. The same results were observed in biomass productivity as shown in Table 1. The decrease of nitrogen (by 50 % and 100 %) decreased the biomass productivity by 19 % and 52 %, respectively. Cultures containing -50 % and -100 % of nitrogen resulted in significant decrease of all measured compounds after 16 days of incubation. A 100 % increase of nitrogen significantly increased protein productivity up to 6 % and decreased carotenoid productivity by to 7 %, while it didn't cause any significant changes on the productivity of lipids, carbohydrates and c-phycocyanin (Table 1).

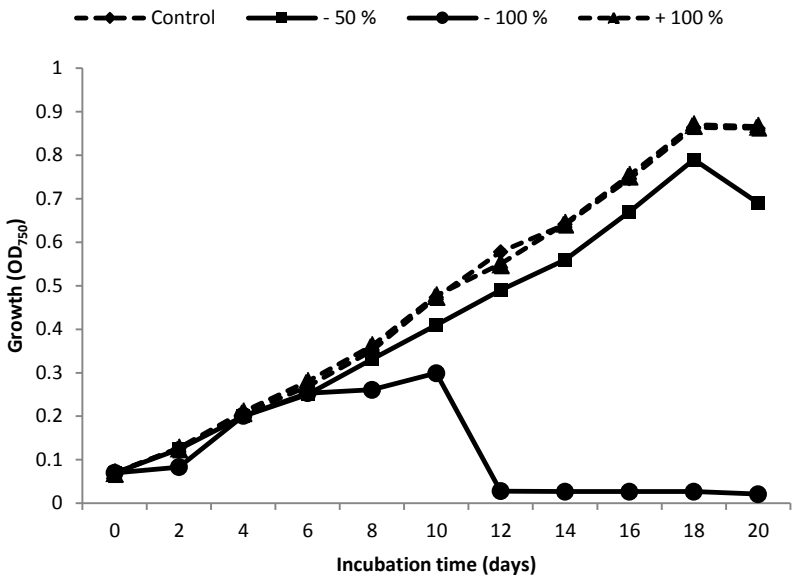

Figure 1 Effect of different sodium nitrate concentrations on growth of *Arthrospira platensis* for 20 days of incubation

Figure 2 shows the effect of different concentrations of phosphorus (mono-potassium phosphate) on *A. platensis* growth. A 50 % and 100 % decrease in phosphorus concentration caused 39 % and 42 % growth inhibition, respectively, after 20 days of incubation. Increasing of phosphorus concentration up to 50 % and 100 % didn't show any significant change in biomass production. Whereas, 50 % and 100 % decrease in phosphorus concentration caused a 15 % and 24 % reduction in biomass productivity (Table 2). However, lipid and carotenoid productivities increased by 128 % and 24 %, respectively, in phosphorus free medium; while total protein, carbohydrates and CPC increased significantly by 7 %, 13 % and 18 %, respectively, by 100 % increase in phosphorus concentration.

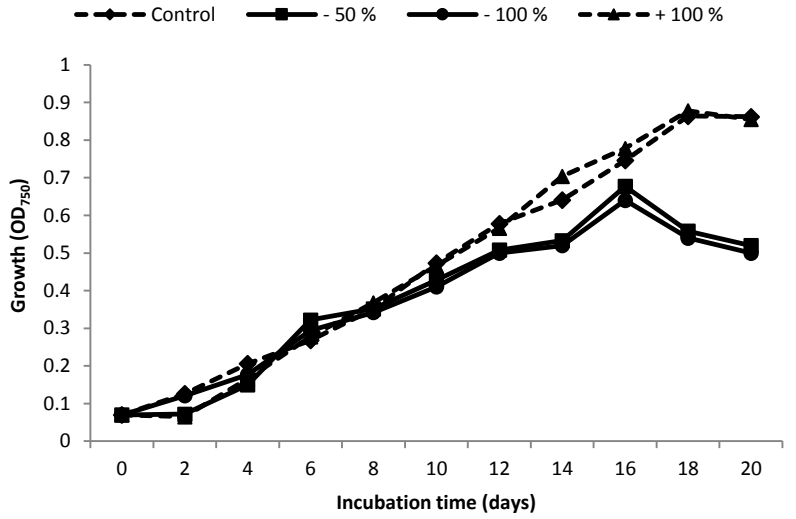

Figure 2 Effect of different potassium dihydrogen phosphate (monopotassium phosphate) concentrations on growth of *Arthrospira platensis* for 20 days of incubation

Table 1 Effect of different nitrogen concentrations on biomass, lipids, proteins, carbohydrates, c-phycocyanin (CPC) and carotenoids productivities of *Arthrospira platensis* after 16 days of incubation

Sodium nitrate	Productivities (mg L^{-1} d^{-1})					
	Biomass	**Lipids**	**Proteins**	**Carbohydrates**	**CPC**	**Carotenoids**
Control (2.5 g L^{-1})	136 ± 10 [a]	7.60 ± 0.29 [a]	78.34 ± 0.50 [a]	16.14 ± 0.70 [a]	2.95 ± 0.28 [a]	0.38 ± 0.03 [a]
-50 %	6 ± 109[b]	6.67 ± 0.30 [b]	62.32 ± 0.28 [b]	11.19 ± 0.50 [b]	2.02 ± 0.11 [b]	0.29 ± 0.03 [b]
-100 %	6 ± 65[c]	1.92 ± 0.22 [c]	21.96 ± 0.11[c]	2.55 ± 0.30 [c]	1.05 ± 0.07 [c]	0.18 ± 0.01 [c]
+100 %	10 ± 138a	7.17 ± 0.43[ab]	86.00 ± 0.07 [d]	15.35 ± 1.08 [a]	3.14 ± 0.22 [a]	0.33 ± 0.01 [d]

Each value is the mean of three readings ± standard deviation
Values in the same column with the same letter are not significant ($p \leq 0.05$)

Table 2 Effect of different potassium dihydrogen phosphate (monopotassium phosphate) concentrations on biomass, lipids, proteins, carbohydrates, c-phycocyanin (CPC) and carotenoids productivities of *Arthrospira platensis* after 16 days of incubation

Monopotassium phosphate	Productivities (mg L^{-1} d^{-1})					
	Biomass	**Lipids**	**Proteins**	**Carbohydrates**	**CPC**	**Carotenoids**
Control (0.5g L^{-1})	136 ± 10 [a]	7.60 ± 0.29[a]	78.34 ± 0.50 [a]	16.14 ± 0.70 [a]	2.95 ± 0.28[a]	0.38 ± 0.03 [a]
-50 %	115 ± 6[b]	7.37 ± 0.42[a]	33.88 ± 0.75 [b]	51.67 ± 0.07 [b]	3.29 ± 0.18[ac]	0.59 ± 0.02 [b]
-100 %	103 ± 6[c]	17.33 ± 0.49[b]	28.01 ± 0.43[c]	12.90 ± 0.06 [c]	1.72 ± 0.16[c]	0.47 ± 0.01 [c]
+100 %	140 ± 6[a]	6.35 ± 0.44[c]	83.78 ± 0.80 [d]	18.22 ± 0.17 [d]	3.48 ± 0.14[c]	0.37 ± 0.01 [a]

Each value is the mean of three readings ± standard deviation
Values in the same column with the same letter are not significant ($p \leq 0.05$)

Effect of sulfur on growth was studied by changing of potassium sulphate concentration (Figure 3). Decreasing and increasing of potassium sulfate showed no significant changes in growth pattern (Figure 3) or biomass productivity (Table 3). Decreasing sulfur concentration with -50 % and -100 % resulted in reduction of lipid and protein productivities by 40 %, 44 % and 27 %, 41.8 % respectively, but increased the carbohydrate productivity by 22.3 % and 279 % respectively. A 100 % increasing of sulfur resulted in 46.7 % reduction of lipid, 20.7 % reduction in protein productivities and 10.5 % increase in carotenoid productivity.

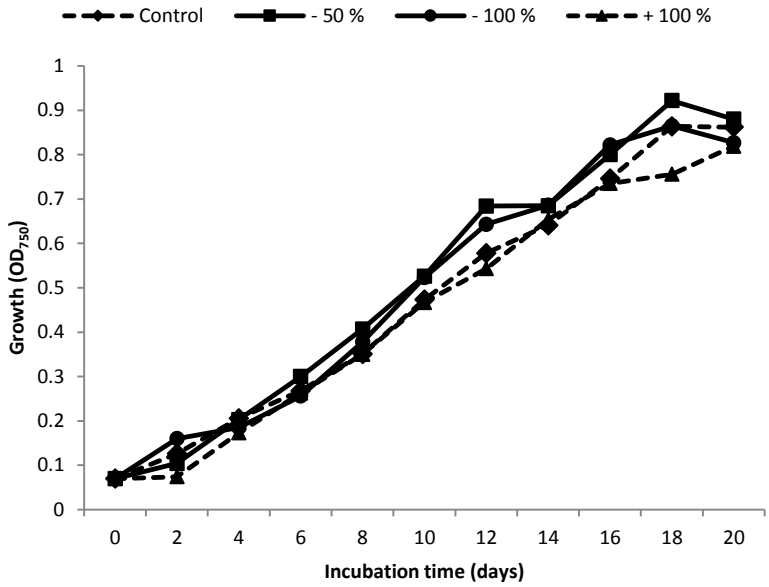

Figure 3 Effect of different potassium sulfate concentrations on growth of *Arthrospira platensis* for 20 days of incubation

Data in Figure 4 showed that a 50 % decrease in salt concentration led to a 14 % growth enhancement of *A. platensis* after 20 days of incubation (Figure 4), with significant increase of 8 % in biomass productivity (Table 4). The 50 % salinity reduction caused an increase in lipid, protein and carbohydrate productivities of 43, 2 and 68 % respectively.; while, CPC and carotenoid productivities were decreased by 16 and 75 %, respectively. Increasing salt concentration (by 200 % and 300 %) increased lipid and carotenoid productivities by 93 %, 25 % and 32 %, 37 % respectively.

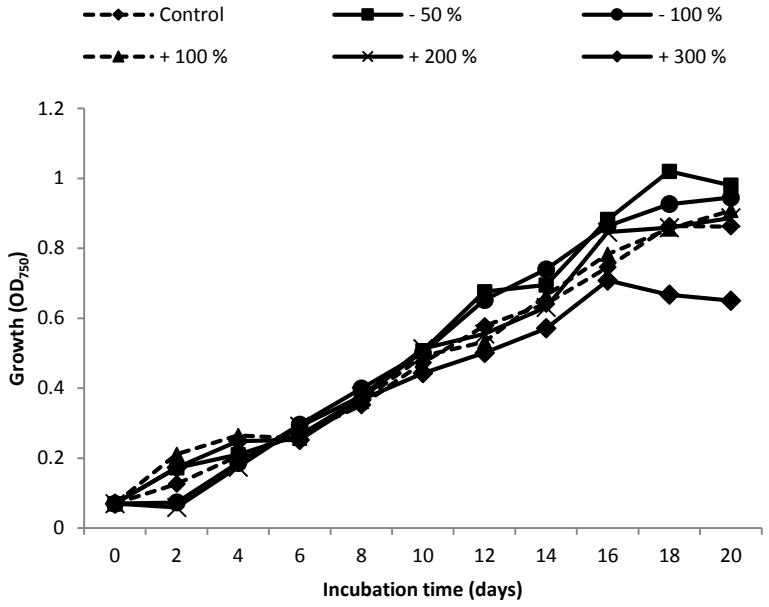

Figure 4 Effect of different sodium chloride concentrations on growth of *Arthrospira platensis* for 20 days of incubation

Table 3 Effect of different potassium sulphate concentrations on biomass, lipids, proteins, carbohydrates, c-phycocyanin (CPC) and carotenoids productivities of *Arthrospira platensis* after 16 days of incubation

Potassium sulphate	Productivities (mg L^{-1} d^{-1})					
	Biomass	**Lipids**	**Proteins**	**Carbohydrates**	**CPC**	**Carotenoids**
Control (1 g L^{-1})	136 ± 10^{a}	7.60 ± 0.29^{a}	78.34 ± 0.50^{a}	16.14 ± 0.70^{a}	2.95 ± 0.28^{a}	0.38 ± 0.03^{a}
- 50 %	136 ± 10^{a}	4.53 ± 0.35^{b}	57.16 ± 0.42^{b}	20.06 ± 0.08^{b}	3.04 ± 0.29^{a}	0.31 ± 0.01^{b}
- 100 %	134 ± 6^{a}	4.19 ± 0.35^{bc}	45.59 ± 0.50^{c}	61.36 ± 0.14^{c}	3.50 ± 0.21^{b}	$0.29 \pm 0.03^{b\,c}$
+ 100 %	142 ± 10^{a}	4.05 ± 0.36^{bcd}	62.12 ± 0.58^{d}	16.50 ± 0.08^{a}	3.09 ± 0.22^{ab}	0.42 ± 0.02^{d}

Each value is the mean of three readings ± standard deviation
Values in the same column with the same letter are not significant ($p \leq 0.05$)

Table 4 Effect of different sodium chloride concentrations on biomass, lipids, proteins, carbohydrates, c-phycocyanin (CPC) and carotenoids productivities of *Arthrospira platensis* after 16 days of incubation

Sodium chloride	Productivities (mg L^{-1} d^{-1})					
	Biomass	**Lipids**	**Proteins**	**Carbohydrates**	**CPC**	**Carotenoids**
Control (1 g L^{-1})	136 ± 10^{a}	7.60 ± 0.29^{a}	78.34 ± 0.50^{a}	16.14 ± 0.70^{a}	2.95 ± 0.28^{a}	0.38 ± 0.03^{a}
-50 %	146 ± 7^{b}	10.84 ± 0.46^{b}	79.71 ± 0.38^{b}	27.04 ± 0.38^{b}	2.48 ± 0.23^{b}	0.16 ± 0.03^{b}
-100 %	134 ± 6^{a}	5.78 ± 0.06^{c}	40.00 ± 0.29^{c}	17.58 ± 0.20^{c}	1.35 ± 0.15^{c}	$0.09 \pm 0.01^{b\,c}$
+100 %	128 ± 6^{c}	7.26 ± 0.33^{a}	43.27 ± 0.26^{d}	29.71 ± 0.46^{d}	2.73 ± 0.20^{ab}	0.29 ± 0.01^{d}
+200 %	119 ± 10^{cd}	14.64 ± 0.37^{d}	35.03 ± 0.37^{e}	21.68 ± 0.31^{e}	2.86 ± 0.26^{a}	0.50 ± 0.02^{ef}
+300 %	113 ± 10^{d}	9.50 ± 0.41^{e}	32.57 ± 0.48^{f}	10.24 ± 0.42^{f}	2.90 ± 0.17^{a}	0.52 ± 0.02^{f}

Each value is the mean of three readings ± standard deviation
Values in the same column with the same letter are not significant ($p \leq 0.05$)

DISCUSSION

Arthrospira has gained importance as human food and pharmaceutical agent for its high content of protein, vitamins, carotenoids and essential fatty acids (**Vonshak *et al.*, 1982; Belay, 2002**), so many studies were established to optimize these important compounds production (**Vonshak *et al.*, 1982**). The present study aimed to enhance the production of these compounds, which were calculated as productivity in mg L^{-1} d^{-1}, by modification of media composition. Different nitrogen ($NaNO_3$), phosphorus (KH_2PO_4), sulfur (K_2SO_4) and salinity (NaCl) concentrations were selected for this purpose. Sodium nitrate was used as the nitrogen source in *Arthrospira* medium, the decrease of sodium nitrate led to remarkable decrease in the growth, biomass, proteins, carbohydrates and phycocyanin productivities. These results were in agreement with finding of **Uslu *et al.* (2011)** who studied the effects of nitrogen deficiency on protein content of *Spirulina* cultivated on Zarrouk medium and recorded 67, 54, 6 % of cellular dry weight protein for groups of control, 50 % and 100 % deficient nitrogen, respectively. Also, **Piorreck *et al.*, (1948)** reported that total lipids and total protein decreased with decreasing nitrogen supply content in *Arthrospira* culture unlike eukaryotic algae and this was attributed to growth inhibition and new lipid synthesis. In contrary, **Bhattacharya and Shivaprakash (2005)** recorded the highest values of biomass, protein and phycocyanin contents in *Spirulina* species in Zarrouk medium containing 0.25 % $NaNO_3$ as nitrogen source. In addition, **Abd El-Baky *et al*. (2003)** stated that *S. platensis* and *S. maxima* responded to nitrogen deficiency by accumulation of carbohydrates and carotenoids. This was attributed to the fact that these compounds do not require nitrogen for their synthesis. However, the recorded decrease of these components productivity under nitrogen deficiency in the present study is attributed to growth inhibition. The growth inhibition under nitrogen deficiency may be explained by findings of **Peter *et al*. (2010)** who confirmed that limitation of nitrogen in the medium altered energy transfer between photosynthetic pigments particularly phycobilisomes, and concluded that phycocyanin is the target pigment protein for nitrogen chlorosis in *Arthrospira platensis.*

Phosphorus is essential macro nutrient for plants and algae as it is required for growth and production of energy molecules. Reduction of phosphorus concentration led to reduction of biomass and protein productivities and enhancement of lipids, carbohydrates and carotenoids productivities. **Leonardos and Lucas (2000)** agreed with our results as they reported that phosphorus limitation decrease protein content of microalga *Chaetoceros muelleri*, also **Kilham *et al* . (1997)** demonstrated that phosphorus starvation reduces protein content in algal cells. **Kumar *et al*. (2012)** recorded that maximum lipid content of *Spirulina* was observed at low phosphorus concentration. In addition, total lipid content in *Scenedesmus* sp. was observed to increase from 23 % to 53 % with a reduction phosphorus concentration from 2 to 0.1 mg L^{-1} (**Xin *et al.*, 2010**). **Phadwal and Singh (2003)** revealed that the increase of β-carotene accumulation in *Dunaliella salina* was obtained with phosphorus starvation. **Kilham *et al*. (1997)** demonstrated that phosphorus starvation increased carbohydrate content of algal cells. In the present study, the increase of lipids, carbohydrates and carotenoids productivities is attributed to accumulation of these compounds under phosphorus deficiency.

Although, sulfur is an essential element for amino acids and lipids synthesis (**Romano *et al.*, 2000**), changes of its concentration in the cyanobacterial media was not well investigated. The present study showed that reduction of sulfur in the growth medium showed insignificant decrease in *A. platensis* growth and biomass productivity. However, lipids and protein productivities were decreased as sulfur concentration decreased; while, sulfur deprivation led to increase of carbohydrate productivity. Further studies are required to explain the exact role of sulfur on lipid and protein production.

Spirulina platensis is halophilic organism that was isolated by **Vonshak *et al*. (1982)** from hypersaline habitats. In our results, decrease of sodium chloride by 50 % below the control showed significant increase of biomass productivity. Decrease or increase of salt concentration increased lipid productivity. Also, carotenoids and carbohydrates increased with increasing salinity. The increase of lipid, carotenoids and carbohydrates productivities resulted from over production of these compounds as no changes were recorded in biomass productivity. **Shalaby *et al.* (2010)** recorded remarkable alteration of *Spirulina platensis* metabolism under salt stress, as it caused increase in carotenoids production at high salinity concentrations. **Vonshak *et al*. (1988)** observed an increase in carbohydrate content of *Arthrospira* under high salinity. **Rafiqul *et al.* (2003)** pointed out that lipid level of *Arthrospira fusiforms* cultivated in nutritional medium of different salinity increased with increasing salinity. **Sujatha and Najarajan (2013)** attributed the increase in some metabolites of *Arthrospira* at high saline levels to excessive formation of free radicals which stimulates the production of some metabolites such as carotenoids in order to protect cells.

CONCLUSION

The present study suggested used a new method for calculation of the valuable compound production of *Arthrospira platensis* depending on "productivity" instead of using the term "content". Results suggested that modification in *A. platensis* growth medium could be used to enhance the production of the nutritional valuable compounds. Reduction of phosphorus by 100 % and increase of salinity by 200 % increased lipid productivity

by 128 % and 93 %, respectively. A 100 % increase in nitrogen or phosphorus resulted in 10 % and 7 % enhancement of protein productivity, respectively. Elimination of sulfate salt from the medium increased the carbohydrate productivity up to 280 %. Phosphate salt elimination and a 300 % increase in salinity resulted in a 64 % enhancement of carotenoids productivity. Whereas, 100 % increase in phosphate concentration caused 18 % enhancement of CPC productivity.

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REFERENCES

ABD EL-BAKY, H. 2003. Over production of phycocyanin pigments in blue green alga *Spirulina* sp. and it's inhibitory effect on growth of Ehrlich ascites carcinoma cells. J *Med Sci*, 3(4), 314-324. http://dx.doi.org/10.3923/jms.2003.314.324

ABD EL-BAKY, H. EL-BAROTY, G. 2012. Characterization and bioactivity of phycocyanin isolated from *Spirulina maxima* grown under salt stress. *Food Funct*, 3(4), 381-388. http://dx.doi.org/10.1039/c2fo10194g

AIBA, S., OGAWA, T. 1977. Assessment of growth yield of a blue-green alga, *Spirulina platensis*, in axenic and continuous culture. *Microbiol*, 102(1), 179-182. http://dx.doi.org/10.1099/00221287-102-1-179

ABOMOHRA, A., EL-SHEEKH, M., HANELT, D. 2013. Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production. *World J Microbiol Biotechnol*, 29(5), 915-922. http://dx.doi.org/10.1007/s11274-012-1248-2

AYEHUNIE, S., BELAY, A., BABA, T.W., RUPRECHT, R.M. 1998. Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). *J Acquir Immune Defic Syndr Hum Retrovirol*, 18(1), 7-12.

BELAY, A. 2002. The potential application of *Spirulina* (*Arthrospira*) as nutritional and therapeutic supplement in health management. *JANA*, 5, 227-48.

BENNETT, A., BOGORAD, L. 1973. Complementary chromatic adaption in filamentous blue-green algae. *J Cell Biol*, 582, 419-435.

BHATTACHARYA, S., SHIVAPRAKASH, M. 2005. Evaluation of three *Spirulina* species grown under similar conditions for their growth and biochemicals. *J Sci Food Agric*, 85, 333-336. http://dx.doi.org/10.1002/jsfa.1998

BISCHOFF, H., BOLD, H. 1963. Phycological studies, IV. Some soil algae from Enchanted Rock and related algal species. University of Texas Publications 6318, 1-95.

BRADFORD , M . 1976. A rabid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, 72(1-2), 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3

COSTA, J., COLLA, L., FILHO, P., KABKE, K., WEBER, A. 2002. Modelling of *Spirulina platensis* growth in fresh water using response surface methodology. *World J Microbiol Biotechnol*, 18(7), 603-607. http://dx.doi.org/10.1023/A:1016822717583

ESTRADA, J. E. P., BESCŐS, P.B., VILLAR, DEL FRENSO, A.M. 2001. Antioxidant activity of different fractions of *Spirulina platensis* protean extract. *Farmaco*, 56(5-7), 497-500. http://dx.doi.org/10.1590/S1516-89132007000100020

FOLCH, J., LEES, M., STANLEY, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*, 226, 497-509.

HAYASHI, K., HAYASHI, T., KOJIMA, I. 1996. A natural sulfated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: *in vitro* and *in vivo* evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. *AIDS Res Hum Retroviruses*, 12(15), 1463-1471. http://dx.doi.org/10.1089/aid.1996.12.1463

HERNÁNDEZ-CORONA, A., NIEVES, I., MECKES, M., CHAMORRO, G., BARRON, B. 2002. Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. *Antiviral Res*, 56(3), 279-285. http://dx.doi.org/10.1016/S0166-3542(02)00132-8

KILHAM, S., KREEGER, D., GOULDEN, C., LYNN, S. 1997. Effects of nutrient limitation on biochemical constituents of *Ankistrodesmus falcatus*. *Freshwat Biol*, 38(3), 591-596. http://dx.doi.org/10.1046/j.1365-2427.1997.00231.x

KOCHERT, G. 1978. Carbohydrate determination by the phenol-sulphoric acid method. In: Ahellebust J, Craigie J (eds) Hand book of phycological methods: physiological and biochemical methods. Cambridge University press, Cambridge. ISBN 0521200490

KUMAR, V., BHATNAGAR, A., SRIVASTAVA, J. 2011. Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound. *J Med Plants Res*, 5(32), 7043-7048. http://dx.doi.org/10.5897/JMPR11.1175

KUMAR, P., SHAHI ,S., SHARMA, P. 2012. Effect of various cultural factors on isolated *Spirulina* strain for lipid and biomass accumulation. *Current Discovery*, 1(1), 13-18.

LEE, J., HAYASHI, K., MAEDA, M., HAYASHI, T. 2004. Antiherpetic activities of sulfated polysaccharides from green algae. *Planta Med*, 70(9), 813-817. http://dx.doi.org/10.1055/s-2004-827228

LEONARDOS, N., LUCAS, I. 2000. The nutritional values of algae grown under different culture conditions for *Mytilus edulis* L. larvae. *Aquaculture*, 182(3-4), 301-315. http://dx.doi.org/10.1016/S0044-8486(99)00269-0

MACKINNEY, G. 1941. Absorption of light by chlorophyll solutions. *J Biol Chem*, 140, 15-322.

OZDEMIR, G., KARABAY, N., DALAY, M., PAZARBASI, B. 2004. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. *Photother Res*, 18(9), 754-757. http://dx.doi.org/10.1002/ptr.1541

PAYNE, J., STEWART , J. 1988. The chemical composition of the thallus wall of *Characiosophon rivularis* (Characiosiphonaceae, Chlorophyta). *Phycologia*, 27(1), 43-49. http://dx.doi.org/10.2216/i0031-8884-27-1-43.1

PELIZER, L., DANESI, E., RANGEL, C., SASSANO, C., CARVALHO, J., SATO, S., MORAES, I. 2003. Influence of inoculum age and concentration in *Spirulina platensis* cultivation. *J Food Eng*, 56(4), 371-375. http://dx.doi.org/10.1016/S0260-8774(02)00209-1

PETER, P., SARMA, A., HASAN, A., MURTHY, S. 2010. Studies on the impact of nitrogen starvation on the photosynthetic pigments through spectral properties of the cyanobacterium, *Spirulina platensis*: Identification of target phycobiliproteins under nitrogen chlorosis. *Bot Res Int*, 3(1), 30-34.

PHADWAL, K., SINGH, P. 2003. Effect of nutrient depletion on beta-carotene and glycerol accumulation in two strains of *Dunaliella* sp. *Bioresour Technol*, 90(1), 55-58. http://dx.doi.org/10.1016/S0960-8524(03)00090-7

PIORRECK, M., BAASCH, K. H., POHL , P. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry*, 23(2), 207- 216. http://dx.doi.org/10.1016/S0031-9422(00)80304-0

QUOC, K., PASCAUD, M. 1996. Effect of dietary gamma-linolenic acid on the tissue phospholipid fatty acid composition and the synthesis of eicosanoids in rats. *Ann Nutr Metab*, 40(2), 99-108.

RAFIQUL, I., HASSAN, A., SULEBELE, G., OROSCO, C., ROUSTAIAN, P., JALAL, K. 2003. Salt stress culture of Blue-green Alga *Spirulina fusiformis*. *Pak J Biol Sci*, 6(7), 648-650. http://dx.doi.org/10.3923/pjbs.2003.648.650

ROMANO, I., BELLITI, M., NICOLAUS, B., LAMA , L., MANCA, M., PAGNOTTA, E., GAMBACORTA, A. 2000. Lipid profile: a useful chemotaxonomic marker for classification of a new cyanobacterium in *Spirulina* genus. *Phytochemistry*, 54(3), 289-294. http://dx.doi.org/10.1016/S0031-9422(00)00090-X

SHALABY, E., SHANAB, S., SINGH, V. 2010. Salt stress enhancement of antioxidant and antiviral efficiency of *Spirulina platensis*. *J Med Plants Res*, 4(24), 2622-2632.

STANIER, R., KUNISAWA, R., MANDEL, M., COHEN-BAZIRE, G. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol Rev*, 35(2), 171–205.

SUJATHA, K., NAGARAJAN, P. 2013. Optimization of growth conditions for carotenoid production from *Spirulina platensis* (Geitler). *Int J Curr Microbiol App Sci*, 2(10), 325-328.

USLU, L., IŞIK, O., KOÇ, K., GÖKSAN, T. 2011. The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*. *Afr J Biotechnol*, 10(3), 386-389. http://dx.doi.org/10.5897/AJB10.1547

VONSHAK, A. 1997. *Spirulina*: Growth, physiology and biochemistry. In: Vonshak A. (eds) *Spirulina platensis (Arthrospira)*: Physiology, Cell-Biology and Biotechnology. Taylor and Francis, London, pp. 43-65. http://dx.doi.org/10.1023/A:1007911009912

VONSHAK, A., ABELIOVICH ,A., BOUSSIBA, S., ARAD, S., RICHMOND, A. 1982. Production of *Spirulina* biomass: Effects of environmental factors and population density. *Biomass*, 2(3), 175-185. http://dx.doi.org/10.1016/0144-4565(82)90028-2

VONSHAK, A., GUY, R., GUY, M. 1988. The response of filamentous cyanobacterium *Spirulina platensis* to salt stress. *Arch Microbiol*, 150(5), 417-420. http://dx.doi.org/10.1007/BF00422279

WALACH, M., BAZIN, M., PIRST, S., BALYUZI, H. 1987. Computer control of carbon-nitrogen ratio in *Spirulina platensis*. Biotechnol Bioeng 29(4), 520-528. http://dx.doi.org/10.1002/bit.260290417

XIN, L., HU, H., KE, G., SUN, Y. 2010. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresour Technol*, 101(14), 5494–5500. http://dx.doi.org/10.1016/j.biortech.2010.02.016

ZARROUK, C. 1966. Contribution à l'étude d'une cyanophycée: Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthése de *Spirulina maxima* (Setch. et Gardner) Geitler. University of Paris, Paris, France.