E.Ü. Su Ürünleri Dergisi 2009
E.U. Journal of Fisheries & Aquatic Sciences 2009
Cilt/Volume 26, Sayı/Issue 2: 139-142

ISSN 1300 - 1590
http://jfas.ege.edu.tr/

Araştırma Notu / Short Note

The Effect of Temperature on Protein and Amino Acid Composition of *Spirulina platensis*

Leyla Hızarcı Uslu[1], Oya Işık[1], Selin Sayın[2], *Yaşar Durmaz[3]
Tolga Göksan[4], Şevket Gökpınar[3]

[1]*Cukurova University, Faculty of Fisheries, Adana, Turkey*
[2]*Mustafa Kemal University, Faculty of Fisheries, Antakya Turkey*
[3] *Ege University, Faculty of Fisheries, İzmir, Turkey*
[4]*Çanakkale Onsekiz Mart University, Faculty of Fisheries, Çanakkale, Turkey*
**E-mail: yasar.durmaz@ege.edu.tr*

Özet: ***Sıcaklığın*** Spirulina platensis***'in protein ve amino asit kompozisyonu üzerine etkisi.*** Bu çalışma, mevsime bağlı iklim değişikliğinin yaz ve kış mevsimlerinde kültüre alınan *Spirulina platensis*'in protein içeriği ve amino asit kompozisyonuna etkilerini incelemek amacıyla yürütülmüştür. Çalışma boyunca ışık yoğunluğu, pH ve tuzluluk günlük olarak ölçülmüş, sıcaklık ve çözünmüş oksijen gün boyunca ve gece ölçülmüştür. Yaz ve kış mevsiminde ortalama gündüz sıcaklığı sırasıyla 33,9±0,4 ºC ve 18,6±0,5 ºC olarak belirlenirken, gece sıcaklığı 29,9±0,2 °C ve 14.4±0.2°C olarak belirlenmiştir. Ortalama ışık yoğunluğu yaz mevsiminde 848,3 µmol $m^{-2}s^{-1}$ iken kışın 506,26±48 µmol $m^{-2}s^{-1}$ olmuştur. Yaz büyüme döneminde protein miktarı (72,9±03 %) ve amino asit konsantrasyonu kış dönemine göre daha yüksek bulunmuştur. Amino asitlerden Prolin, Sistin ve Arjinin sadece kış mevsiminde gözlenmiştir.

Anahtar Kelimeler: *Spirulina platensis*, sıcaklık, protein, amino asit.

Abstract: The purpose of this study was to clarify the seasonal variation of protein content and amino acid composition of *Spirulina platensis* grown in summer and winter. During the study, while the light intensity, pH and salinity were measured daily, the temperature and dissolved oxygen were measured during daytime and at night. While the mean day temperatures were recorded as 33.9±0.4 ºC and 18.6±0.5 ºC, the mean night temperatures were found to be 29.9±0.2 ºC and 14.4±0.2 ºC in summer and winter, respectively. The mean light intensity of 848.3 µmol $m^{-2}s^{-1}$ was determined in summer. It was 506.26±48 µmol $m^{-2}s^{-1}$ in winter. The protein amount (72.9±03 %) and the amino acid concentrations of *S. platensis* grown in summer were found to be higher than in winter. Some of the amino acids, Prolin, Sistin, and Arginine observed in winter, only.

Key Words: *Spirulina platensis*, temperature, protein, amino acid.

Introduction

Spirulina platensis, a filamentous cyanobacterium, is widely used in many countries as health food due to its protein content and biochemical substances for immune system. It is known that the environmental conditions, especially culture temperature and light intensity greatly influence the composition and physiological state of phytoplankton (Reynolds, 1984), in particular, protein and amino acid metabolism in the cells (Borowitzka, 1988; Becker, 1993; Olguin, *et al*., 2001). The optimum temperature for *Spirulina* growth lies in the range of 30 to 35 ºC. In winter, *Spirulina* does not grow significantly in open ponds (except in tropics), resulting in lower yields (Richmond, 1992). Therefore, the regions where winter temperatures are below 15 ºC are not suitable to grow *Spirulina* (Richmond, *et al*., 1990). In order to enhance culture conditions and to lower the costs, algae manufacturers frequently cover the ponds with transparent polyethylene to keep the medium warmer and to reduce the risk of contamination (Vonshak, 1992). *Spirulina* contains high levels of protein (50-70 %) that is associated with health food, pharmaceuticals and nutraceuticals (Cohen, *et al*., 1987) Low temperatures cause a decrease in growth and the protein content of *Spirulina* (Tomaselli, *et al*., 1988).

The protein source and food supplement *Spirulina platensis* is cultivated widely in tropical and subtropical areas in general. The aim of this work was to determine the influence of physico-chemical variations, temperature, light intensity, pH, dissolved oxygen and salinity, on the protein content and the amino acid composition of the cyanobacterium *Spirulina platensis*, grown in the subtropic region.

Materials and Methods

Microalga *Spirulina platensis* stock cultures were maintained at 24±2 °C on continuous illumination with flourescent (Philips white, 36 watt) lights in erlenmayers (250 mL, 500 mL, 2 L) and carboys (5 L and 10 L) in laboratory conditions. The irradiance, as measured by a Radiation Sensor LI-COR (LI-250), was 80 µmol.m^{-2}.s^{-1}. The stock cultures in 10 L volume were adapted to outdoors before inoculation to the ponds.

The cultures were grown in *Spirulina* medium. The content of the medium consists of the following composition

(g/L): 18.6 $NaHCO_3$, 8.06 Na_2CO_3, 1.00 K_2HPO_4 5.00 $NaNO_3$, 2.00 K_2SO_4, 2.00 NaCl, 0.40 $MgSO4.7H_2O$, 0.02 $CaCl_2.2H_2O$, 0.02 $FeSO_4.7H_2O$, 0.16 $EDTANa_2$ and micronutrient elements (0.001 $ZnSO_4.7H_2O$, 0.002 $MnSO_4.7H_2O$, 0.01 H_3BO_3, 0.001 $Na_2MoO_4.2H_2O$, 0.001 $Co(NO_3)_2.6H_2O$, 0.00005 $CuSO_4.5H_2O$, 0.7 $FeSO_4.7H_2O$, 0.8 $EDTANa_2$) were added 10 mL to 1L, (Işık, *et al.,* 2006).

The experiments were carried out in four fiberglass ponds, $1m^3$ capacity for each, in the greenhouse in summer (July) and winter (January). The cultures were circulated by the paddle wheels at a flow rate about 20 cm s^{-1} continuously. Culture depth was maintained at 10 cm in the ponds. The experiment lasted 38 days (January and February) . Culture medium was added to the ponds to compensate the lost water via evaporation on the 23th day of the experiment. In summer (July), the cultivation was completed in nine days. The temperature, pH, light intensity, salinity and chlorophyll *a* (chl *a*) were measured daily. 5 mL of the culture samples were filtered through GF6 glass fiber filter papers (Schleicher & Schuel) for the chl *a* measurements. Concentrations were measured by a UV-VIS SHIMADZU-1240 Spectrophotometer after extraction in 90 % acetone and held at 4 °C 24h in the darkness. The chl *a* was calculated from the following equation,

$Ca = 11.6.\ D_{665}-1.31\ D_{645}-0.14\ D_{630}$, Chl *a* (µg/L) = Chl *a*.v / V.l

V = Volume of water filtered for extraction
v = Volume of acetone used
l = pathlenght (in cm) of cuvette (Parsons, *et al*., 1963).

The amounts of cellular chl *a* were calculated using the highest cell density and chl *a* values. The chl *a* quantity (µg/L) was divided by cell number (cell/L).

The temperature and dissolved oxygen (DO) were measured twice a day; (12:00 and 24:00). pH of the growth medium was measured using WTW-330 pH meter daily. The salinity was recorded by using YSI 30 salinometer. Wet and dry weight of the biomass and the ash amounts were determined. Water content was determined by the weight loss of 1 g of wet material, maintained at 105 ºC for 4 hour while ash content was obtained by the weight loss of 1 g wet material, maintained at 550 ºC for 4 h (Soeder, *et al*., 1969). The daily sampling for filament counting was made using 5 mL culture, and samples were fixed in 4 % formaldehyde solution. The filaments were counted in triplicate by sedgwick-rafter counting chamber. The growth rates (µ) were determined as in the following formula;

(div./day) = $\log(N_1/N_0)$ x (3.322/t), N_1 and N_0 are the cell concentrations at the end and beginning of a period of time (t), days (Guillard, 1973).

The protein and amino acid amounts were analyzed at The Scientific and Technical Research Council of Turkey, TUBITAK-Marmara Research Center-Food Institute.

When the cultures reached to the stationary phase which was measured with the growth parameters of cell density and the chl *a* contents, the *Spirulina* filaments were harvested with the 40 µm mesh size cloth. The wet algal slurry was kept at -20 ºC for protein and amino acids analyses. The analyses were made with two replicates for each pond.

Statistical evaluations were carried out with SPSS Windows version 10.0. A one-way ANOVA test was used to compare means at 0.01 and 0.05 probability levels.

Results

Temperature, light intensity, DO, pH, salinity determined for four ponds in the greenhouse in two different seasons are shown in Table 1. The mean temperature, light intensity, DO and pH values were found different between summer and winter ($p<0.05$), except salinity. The same culture medium was used in both experimental periods.

Table 1. The growth conditions, temperature, light, DO, pH, salinity of *S. platensis* cultures in summer and winter.

	Summer (July-9 days)		Winter (January-38 days)	
	Day	Night	Day	Night
Temp.(ºC)(mean)	33.9±0.4*	29.9±0.2*	18.6±0.5*	14.4±0.2*
(max.)	36.5±0.2	30.9±0.1	24.2±0.1	17.1±0.1
(min.)	31.7±0.4	28.5±0.2	11.6±0.04	10.4±0.02
Light (µmol/m^2/s) (mean)	848.36±52*	-	506.26±48*	-
(max.)	1085.25±28	-	1084.72	-
(min.)	481.95±44	-	23.6±0.4	-
DO (mg/L)(mean)	24±3.5*	3.8±0.2*	18.7±1.7*	5.9±0.1*
(max.)	35.77±0.6	4.9±0.1	41.6±0.9	7.4±0.04
(min.)	6.65±0.06	2.53±0.2	6.6±0.04	4.8±0.1
pH(mean)	9.25±0.006*	-	9.52±0.004*	-
(max.)	9.77±0.01	-	9.84±0.001	-
(min.)	9.22±0.007	-	9.52±0.004	-
Salinity (max.)	29.85±0.04	-	28.4±0.2	-
(min.)	19.5±0.6	-	22.8±0.2	-

*$p<0.05$

At the end of the growth periods in summer and winter, the filament densities and dry weights were found to be different ($p<0.05$). The filament densities were $123x10^3$ ± 16 and $94x10^3$ ±10 in summer and winter, respectively. The dry weight obtained in winter was found to be almost one quarter of that in summer. Although the environmental factors were far from optimum for *S. platensis*, the yield could be obtained in winter. The growth rate was higher in summer than winter. But the chl *a* quotas of *Spirulina* cultures grown in winter were found to be higher than in summer (Table 2).

In these conditions, protein contents of *S. platensis* biomass grown in summer and winter were found to be %72.9±0.3 and %33.16±0.2, respectively ($p<0.05$).

It was also determined that total amino acid concentration of *S. platensis* grown in summer, 33.09 mg/100g wet biomass, was found to be higher than in winter, 20.88 mg/100g wet biomass. The amino acids of Prolin, Sistin, and Arginine were recorded in summer only.

Table 2. The filament density, chl *a* quota, growth rate, dry weight and ash weight of *S. platensis*.

Growth parameters / Biomass data	Summer (July-9 days)	Winter (January-38 days
Filament density (max.)	$123x10^3$±16*	94.10^3±10*
(filament/mL) (the beginnig)	$1.6x10^3$±5*	$1.7x10^3$±2*
Chl *a* quota (max.) (pg/filament)	7.451*	13.09
Growth rate (mean)	0.78*	0.19
(max.)	1.57	0.47
Dry weight (mg/L)(max.)	1375±0.009*	360±0.01
Ash weight (%)	16.7±0.01*	16.6±0.003
Protein content (%)	72.9±0.3*	33.16±0.2*

*$p<0.05$

Amino acids concentrations of *S. platensis* (mg/100 g wet samples) grown in summer and winter are shown in Table 3.

Table 3. Amino acids composition of S. platensis (mg/100 g wet samples) grown in summer and winter.

Amino Acids (mg/100 g)	Summer	Winter
Threonine	2009.7	1106.5
Valine	2381.7	1491.4
Methionine	551.6	318
Isoleucine	2261.1	1311.9
Leucine	3198.5	1862.3
Phenylalanine	1716.6	1043.2
Lysine	2280.3	1105.3
Aspartic acid	3791.3	1889.8
Serine	1869.4	907.9
Glutamic acid	6747.4	3782.2
Glycine	2163.2	1210.2
Alanine	2179.3	1774.8
Tyrosine	1121.5	871.6
Histidine	818.5	471.6
Proline	-	465.2
Cystine	-	117.2
Arginine	-	1153.5
Total	33.09	20.88

Discussion and Conclusion

The protein content was influenced by ambient factors varied according to the seasons. The cellular response to increasing light intensity and temperature are to reduce protein content, amino acid composition, chlorophyll a and other light-harvesting pigments such as chlorophyll b, chlorophyll c, phycobiliproteins and primary carotenoids (Tomaselli, *et al*., 1997). Torzillo and *et* al. (1984) observed that the protein content of *Spirulina* was at the highest level in summer in relation to ambient factors especially temperature and light intensity. Tomaselli and *et* al. (1988) were studied on the influence of temperature on *Spirulina platensis* M2 and determined the protein content of 58.6, 58, 57.7, 55.5 and 45.5 per cent at the different temperatures of 30, 35, 38, 40 and 42 °C, respectively. Richmond (1986) reported that the cellular content of protein and amino acids is related to dissolved oxygen. In *Spirulina*, one effect of dissolved oxygen concentration relates to the protein content: A treatment of 45% O_2 in the gas phase applied to a *Spirulina* culture greatly reduced protein content, from 48% (of dry weigth) to 22% (Torzillo, *et al*., 1984). Obgonda and et al., (2006) studied the influence of temperature on biomass and protein biosynthesis on *Spirulina* sp. and measured the protein content and amino acid compositions of *Spirulina* at the different temperatures, e.g., 25, 30, 35 and 40 ºC. They observed that the highest amounts of protein (46.39 g / 100g) and amino acid (76.09 g / 16 gN) were obtained at temperature of 30 ºC. Koru and Cirik (2002) were studied on the biochemical composition of *Spirulina* biomass in open-air system. They determined the protein content at the different temperatures, e.g., 35, 37 and 42 ºC. They reported that when the temperature increased from 35 (64.7% proteins) to 42 ºC (43.1% proteins) marked changes in the macromolecular composition of the *Spirulina* occurred.

Conclusions

The results of this study indicated that the biochemical composition, especially protein amounts and composition of amino acids of *Spirulina* cells was influenced by the culture conditions depending on the environmental factors, clearly.

Acknowledgements

This study was supported by the Cukurova University Research Fund.

References

Becker, E.W., 1993. Development of *Spirulina* research in a developing country India. Bulletin de I, Institut Oceanographique (Monaco). (Spec. Issue 12) 65-75.

Borowitzka, M.A., 1988. Vitamins and fine chemicals from micro-algae. In:Borowitzka (Eds.), Micro-Algal Biotechnology, Cambridge University Pres, Cambridge, UK. pp 153-196.

Cohen, Z., A. Vonshak, A. Richmond, 1987. Fatty acid composition of *Spirulina* strains grown under various environmental conditions. Phytochemistry. 26 (8) 2255-2258.

Guillard, R.R.L., 1973. Culture Methods and Growth Measurements, Division Rates in Handbook of Phycological methods J.R. Stein (Ed.), Chambridge University Pres, Chambridge. pp. 289-311.

Işık, O., L. Hızarcı, S. Sayın, S. Gökpınar, Y. Durmaz, T. Göksan. 2006. The Effect of the Environmental Factors on the Vitamin C (Ascorbic Acid), E (Alpha-tocopherol), β-carotene Contents and the Fatty Acid Composition of *Spirulina platensis*. E.U. Journal of Fisheries & Aquatic Sciences Vol; 23 (3-4): 257–261.

Koru, E., S. Cirik, 2002. Biochemical composition of *Spirulina* biomass in open-air system. Proceedings of ICNP. Trabzon. pp 97-100.

Ogbonda, K.H., R.E. Aminigo, G.O.Abu, 2006. Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina* sp. Bioresource Technology.

Olguin, E., S. Galicia, O. Angulo-Guerrero, E. Hernndez, 2001. The effect of low light flux and nitrogen deficiency on the chemical composition of *Spirulina* sp. (Arthrospira) grown on digested pig waste. Biores. Technol. *77,* 19-24.

Parsons, T.R., J.D.H. Strickland, 1963. Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J. Marine Research. *21*No: 3, p.115-163.

Reynolds, C.S., 1984. The ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge. 384 pp.

Richmond, A., 1986. Outdoor Mass Cultures of Microalgae. (A. Richmond Editör). Handbook of Microalgal Mass Cultures of Microalgae. CRC Press, INC. Boca Raton, Florida. 285-329.

Richmond, A., 1992. Mass culture of cyanobacterium. In: Photosynthetic Prokaryotes, N.H. Mann, N.G. Carr (Eds.), Plenum Press, New York pp 181-209.

Richmond, A., E. Lichtenberg, B. Stahl, A. Vonshak, 1990. Quantitative assessment of the major limitations on productivity of *Spirulina* platensis in open raceways. *J.* Applied Phycology. *2* 195-206.

Soeder, C.J., J.F. Talling, I. Baak, 1969. Chemical components, Dry weight and ash content. A manual on methods for Measuring Primary Production in Aquatic Environments, A.R. Vollenweider (Ed.), Blackwell Scientific Publications, Edinburgh Melbourne.

Tomaselli, L., G. Boldrini, M.C. Margheri, 1997. Physiological behaviour of Arthrospira (*Spirulina*) maxima during acclimation to changes in irradiance. Journal of Applied Phycology 9 37-43.

Tomaselli, L., L. Giovannetti, A. Sacchi, F. Bocci, , 1988. Effects of temperature on growth and biochemical composition in *Spirulina platensis* strain M2. In: Algal Biotechnology, T. Stadler, J. Mellion, M.C. Verdus, Y. Karamanos, H. Morvan, D. Christiaen (Eds.), Elsevier Applied Science, London. pp 303-314.

Torzillo, G., F. Giovanetti, R. Bocci, L. Materassi, 1984. Effect of oxygen concentration on the protein content of *Spirulina* biomass.

Vonshak, A., 1992. Microalgal biotechnology: is it an economical success?. In Biotechnology:Economic and Social Aspects, E.J. Da Silva, C. Ratledge, A, Sasson (Eds.), Cambridge University. pp 70-80.