

**THE EFFECTS OF DIFFERENT SALINITY RATES ON FAT AND FATTY ACID COMPOSITION OF *Spirulina platensis***Hilal Kargın Yılmaz<sup>1</sup>, Deniz Ayas<sup>1\*</sup>, Harun Yılmaz<sup>1</sup>, Yeşim Özoğul<sup>2</sup><sup>1</sup> Faculty of Fisheries, University of Mersin, Mersin, Turkey<sup>2</sup> Faculty of Fisheries, University of Çukurova, Mersin, Turkey**Abstract:**

The present study investigated the effects of different salinity ratios on the fat and fatty acid composition of *Spirulina platensis*. *Spirulina platensis* cyanobacteria was cultivated in a helical photobioreactor and in a continuous system. Cultivation continued in medium of different salinity (10‰, 20‰, 30‰) for 10 days. Results of this study showed that lipid values increased in parallel with the increase in salinity. The lowest lipid content was obtained from 10‰ salinity (7.4%) whereas the highest level of lipid was found at 30‰ salinity (9.4%). Variations in fatty acid composition of *S. platensis* occurred due to the different rates of salinity. The rates of saturated fatty acids (SFA) ranged from 45.0% to 45.3%, monounsaturated fatty acids (MUFAs) from 7.1 to 7.2%, and polyunsaturated fatty acids (PUFAs) from 32.6 to 33.0% in medium of different salinity. The major fatty acids found in *S. platensis* were decanoic acid (C10:0, 18.5-18.9%), palmitic acid (C16:0, 25.1%), stearic acid (C18:0, 1.3-1.4%), palmitoleic acid (C16:1, 5.2-5.3%), oleic acid (18:1 n-9, 1.9%), linoleic acid (C18:2 n-6, 13.5-13.7%) and gamma-linolenic acid (C18:3 n-6, 19.1-19.3%). Stearic, oleic, linoleic and gamma-linolenic acid values increased; decanoic and palmitoleic acid levels decreased in parallel with the increase in medium salinity.

**Keywords:** *Spirulina platensis*, Lipid, Fatty Acids Composition, Salinity Stress**Özet:****Farklı Tuzluluk Oranlarının *Spirulina platensis*'in Yağ ve Yağ Asitleri Kompozisyonuna Etkileri**

Çalışmada tuzluluk stresine bağlı olarak *Spirulina platensis*'in yağ ve yağ asitleri kompozisyonunda meydana gelen değişimlerin belirlenmesi amaçlanmıştır. *Spirulina platensis*'in sarmal fotobiyoreaktörde sürekli sistemde kültürü yapılmıştır. Deneme farklı tuzluluk ortamlarında (‰10, ‰20, ‰30) 10 gün süresince sürekli sistemde yürütülmüştür. Çalışmada lipid düzeylerinin ortam tuzluluğunun artışına paralel olarak artış gösterdiği belirlenmiştir. En düşük lipid düzeyi (%7.4) ‰10 tuzlulukta gerçekleşirken; en yüksek lipid düzeyi (%9.4) ‰30 tuzlulukta

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elde edilmiştir. Farklı tuzluluk oranlarına bağlı olarak *S.platensis*'in yağ asidi kompozisyonunda değişimler meydana gelmiştir. Farklı tuzluluk ortamlarında doymuş yağ asitleri %45.0-%45.3, tekli doymamış yağ asitleri %7.1-%7.2, çoklu doymamış yağ asitleri %32.6-%33.0 aralığında bulunmuştur. *S.platensis*'in temel yağ asitleri dekanolik asit (C10:0, %18.5-%18.9), palmitik asit (C16:0, %25.1), stearik asit (C18:0, %1.3-%1.4), palmitoleik asit (C16:1, %5.2-%5.3), oleik asit (18:1 *n*-9, %1.9), linoleik asit (C18:2 *n*-6, %13.5-%13.7) ve gama-linolenik asit (C18:3 *n*-6, %19.1-%19.3) olarak belirlenmiştir. Ortam tuzluluğunun artışına bağlı olarak; stearik, oleik, linoleik and gama-linolenik asit düzeylerinde artış, dekanolik ve palmitoleik asit seviyelerinde düşüş görülmüştür.

**Anahtar Kelimeler:** *Spirulina platensis*, Lipit, Yağ asidi kompozisyonu, Tuzluluk stresi

## Introduction

Microalgae have been widely researched in recent years due to their abundant content of fat and fatty acids. *Sprulina platensis*, a member of the blue-green algae class (*Cyanophyceae*) is a filamentous, spiral prokaryotic organism composed of microscopic cells. *S.platensis* is a highly important cyanobacteria species in that it contains high levels of gamma-linoleic acid. Therefore, it is widely cultivated to be used commonly as a human and animal food source, and in cosmetics, pharmaceuticals, and other various industrial fields (Cirik, 1989; Chen *et al.*, 1996; Glazer, 1999). It is produced commercially as a food source in health-foods and the pharmaceutical industry, especially in developing countries (Richmond, 1988; Zeng and Hu, 1992). Microalgae are principal organisms in that they produce a distinct range of chemical and biological compounds, principally vitamins, pigments, proteins, minerals, lipids and polysaccharides. In comparison to other living sources, algae are very rich in some kinds of fatty acids such as polyunsaturated fatty acids (PUFA),  $\delta$ -linoleic acid (GLA) (Borowitzka, 1992; Cohen, 1997).

To find out the changes in fat and fatty acid composition of *S.platensis* stemming from various stress sources (Salinity, temperature, etc.), a lot of studies have been carried out by Vonshak *et al.* (1996), Rafigul *et al.* (2003), Koru and Cirik (2003), Işık *et al.* (2006), Ayachi *et al.* (2007). The present study investigated the effects of different salinity rates on fat and fatty acid composition of *S.platensis* in a helical photobioreactor.

## Material and Methods

### Material production

The experiment was conducted in the Plankton Laboratory of Faculty of Fisheries in Mersin University. A helical tubular photobioreactor was utilized in the experiment. The helical photobioreactor consisted of a transparent hose with inner diameter, length and volume of 1.8 cm, 80 m and 20.3L respectively. A stock tank which contained a constant volume of 4L of alga was located on a platform above the helical part. The total volume of the system was 24.3L. A peristaltic pump was used to ensure the circulation of the alga in the bioreactor. Four 40w daylight fluorescent lamps were used to provide constant lighting during the study. *Spirulina* requires CO<sub>2</sub> for photosynthesis and it is necessary to prevent accumulation of dissolved O<sub>2</sub>, condensing in the system as a result of the photosynthesis, to cause increasing pH levels to excessive levels. Therefore, CO<sub>2</sub> was continually added to the system. The helical photobioreactor used in the experiment was designed for continuous production under laboratory conditions. The aim was to sustain the same growth rate of the culture which has reached the maximum growth rate through production in continuous system. The strain of *S.platensis* that was used as the experiment material was supplied by Çukurova University, Faculty of Fisheries. *Spirulina* medium (Schlösser, 1982) was utilized as the nutritional medium. *S.platensis* was cultured in the helical photobioreactor at three salinity levels (10‰, 20‰, 30‰) and continuous production lasted for a period of 10 days. Nutrient was added to the system through a dosage pump at a flow rate of 7 mL/min and 10 L of algae was harvested daily from the stock tank at the same flow rate.

The temperature was kept within the range 25.71 to 26.77°C and pH was kept within the range 9.27 to 9.52. Fat and fatty acids analyses of the obtained samples were performed using dry matter.

### Fat and fatty acid analysis

Lipid extraction was carried out according to the method Bligh and Dyer (1959). Fatty acid profiles of fat extracted from the *Spirulina* samples were determined by gas chromatography (GC). The fatty acid composition was analysed by the GC Clarus 500 with autosampler (Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m 0.32 mm, ID 0.25 mm, BP20 0.25  $\mu$ m; SGE Analytic Science Pty Ltd, Victoria, Australia). The oven temperature was 140 °C, held for 5 min, raised to 200 °C at a rate of 4 °C/min and to 220 °C at a rate of 1 °C/min, while the injector and the detector temperature were set at 220 °C and 280 °C, respectively. The sample size was 1  $\mu$ L and the carrier gas was controlled at 16 ps. The split ratio used was 1:50. Fatty acids were identified by comparing the retention times of fatty acid methyl esters with a standard 37-component fatty acid methyl ester mixture (catalog no 18919; Supelco). Tri replicate GC analyses were performed and the results were expressed in GC area % as the mean value  $\pm$  standard deviation.

### Statistical analysis

Statistical analysis of data was carried out with the SPSS 16.0. All data were checked for outliers (Z values were checked) and homogeneity of variance (Duncan test was used) was also tested. One-way ANOVA (Analysis of Variance) was used to evaluate the effect of different salinity on fatty acid composition.

### Results and Discussion

The lipid levels of *Spirulina* produced at 10 ‰, 20 ‰ and 30 ‰ salinity were found to be 7.4%, 8.4% and 9.4% respectively. The present study found that lipid values increased in parallel with the increase in salinity. Ungsethaphand *et al.* (2009) pointed out that lipid level of *S.platensis* decreased when sodium bicarbonate added in nutritional medium. This finding by Ungsethaphand *et al.* (2009) does not support our finding.

Rafigul *et al.* (2003) pointed out that the lipid level of *S.fusiformis* cultivated in nutritional medium of different salinity increase with increasing

salinity. Ayachi *et al.* (2007) stated that the total fatty acids (TFA) level of *S.platensis* increased with increase in medium salinity. The results presented by the researchers were similar to those obtained in the present study. Likewise, in the present study, the lipid metabolism increased because of stress from salinity.

Fatty acids -SFAs, MUFAs, PUFAs- in *S.platensis* are presented in Table I. The rates of saturated fatty acids (SFA) ranged from 45.0% to 45.3%, monounsaturated fatty acids (MUFAs) from 7.1 to 7.2%, and polyunsaturated fatty acids (PUFAs) from 32.6 to 33.0% in medium of different salinity. The major fatty acids found in *S.platensis* were decanoic acid (18.5-18.9%), palmitic acid (25.1%), stearic acid (1.3-1.4%), palmitoleic acid (5.2-5.3%), oleic acid (1.9%), linoleic acid (13.5-13.7%) and gamma-linolenic acid (19.1-19.3%). Işık *et al.* (2006) reported that the amount of decanoic, palmitic, linoleic and gamma-linolenic acid contents in *S.platensis* changed between 0.3%-8.9%, 28.0%-36.9%, 20.2%-23.0% and 11.4%-22.2% respectively. Işık *et al.* (2006) also reported that the values of gamma-linolenic acid in *Spirulina* were similar to our study.

Table 1. Variations in fatty acid composition of *S. platensis* in different nutritional medium.

Fatty Acid	Salinity		
	10‰ $\bar{X} \pm S_x$	20 ‰ $\bar{X} \pm S_x$	30‰ $\bar{X} \pm S_x$
C10:0	18.91 $\pm$ 0.05 <sup>c</sup>	18.73 $\pm$ 0.04 <sup>b</sup>	18.51 $\pm$ 0.03 <sup>a</sup>
C16:0	25.09 $\pm$ 0.03 <sup>a</sup>	25.11 $\pm$ 0.03 <sup>a</sup>	25.09 $\pm$ 0.04 <sup>a</sup>
C18:0	1.32 $\pm$ 0.03 <sup>a</sup>	1.39 $\pm$ 0.01 <sup>b</sup>	1.40 $\pm$ 0.01 <sup>b</sup>
$\Sigma$ SFA	45.32	45.23	45.00
C16:1	5.30 $\pm$ 0.03 <sup>b</sup>	5.22 $\pm$ 0.02 <sup>a</sup>	5.17 $\pm$ 0.03 <sup>a</sup>
C18:1n9	1.88 $\pm$ 0.01 <sup>a</sup>	1.92 $\pm$ 0.01 <sup>b</sup>	1.92 $\pm$ 0.01 <sup>b</sup>
$\Sigma$ MUFA	7.18	7.14	7.09
C18:2n6	13.47 $\pm$ 0.04 <sup>a</sup>	13.64 $\pm$ 0.06 <sup>b</sup>	13.74 $\pm$ 0.05 <sup>b</sup>
C18:3n6	19.12 $\pm$ 0.02 <sup>a</sup>	19.26 $\pm$ 0.04 <sup>b</sup>	19.29 $\pm$ 0.05 <sup>b</sup>
$\Sigma$ PUFA	32.59	32.90	33.03
Unidentified	14.91	14.73	14.88

\*There is a significant difference at the level of  $p < 0.05$  among those which are shown by different letters in the same line.  $\bar{X} \pm S_x$ : Standard deviation

Variations in fatty acid composition of *S.platensis* occurred due to the different rates of

salinity (Table 1). Stearic, oleic, linoleic and gamma-linolenic acid values increased; decanoic and palmitoleic acid levels decreased in parallel with the increase in medium salinity. Even though decanoic acid levels differentiated statistically ( $p < 0.05$ ), palmitic acid levels did not indicate any variations statistically among all three sample groups. Regarding stearic, oleic, linoleic and gamma-linolenic acids, samples of 10‰ group were different from samples of 20‰ and 30‰ groups statistically ( $p < 0.05$ ). Ayachi et al. (2007) stated that fatty acid composition of *S.platensis* showed differences depending on culture age and medium salinity. The researchers found out that gamma-linolenic acid reached optimum level at salinity of 30‰ in 15 days cultures. These results support the findings of the present study.

### Conclusion

In the present study, it was found that the increase of medium salinity caused a significant increase in lipid levels of *S.platensis*. In addition, linoleic and gamma-linolenic acids levels also significantly increased stemming from the increase of medium salinity, considering the importance of these fatty acids in human and animal feeding; this study shows that this species should be produced in high salinity.

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