

Utilization of Geothermal Water (Şanlıurfa-Karaali) Medium to Enhance DNA Protection and Phycocyanin in *Spirulina platensis* Production

Betül Güroy¹ , Sibel Bayıl Oğuzkan^{2,*} , Derya Güroy³ 

¹Yalova University, Armutlu Vocational School, Food Processing Technology Department, Yalova, Turkey.

²Gaziantep University, Vocational School of Health Services, Department of Medical Services and Techniques, Gaziantep, Turkey.

³Yalova University, Armutlu Vocational School, Aquaculture Department, Yalova, Turkey.

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Corresponding Author

Tel.: +903423172188

E-mail: bayil@gantep.edu.tr

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Abstract

Spirulina's industrial use depends on its sustainability and economic production. Geothermal water sources are well known potential water sources for *Spirulina*'s slow-cost production. This research aimed to study the effects on *Arthrospira (Spirulina) platensis* culture of Sanlıurfa (Karaali) geothermal water. *Spirulina* was cultured in a 2000 mL Erlenmeyer flask for three weeks. The Schlösser medium (SM) was substituted with 50% and 100% of the volume of the culture medium with the Sanlıurfa geothermal water. The results determined that 3.25 phycocyanin purity ratio (A_{620}/A_{280}), 8% phycocyanin and 38.3% protein in the nutrient medium containing 50% Sanlıurfa (Karaali) geothermal water. In the Schlösser *Spirulina* nutrient medium, defined as the control group, the protein content of 55% and a content of phycocyanin of 16.9% was achieved a phycocyanin purity ratio of 4.43. Although the protein and phycocyanin ratios of *Spirulina* produced with geothermal water replaced are lower than in Schlösser medium, DNA protective activity of *Spirulina* produced in the geothermal water of Sanlıurfa Karaali showed better protective activity than those produced in the Schlösser medium.

Introduction

Spirulina is a microalga systematized in the class of cyanobacteria called blue-green microalgae, containing high-value molecules such as essential amino acids, vitamins, minerals, essential oils, beta-carotene, phycocyanin, chlorophyll and other bioactive components. *Spirulina* is effectively produced in open ponds and photobioreactors. The production strategy affects *Spirulina's* cost and is produced at 3.8-9.59 €/kg in outdoor pools. *Spirulina* production costs may increase up to 20-fold in the photobioreactor system and range from 18.7 to 74.29 €/kg (Delrue et al., 2017).

Although it is a preferred food in human nutrition, it is an expensive ingredient in the feed sector (Sugiharto et al., 2018). It is emphasized that *Spirulina platensis*, which has a significant health effect, inhibits inflammatory cells in the lungs and is beneficial to health (Xiong et al., 2018). Several kinds of research have been carried out that show that *Spirulina*-supplemented foods are richer in protein, fat and mineral content (Güroy, 2017; Lucas et al., 2018). It has also been reported that dietary *Spirulina* enhances disease resistance (Yuand et al., 2018; Lokapinrasaria et al., 2016) and prevents weight loss during starvation in fish (Güroy et al., 2011). Although it is an expensive

ingredient in feeds, it is stated that even small amounts of *Spirulina* addition provide significant benefits (Mahmoud et al., 2018; Sugiharto et al., 2018).

Spirulina is an expensive product due to its high production cost, and research on *Spirulina* has focused on reducing *Spirulina* operating costs. The use of the different mediums as nutrients for algae production (Markou et al., 2018), the application of production strategies such as various light sources (Prates et al., 2018) and temperature change (de Jesus et al., 2016) have positive effects on low-cost algal production. Nutrient mediums formulated by Zarrouk (1966), Paoletti et al. (1975) and Schlösser (1994) are used to produce *Spirulina* in the world as they are sufficient in terms of micro and macronutrients. Since these nutrient media are generally expensive, alternative nutrient media that can be used instead of them are being investigated. Research conducted is often compared to these mediums (Pelizer & Moraes, 2014). Since geothermal waters are similar mineral contents throughout the year, they can be considered an alternative nutrient medium. Geothermal water can be used directly or substitutable of these nutrient mediums depending on mineral content to achieve optimal algal productivity. In Turkey, two hundred twenty-seven geothermal areas are determined temperatures ranging from 20-287 °C (Lund & Boyd, 2016). Şanlıurfa (Karaali) geothermal water in the Southeastern Anatolia Region has a source water temperature of 48°C and 236.6 mg.L⁻¹ bicarbonate that it has content (Çiftçi, 2015).

One of the most important potential advantages of geothermal waters isto be veryrich in minerals to support When geothermal nutrients are used in *Spirulina* cultivation, the mineral structure of geothermal waters may cause the content of *Spirulina* nutrient levels and bioactive components to decrease or increase (Guroy & Guroy, 2018; Bayil Oguzkan et al., 2018). This advantage and disadvantage of protein quantities can broaden the spectrum of economic use of *Spirulina*. The bioactive components in natural products could eliminate the harmful effects of free radicals on the cell with their antioxidant character. The hydroxyl and hydrogen peroxide radicals are the most influential radicals in metabolism. This radical can quickly attack nucleotides and cause permanent damage to the structure of DNA, which is the most critical genetic nucleotide (Gutteridge & Halliwell, 2000). Therefore, it was also aimed to determine the oxidant status and DNA protective activities of *Spirulina* in this present study. In a previous study, *Spirulina* produced in Shössler medium showed antioxidant activity and had protective effects on DNA (Bayil Oguzkan et al., 2018). In this study, Şanlıurfa (Karaali) geothermal water in the Southeastern

Anatolia Region as a nutrient medium was investigated for the first time in *Arthrospira (Spirulina) platensis* cultivation.

Material and Method

Geothermal Water and *Spirulina* mediums

Şanlıurfa geothermal water was transported to Algal Production Unit of Yalova University with the support of Armutlu District Governorship of Yalova and Haliliye Municipality of Şanlıurfa. Schlösser medium (Schlösser, 1994), containing the following nutrients (g.L⁻¹): 13.61 (NaHCO₃); 4.03 (Na₂CO₃); 0.5 (K₂HPO₄); 2.50 (NaNO₃); 1 (K₂SO₄); 1 (NaCl); 0.20 (MgSO₄.7H₂O); 0.04 (CaCl₂.2H₂O); 0.01 (FeSO₄.7H₂O); 0.08 (Na₂EDTA.2H₂O). Trace metal solution was followed as; 2.15 µM (Na₂EDTA); 2.52 µM (FeSO₄.7H₂O); 8.97 nM (MnSO₄.7H₂O); 3.48 nM (ZnSO₄.7H₂O); 0.162 µM (H₃BO₃); 3.44 nM (Co(NO₃)₂.6H₂O); 4.13 nM (Na₂MoO₄.2H₂O); 0.02 nM (CuSO₄.5H₂O). All nutrients were dissolved in distilled water containing as according to Schlösser (1994).

In this study, bicarbonate values in nutrient media were taken into account. The bicarbonate content of the experimental groups is presented in Table 1.

Culture Conditions of *Spirulina (Arthrospira) platensis*

Spirulina (Arthrospira) platensis was obtained from the Algal Production Unit of Yalova University. Starting cultures were prepared by inoculating *Spirulina platensis* in Schlösser *Spirulina* medium (Schlösser, 1994) at 30°C under air feed and average 1000 lux illumination. The experiment was run with three replicates in a 2000 mL Erlenmeyer flask for three weeks. The groups were as follows.

Group 1: containing 1800 mL sterilized Schlösser medium, Group 2: containing 1800 mL geothermal medium, Group 3: containing 900 mL geothermal media and 900 mL Schlösser medium.

A starting culture containing 70 x 10⁴ cells/mL in a ratio of 1: 6 (300 mL) of the volume of the prepared nutrient medium was inoculated. At the beginning of the experiment, the groups were 8 x 10⁴ cells/mL. The temperature (°C), pH, optical density and cell counts of culture in the Erlenmeyer flask were measured every three days a week during the trial. All groups were inoculated with *Spirulina platensis* culture (0.02 mg/mL = initial optical density of 0.238 at absorbance 750 nm and the optical density read at A₇₅₀ (Lakshmanan et al., 2013). Algae cells were filled in the Neubauer counting

Table 1. The bicarbonate content of the experimental groups.

<i>Spirulina</i> Mediums	HCO ₃ (g/L)
Schlösser medium 100%	13.6
Geothermal water 100%	0.236
Geothermal water 50%	6.918

chamber and covered with lamellae. Cells were counted under a microscope and calculated with the following expression (Radhakrishnan et al., 2017).

$$\text{Cell/mL} = \text{Total number of cells} / 10 \times 4 \times 10^{-6}$$

At the end of cultivation, *Spirulina* biomass was dried after filtration from 45 μ plankton mesh.

Phycocyanin Analysis

40 mg samples were placed in a 10 mL centrifuge tube containing 100 mM phosphate buffer (10.64 g K₂HPO₄ and 5.29 g KH₂PO₄ per L, 10 mL, pH 7.0) added and stored in the refrigerator overnight after vortex. The blue supernatant was separated from the cell residue after centrifugation. C-phycocyanin calculations were determined using spectrophotometry-based methods on the absorbance ratio. The content of C-phycocyanin was calculated according to Setyoningrum and Azimatun Nur (2015) and Boussiba and Richmond (1979). Phycocyanin has a single visible absorbance maximum between 615 and 620 nm.

$$\% \text{Phycocyanin} = [A_{620} \times V \times 100] / [7.3 \times \text{Sample(mg)} \times \% \text{Dry matter}] \text{ (Setyoningrum \& Azimatun Nur, 2015).}$$

7.3 is the extinction coefficient of C-PC at 620 nm; V is total volume; 100 represents 100%.

The purity of C-PC preparations was evaluated based on the ratio between absorbencies from phycocyanobilin at 620 nm and aromatic amino acids in all proteins in the preparation at 280 nm (Antelo et al., 2010).

$$\text{Purity ratio} = A_{620} / A_{280}$$

The yield of the phycocyanin was calculated according to (Silveira et al., 2007).

$$[\text{C-PC(mg/g)} * \text{solvent volume (mL)}] / (\text{dry biomass(g)})$$

The concentration of the phycocyanin was calculated according to Bennett and Bogorad (1973).

$$\text{C-PC (mg/mL)} = [A(615) - 0.474 * A(652)] / 5.34$$

Protein was determined according to the Kjeldahl method (AOAC, 2000).

DNA Protective Activity of *Spirulina*

A pBR322 plasmid DNA (vivantis) was used to determine the DNA protection activity of *Spirulina* samples from damages caused by UV and oxidative stress. According to the method specified by Vanella et al. (2000) and imaging was performed on 1.25% agarose gel. Plasmid is a small, circular, double-stranded DNA molecule clearly showed the band on agarose gel electrophoresis. Pbr 322 DNA was often used DNA protective analysis that was optimized experimentally (Vanella et al., 2000; Kumar et al., 2001). Plasmid DNA was exposed to damage by applying H₂O₂ and UV in the presence of *Spirulina* in the component.

A 3.0 μ l pBR322 plasmid DNA (172ng. μ l) and 1 μ l 30% H₂O₂ were placed in tubes. A light source was used AS a UV transilluminator (DNR-IS) device that generates light at 302 nm wavelength and 8000 μ W/cm intensity at ambient temperature. After gel electrophoresis was conducted for 45 minutes, the photograph was obtained through imaging in the gel documentation system (DNR-IS, Mini BISA Pro).

Statistical Analysis

Data were subjected to a one-way analysis of variance (ANOVA). LSD test was used to determine the difference between the groups. The statistical evaluation of the data was provided by Statgraphics Centurion XVI (Manugistics Incorporated, USA). The mean values obtained are given as "Mean \pm Standard Error". All tests were performed at a 95% confidence interval.

Results

The cell concentrate, optical density, pH, and dry matter values in all groups were measured every three days a week during the trial (Figure 1, 2, 3, 4). The

Table 2. Phycocyanins and protein values of *Arthrospira (Spirulina) platensis* grown with Schlösser's and geothermal media.

	% C-PC	Purity ratio A ₆₂₀ /A ₂₈₀	C-PC mg/mL	The yield of C-PC mg/g	% Protein
%100 Schlösser medium	16.91 \pm 0.51	4.43 \pm 0.28	0.24 \pm 0.02	54.26 \pm 1.5	55.00 \pm 1.01
Geothermal medium % 100	4.66 \pm 0.65	1.22 \pm 0.45	0.10 \pm 0.01	18.25 \pm 0.58	28.37 \pm 0.98
Geothermal medium %50	8.08 \pm 0.74	3.26 \pm 0.72	0.11 \pm 0.01	24.52 \pm 0.51	38.35 \pm 0.88

Table 3. Bicarbonate cost for obtaining 1 kg *Spirulina*

<i>Spirulina</i> Mediums	HCO ₃ (kg/m ³)	Biomass kg/€
Schlösser 100%	12.8	3
Geothermal water 100%	0.28	0.07
Geothermal water 50%	5.4	1.35

highest protein, phycocyanin and dry biomass content among the groups were obtained with the Schlösser medium. ($p \leq 0.05$) (Table 2). In this experiment, the purity of the phycocyanin was achieved at 3.25 with 50% geothermal water. However, in the Schlösser medium, phycocyanin was obtained at an analytical purity of over 4. Schlösser medium is contained 13.6 g L^{-1} (Schlösser, 1994) of bicarbonate while the content Sanliurfa-Karaali in Turkey Geothermal Water 0.236 g L^{-1} (Çiftçi, 2015) comprising bicarbonate. In this research, with 50% geothermal water was obtained 38% protein, resulting in 8% phycocyanin (Table 2) and 1.27 g L^{-1} dry matter. In the group containing 100% geothermal nutrient medium, 48.35% protein was obtained, but the dry matter values ($p \leq 0.05$) were lower than the other groups. However, when dry matter values were taken into consideration, less biomass was produced than in the other groups (Table 2). In the Schlösser medium, 55% protein and 1.75 g L^{-1} *Spirulina* were provided in 16% phycocyanin. In the cost analysis (Table 3), 5.4 kg of HCO_3 was consumed to produce 1 kg *Spirulina* with %50 geothermal water, while in the control group, 1 kg

Spirulina was obtained with 12.84 kg HCO_3 . The highest phycocyanin concentration (0.24 mg mL^{-1}) was received with the Schlösser medium. In the group containing 50% geothermal water, 0.11 mg mL^{-1} phycocyanin concentration was obtained. The phycocyanin yield of the Schlösser medium was received at 54 mg g^{-1} , but geothermal groups reduced the phycocyanin yield.

In the study, the pBR322 plasmid DNA to detect the effects of the *Spirulina* samples in protecting the DNA against oxidative and UV induced damages. Three types of forms are seen in the plasmid DNA agarose gel imaging system. According to the separation state, these are Nicked DNA, Linear DNA, and Supercoiled DNA. The supercoiled structure is observed because only the plasmid loadings do not have DNA cleavage or opening. DNA protective activity is an indicator of this protective effect in band lustre up to the form of the band in evaluations. This study obtained band images by loading three different agarose gels. In Figure 5 (a), the DNA bands of *Spirulina* produced in the control groups and Schlösser media were visualized. Figure 5 (b) was shown that the band obtained by using geothermal

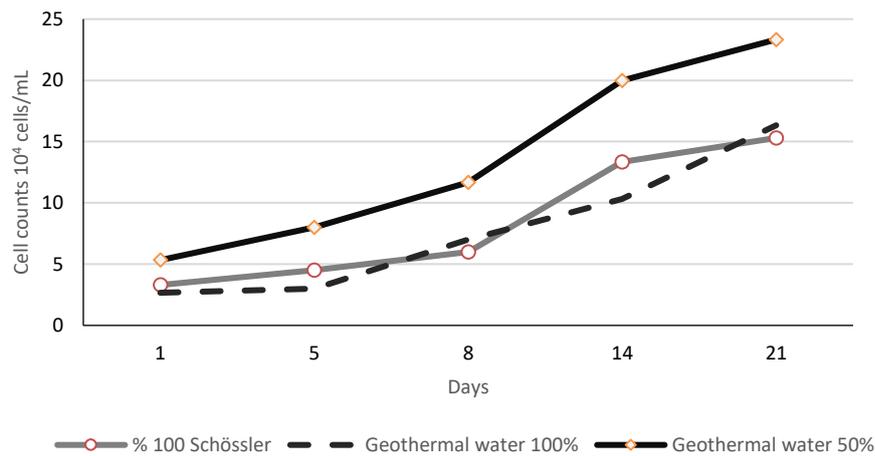


Figure 1. Cell concentration of *Arthrospira (Spirulina) platensis* grown in with Schlösser’s and geothermal media.

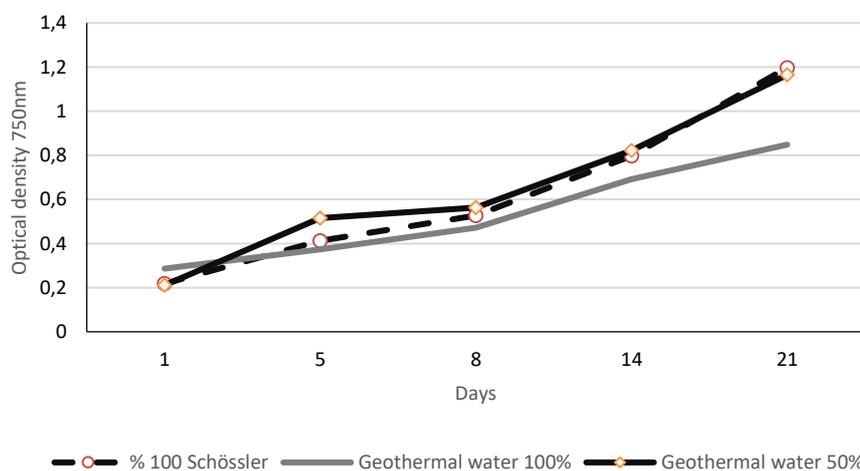


Figure 2. Optical density of *Arthrospira (Spirulina) platensis* grown in with Schlösser’s and geothermal media.

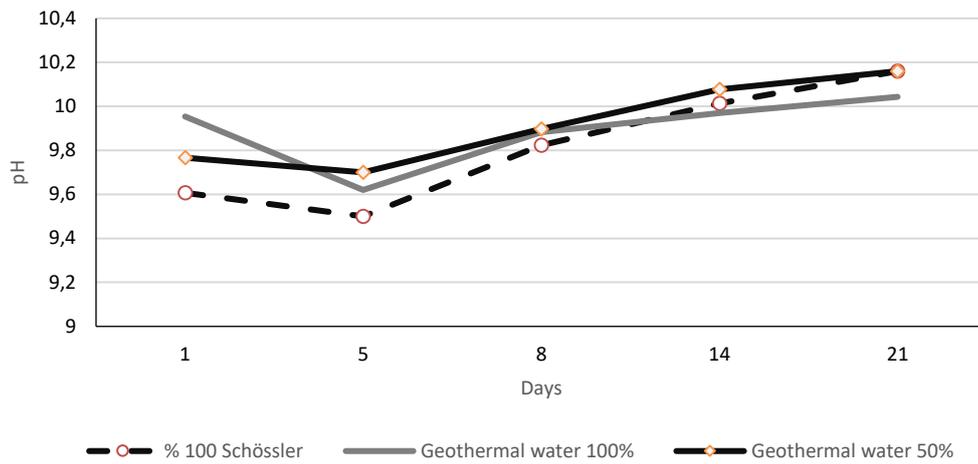


Figure 3. pH of *Arthrospira (Spirulina) platensis* grown in with Schlössler's and geothermal media.

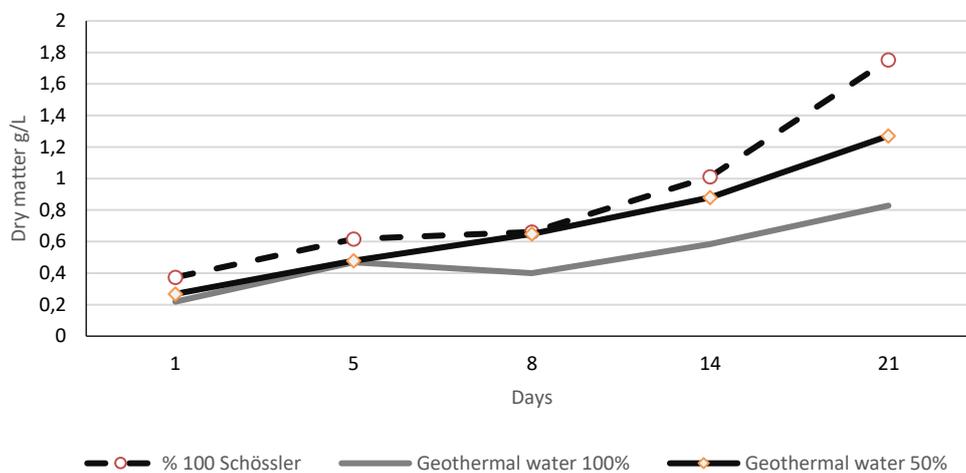


Figure 4. Dry matter of *Arthrospira (Spirulina) platensis* grown in with Schlössler's and geothermal media.

water at a rate of 50% and 100% is the band images.

DNA protection activities of producing at Schlössler medium (code of A1) and control group (code of K1 and K2) compare with different ratios of geothermal water (%50 A and %100 A2). The *Spirulina* produced in the Shössler medium shows DNA protective activity compared to the control groups, as shown in Fig. 5 (a). When the DNA band results in Fig. 5 (b) was examined, it was found that the A and A2 had higher activity than the Schlössler medium (A1). When we compare the bands in Figures 5 (b), it was seen that sample A2 produced in 100% geothermal water gives a better band appearance compared to 50% geothermal water.

Discussion

The purity of the phycocyanin is determined by the ratio of the absorbance at 620 nm to the absorbance at 280 nm (A620/A280). The application area of the phycocyanin is determined according to the obtained

purity ratio. According to this, it is accepted that phycocyanin has a purity ratio of 0.7 or more, suitable for food, 3.9 for reactive, and four or more for analytical purity (Kuddus et al., 2013). In this research, phycocyanin purity was achieved over three in the 50% geothermal water and the Schlössler medium. These results have shown the idea that geothermal waters are attractive for phycocyanin production. Phycocyanin obtained from *Spirulina* is sold in international markets for \$ 200-1800 per kg, depending on the degree of purity. Jespersen et al. (2005) reported that the ideal stability between the gardenia blues, phycocyanin (lina blue), and indigo blue pigments for use in food was provided by the phycocyanin. The phycocyanin content of the Schlössler medium (16%) was higher than the 50% geothermal water (8%) and the 100% geothermal water (4%). There has been reported that the *Spirulina* dry weight may include between 10-20% of phycocyanin (Vonshak, 1997; Chaiklahan et al., 2011). Even if a minimum of 10% of phycocyanin is obtained with *Spirulina*, the cost of phycocyanin is on average 180

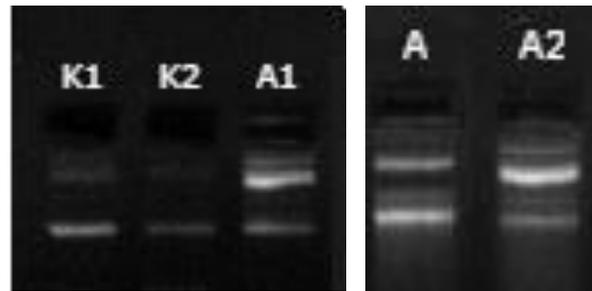


Figure 5 (a): Control and Shössler medium **(b):** % 50 (A) and %100 (A2) Şanlıurfa Karaali geothermal water

K1: Plasmid DNA (3 μ l) + distilled water (6 μ l)

K2: Plasmid DNA (3 μ l) + UV (5 minutes) + H₂O₂ (1 μ l)

A1: Plasmid DNA (3 μ l) + %100 Shössler 40 °C (5 μ l) + UV (5 minutes) + H₂O₂ (1 μ l)

A: Plasmid DNA (3 μ l) + % 50 Şanlıurfa Karaali geothermal water 40°C dried (5 μ l) + UV (5 minutes) + H₂O₂ (1 μ l)

A2: Plasmid DNA (3 μ l) + %100 Şanlıurfa Karaali geothermal (5 μ l) + UV (5 minutes) + H₂O₂ (1 μ l)

kg/€. The phycocyanin content of *Spirulina* varies depending on many factors. For instance, it is known that different light sources alter the C-PC productivity of *Spirulina platensis* (Prates et al., 2018) recommends a green LED to achieve maximum phycocyanin (126.39 mg/g). Ho et al. (2018) reported that the addition of 50% nitrate obtained 14% phycocyanin and 86.6 mg L⁻¹ phycocyanin concentrate. The highest phycocyanin concentration (240 mg L⁻¹) was achieved with the Schlösser medium, while in the group containing 50% geothermal water, 110 mg/L phycocyanin was obtained. This result is higher than the findings of Ho et al. (2018), although it is lower than the value found by Prates et al. (2018). In the control group, 54 mg/g phycocyanin yield was obtained, but geothermal water more reduced the yield of phycocyanin. Further research should be carried out on this issue since light and temperature on the phycocyanin are affected.

The highest protein (55%) and dry biomass (1.75 g L) content among the groups were obtained in the Schlösser medium. ($p \leq 0.05$). In this research, with 50% geothermal water was achieved 38% protein and 1.27 g L⁻¹ dry matter. In the group containing 100% geothermal nutrient medium, 28.37% protein was obtained, but the dry matter values ($p \leq 0.05$) were lower than the other groups. In Turkey, geothermal water obtained from the Armutlu region was evaluated for *Spirulina* production. In the summer period in the greenhouse, 1.286 g L⁻¹ dry matter content and 59% protein ratio were provided in 75% geothermal water substituted groups instead of Schlösser *Spirulina* medium in 500 L algal ponds (Guroy et al., 2018). In another research, geothermal water obtained from the Armutlu region was replaced with 20% distilled water, and the *Spirulina* medium was prepared. According to the results of studies examining the effects of different inoculation rates, the 1:6 inoculation rate provided the best development. In the group containing geothermal water, 48.42% protein and 22.48% phycocyanin content were detected, and it was reported that 61% protein was obtained with Schlösser medium (Guroy & Guroy, 2017).

Geothermal energy, together with economic benefits in the heating of photobioreactors and greenhouses, also reduces algal biomass drying costs (Godlewska et al., 2015). Cost analysis has shown that geothermal waters may be a low-cost alternative in *Spirulina* production when dry matter values are considered. Macro and microelements in geothermal waters can reduce the cost of commercial compounds added to the water environment to prepare *Spirulina* media. Geothermal water can be used directly or substituting these nutrient mediums depending on mineral content to achieve optimal algal productivity.

Detailed studies have been conducted on various medicinal plants extracts that control oxidative DNA damage causing cancers (Bayil Oguzkan et al., 2016). DNA damage can arise from the harmful effects of UV rays leading to a destruction of the stratosphere layer and adverse effects on living organisms. Moreover, UV rays can cause serious diseases, including skin cancer and skin ageing. The topical application of both enzymatic and non-enzymatic skin antioxidants is a practical approach to protect the skin against the harmful effects of UV rays (Hitoshi, 2010). For this reason, the use of *Spirulina platensis* as alternative products of the healthcare sector, especially in cosmetics and medicine, should be assessed by testing the protective activity on DNA. In this study, *Spirulina platensis* produced on geothermal water has been demonstrated to be an excellent protective activity, as shown in Fig. 5 (b). The literature also reported that it plays a critical role in antioxidants in eliminating the harmful effects of UV rays that cause DNA damage (André et al., 2017). Human skin has several mechanisms to reduce the detrimental impact of VIS (visible rays) and UV rays. However, high levels of exposure to UV rays can reduce the number of cellular antioxidants and ultimately lead to UV-induced oxidative DNA damage caused by reactive oxygen species (Tepe et al., 2011). In addition to UV rays, free radicals can cause DNA damage. Also, we have been demonstrated that how geothermal water affects *Spirulina* samples' DNA protective activities and

antioxidant levels. *Spirulina platensis* produced with different ratios of geothermal water has been the better properties to compare the Schlösser medium.

Conclusion

This study results have shown that geothermal waters can be used to grow *Spirulina (Arthrospira) platensis* and geothermal waters may be a low-cost alternative in *Spirulina* production when dry matter values are considered. Since the nutrient medium, light and temperature affect the *Spirulina* nutrient components and bioactive components, the investigations in this area should be increased.

Ethical Statement

Spirulina, used as an organism in this study, is a symbiotic, multicellular and filamentous blue-green microalga and is not subject to the experimental animal ethical committee review since it is not considered an experimental animal.

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Author Contribution

BG: Investigation, Methodology, Conceptualization, Writing -review and editing; SBO: Data Curation, Formal Analysis, and Writing -original draft; DG: Formal Analysis, Investigation, Writing -review and editing

Conflict of Interest

The authors declare that there were no conflicts of interest.

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