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Research Article

Geothermal water as a nutrient medium source on the biomass productivity and biochemical composition of Spirulina (*Arthrospira platensis*) under the greenhouse conditions

Betül Güroy[1,*], Derya Güroy[2], Serhan Mantoğlu[1], Onur Karadal[3] and Sibel Bayıl Oğuzkan[4]

[1] Department of Food Processing, Armutlu Vocational School, Yalova University, 77500 Armutlu, Yalova, Turkey
[2] Department of Aquaculture, Armutlu Vocational School, Yalova University, 77500 Armutlu, Yalova, Turkey
[3] Department of Aquaculture, Faculty of Fisheries, İzmir Katip Çelebi University, 35620 Çiğli, İzmir, Turkey
[4] Department of Medical Services and Techniques, Vocational School of Health Services, Gaziantep University, 27310 Sehitkamil, Gaziantep, Turkey

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Abstract – Spirulina (*Arthrospira platensis* Gomont, 1892) is grown in highly alkaline waters and is an important ingredient for many industries. However, analytical grade-based media in used commercial-scale production of Spirulina is quite expensive. Spirulina cultivation was studied by replacing the Schlösser medium with geothermal water in terms of its biomass, biochemical content, amino acid and mineral production. Spirulina was cultured in a semi-open raceway system with a 420 L culture volume for three weeks. Then, the geothermal water (GW) was replaced with 25% (GW25), 50% (GW50), 75% (GW75) and 100% (GW100) of the volume of the Schlösser culture medium (SM). The biomass concentration was determined to the higher in the GW50 (1.324 g/L) than in other groups (GW25, GW75, GW100 and SM), while the minimum yield was in GW100 (0.624 g/L) group. The highest protein content and phycocyanin purity ratio were found in the GW75 medium containing 116.6 times higher phosphate than the SM. The chlorophyll content of SM, GW25 and GW50 was higher than that of the GW100 ($P < 0.05$). The highest lipid content was determined in the GW100 ($P < 0.05$). Isoleucine, leucine and valine levels of the GW50 and GW75 were higher than those of the other groups ($P < 0.05$). The iron levels of the GW50 and GW75 groups, which have the highest nitrate content in the culture mediums, were significantly lower. The chlorophyll, phycocyanin, protein, dry biomass, mineral and amino acid in groups (GW25, GW50 and GW75) prepared by mixing Schlösser medium and geothermal water mix were better than geothermal water (GW100) and SM. As a result, it is concluded that it is possible to use partial geothermal water in Spirulina production under this study conditions.

Keywords: Microalgae / natural mineral water / potential culture mediums / bioactive components / biomass economic index

1 Introduction

Algae, which has superior properties thanks to the benefits offered to the human body than many foods or pharmaceuticals (Sabarinathan and Ganesan, 2008; Bhowmick *et al.*, 2020), is one of the sustainable foods that are easy to grow and consume. Algae can complement traditional agriculture in a more sustainable way for our planet. Because algae can convert carbon dioxide into organic matter, it has the potential to create a balanced ecosystem in the future. Algae cultivation will be an important field of work to meet the fuel and food that people will need future (Slate *et al.*, 2019; Torres-Tiji *et al.*, 2020). Besides, algae contain valuable bioactive and food components.

Microalgae have been an important economic activity for the renewable energy, food, pharmaceutical and feed industries because they contain valuable bioactive and food components. Spirulina (*Arthrospira platensis*) is one of the cyanobacterium (Cyanophyceae: Microcoleaceae) with the highest nutritional profile among all foods for humans and animals that are culturable in alkaline water using sunlight, water, inorganic nutrients and carbon dioxide (Capelli and Cysewski, 2010). It is sold as a highly bioavailable functional food due to its content of high-value materials such as protein, amino acids, fatty acids, vitamins and minerals. Spirulina is generally

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*Corresponding author: `betulguroy@yahoo.com.tr`

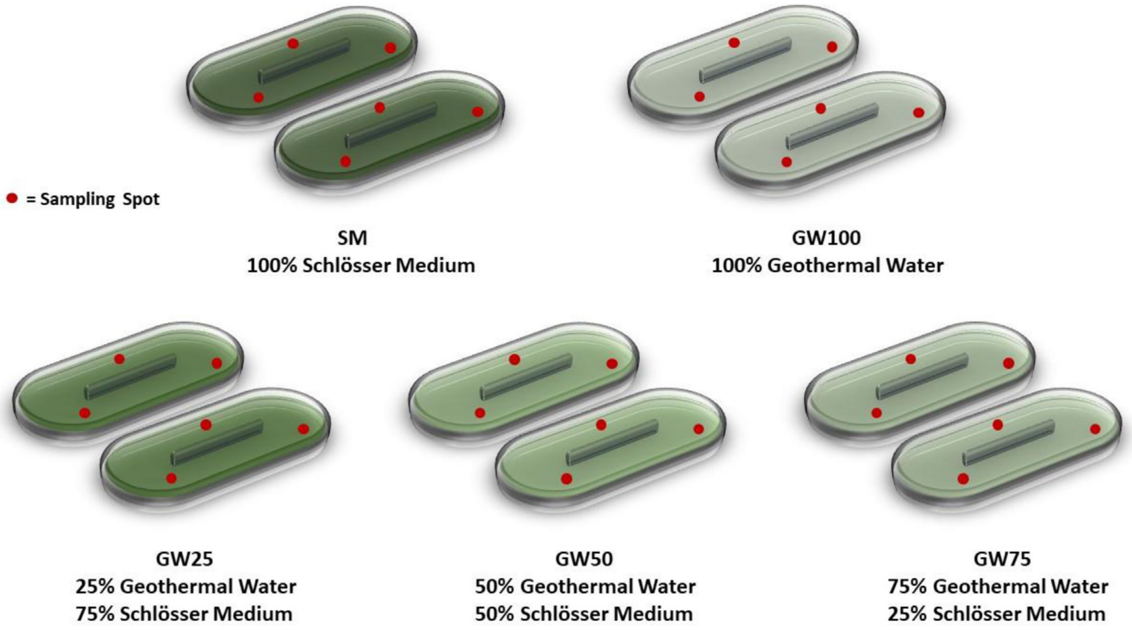

Fig. 1. Schema of the experimetal setup.

accepted as safe by the European Food Safety Authority (Chacón-Lee and González-Mariño, 2010). Spirulina is an important antioxidant and antimicrobial source for living organisms (Bhowmick *et al.*, 2020) due to its high-valued cell compounds such as pigments, polyphenols, Vitamin E and Vitamin D (Michael *et al.*, 2018; Bhowmick *et al.*, 2020; Lafarga *et al.*, 2020; Ragaza *et al.*, 2020). For this reason, the production amounts of Spirulina have increased globally in recent years (FAO, 2021).

Spirulina is successfully grown in a highly alkaline culture medium that minimises contamination. It is generally produced using Zarrouk's or Schlösser's *etc*. mediums (Zarrouk, 1966; Schlösser, 1982). However, these media are considerably expensive due to their agricultural-grade mineral salts. Providing an inexpensive nutrient medium sufficient to promote growth during cultivation is essential to the commercial spread of Spirulina worldwide. Recent research on Spirulina cultivation has focused on increasing productivity or reducing production costs without reducing its nutrient content (Soni *et al.*, 2017). The culture medium or alternative materials such as seawater, natural mineral water, manure water and industrial wastes are successfully used to reduce the production cost of Spirulina (Sandeep *et al.*, 2013; Dineshkumar *et al.*, 2016; Trinh and Nguyen, 2020). One of the methods to reduce the cost of Spirulina production could be the use of geothermal water resources (Godlewska *et al.*, 2015; Trinh and Nguyen, 2020). Geothermal resources are used in many different fields such as heating, electricity generation, tourism and agriculture (Lund *et al.*, 2005, 2011; Godlewska *et al.*, 2015). The water from geothermal sources is naturally rich in minerals. These waters contain minerals that can be applied to produce microalgae at a high rate. Geothermal water can reduce the cost of mineral salts in Spirulina's production in the algae culture medium preparation. The integration of microalgae production technologies into geothermal resources around the world is seen as an essential step. Therefore, the present study aimed to evaluate the effect of different geothermal water levels as alternative culture media on the growth performance, biochemical content, amino acid and minerals profiles in Spirulina (*A. platensis*).

2 Materials and methods

2.1 Geothermal water and Spirulina culture conditions

The study was carried out in Algae Culture Unit, Armutlu Vocational School, Yalova University, Yalova, Turkey. Geothermal water was delivered from the thermal springs in Armutlu, Yalova, with the contributions of Armutlu Municipality. The axenic culture of Spirulina (*A. platensis*) was obtained from UTEX 3086. The cultures were cultivated pre-trial and stocked in fiberglass race-way type algae tanks with 2000 lx illuminance and photoperiod of 12:12 (light:dark) of fluorescents. The temperature was recorded as 26.5 ± 1.1 °C during the study. Initially, the axenic culture of Spirulina was inoculated in an 80 L plexiglass algae tank containing Schlösser's (1982) medium. After that, secondary culture with scale-up was performed into four 500 L fiberglass algae tanks in duplicate when the culture concentration of *A. platensis* reached 0.8–0.9 g/L. Spirulina samples were taken from three different regions of each algae tank and six samples ($n = 6$) were used for the experimental analysis (Fig. 1). This culture was used as a stock culture for experiments at the 420 L scale.

2.2 Experimental design

Schlösser's (1982) medium was used as the standard control medium (SM). The constituents presented in Schlösser's medium were substituted with geothermal water to prepare the new media. As the experimental groups, the geothermal water was replaced with 25% (GW25), 50% (GW50), 75% (GW75) and 100% (GW100) of the volume of the Schlösser culture medium (Fig. 1). The nutrient mediums of all culture groups were designed as 360 L. Spirulina strain (60 L) was inoculated at 1:6 of the volume of prepared nutrient medium. The initial mean biomass yield of the Spirulina medium was measured as 0.19 ± 0.03 g/L. The culturing of all groups was prepared with a 420 L culture volume. Spirulina culture tanks were provided with a 4500 L minimum water capacity submersible pump (Aqua Magic WP6000). The growth of culture was measured three times a week spectrophotometrically (A_{750}) (Hach Lange DR 2800), culture conditions (pH, temperature and illuminance) and greenhouse conditions (Fig. 2) followed for three weeks in June.

2.3 Spirulina harvesting

The biomass concentration was estimated by sampling and filtering 20 mL of each culture using a vacuum pump and GF/C filter paper (Whatman) before harvesting. The filter paper was dried in a dry oven (75 °C) for 4–6 h, followed by biomass measurement. At the end of the trial, Spirulina was harvested with 45 μ plankton mesh, washed with tap water and filtered to obtain fresh Spirulina. Fresh Spirulina was dried in a spray dryer (Shanghai Pilotech Instrument & Equipment Co. Ltd. YC-015 model spray dryer with 1500 mL/h capacity).

2.4 Analyses

2.4.1 Water analysis

Physicochemical water analyses for all culture mediums (Tab. 1) were carried out in the Department of Medical Ecology and Hydroclimatology, İstanbul Faculty of Medicine, İstanbul University, Turkey. The concentrations reported in Table 1 were freshly prepared. It is the analysis of nutrient media obtained by replacing 25%, 50%, 75% and 100% of Schössler nutrient media with geothermal water. The concentrations reported in this table were determined as a result of the analysis of freshly prepared nutrient media. Before *A. spirulina* inoculation, Schlösser medium as a control group prepared in the laboratory and physicochemical content of experimental nutrient media containing geothermal water were determined. Physicochemical, anion, cation, insoluble matter and trace element analysis of all culture mediums were performed according to Standard Methods for the Examination of Water and Wastewater (Clesceri *et al.*, 1989).

2.4.2 Spirulina productivity

The harvested culture was filtered through the filter paper, weighed and dried at 75 °C for 4–6 h. The biomass efficiency was calculated according to the difference between the first and last weight after the filter paper was cooled (Jain and Singh, 2012). Chlorophyll-a (mg/g) was estimated by the procedure and equation suggested by Parsons and Strickland (1965). Optical density was determined by absorbance measurement at 750 nm on a spectrophotometer. Phycocyanin yield (mg/g) was determined according to Moraes *et al.* (2011). The phycocyanin purity ratio ($A_{620/280}$) was determined according to Abalde *et al.* (1998).

2.4.3 Economic analysis

In calculating the media cost, the prices of all the components that make up the media have been calculated by taking into account the local chemical companies (*i.e.*, Türkiye Sisecam, Gübretas, Akdas Kimya). The geothermal water price (0.50 €/m^3) was listed from Armutlu Municipality. However, biomass economic index (BEI) was assessed with the formula below:

BEI (€/kg dry Spirulina) = 1000 × Biomass yield × Medium cost (€/g).

2.4.4 Proximate analysis

Analysis of the chemical compositions in the Spirulina was performed according to standard procedures of AOAC (2000). Dry matter was determined by drying at 105 °C until a constant weight was obtained. Ash content was determined by burning in a muffle furnace at 525 °C for 12 h. Crude protein (N*6.25) was analysed by the Kjeldahl method after acid digestion using the Gerhardt system. According to Folch *et al.* (1957), crude lipid was extracted with chloroform/methanol (2:1 v/v).

2.4.5 Amino acid analysis

60 mg of Spirulina was hydrolysed in 10 mL of 6 M HCl in screw-capped tubes to analyse amino acids. The tubes were flushed with N and then heated at 110 °C for 24 h. The hydrolysates were rotary-evaporated to dryness under vacuum at 40 °C and then re-dissolved in a sodium citrate buffer at pH 2.2. The amino acids were separated by ion-exchange chromatography on a sodium column and detected following post-column derivatisation with ninhydrin by measuring absorbance at 350–450 nm. Identification and quantification of the detected amino acid were performed using external standards after adjustments by linear regression. The standard amino acid was purchased from Sigma-Aldrich Co, the USA, as a synthetic mixture of AAs.

2.4.6 Mineral analysis

The concentrations of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe), zinc (Zn), copper (Cu), selenium (Se) and manganese (Mn) in the Spirulina biomass were conducted according to AOAC (2000). The individual minerals of the prepared samples were determined using Atomic Absorption Spectrophotometer (Thermo iCE 3000 AAS).

2.5 Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA), after proving the normality and homogeneity of the data, followed by Duncan's multiple range test for analysing group means by using Statgraphics Centurion XVI (Statpoint Technologies Inc., The Plains, VA) statistical software

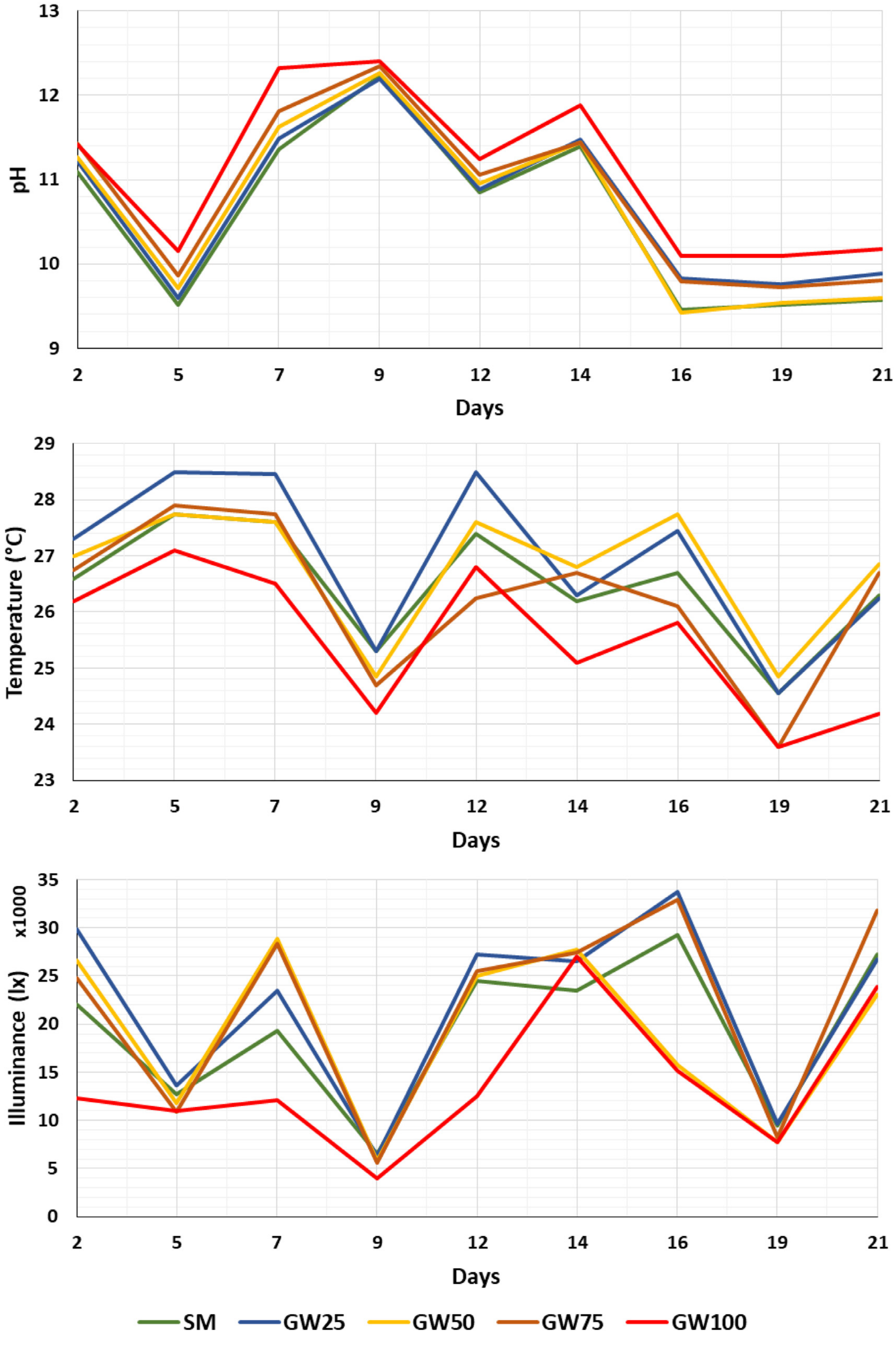

Fig. 2. Temperature (°C), pH, and illuminance (lx) of the experimental Spirulina culture mediums under the greenhouse conditions during the 21 days.

Table 1. Physicochemical properties of experimental mediums.

		SM	GW25	GW50	GW75	GW100
Physical parameters						
Turbidity	NTU	2.00	13.10	18.40	19.10	17.90
Physicochemical parameters						
pH	pH	9.27	9.14	8.93	8.57	8.23
Electrical Conductivity	EC (μS/cm)	21.75	17.27	12.27	7.54	2.83
Density	Density (g/cm^3)	1.02	1.01	1.01	1.00	1.00
Chemical parameters						
Salinity	Salinity (‰)	13.00	10.10	7.20	4.20	1.50
Hardness	Hardness (Fr°)	13.60	17.40	18.90	21.60	83.60
Anions (mg/L)						
Carbonate	CO_3^{-2}	558.00	393.00	246.00	65.40	184.80
Bicarbonate	HCO_3^-	14945.00	11437.50	7442.00	3464.80	579.50
Nitrate	NO_3^-	66.00	70.40	162.80	250.80	18.92
Nitrite	NO_2^-	0.89	0.09	0.06	0.07	0.03
Sulphate	SO_4^{-2}	640.00	630.00	680.00	620.00	840.00
Phosphate	HPO_4^{-2}	0.09	10.00	0.19	10.50	0.74
Chloride	Cl^-	760.40	622.48	506.94	435.04	256.23
Fluoride	F^-	18.70	17.70	5.75	3.30	3.25
Bromide	Br^-	3.00	3.70	3.00	2.80	1.90
Iodide	I^-	0.41	0.02	0.02	0.03	0.02
Cations (mg/L)						
Sodium	Na^+	6322.25	4919.86	3149.63	1839.20	390.83
Potassium	K^+	136.85	136.85	113.39	91.89	11.73
Magnesium	Mg^{+2}	27.35	37.68	40.72	46.80	196.91
Calcium	Ca^{+2}	9.32	7.46	8.39	9.32	10.25
Manganese	Mn^{+2}	0.01	0.02	0.01	0.02	0.23
Iron	Fe^{+2}	2.20	0.53	0.22	0.09	0.31
Insoluble Matters						
Metasilicic acid	H_2SiO_3	3.90	2.60	22.09	83.17	116.96
Total Mineralization		22936.00	17891.60	11635.20	6857.82	2427.81
Trace Elements						
Arsenic	$H_3AsO_4^{-2}$	0	0	0	0	0
Cadmium	Cd^{+2}	0.0002	0.0011	0.0009	0.0007	0.012
Chromium	Cr^{+3}	0.02	0.01	0.01	0.01	0.02
Mercury	Hg^{+2}	0	0	0	0	0
Nickel	Ni^{+2}	0.003	0.002	0.001	0.005	0.004
Lead	Pb^{+2}	0.042	0.029	0.033	0.021	0.009
Antimony	Sb^{+5}	0	0	0	0	0
Selenium	Se^{-2}	0.5	0.01	0	0	0
Barium	Ba^{+2}	<2	<2	<2	<2	<2
Copper	Cu^{+2}	0.10	0.21	0.02	0.12	0.24
Zinc	Zn^{+2}	0.18	0.20	0.28	0.36	0.24
Aluminum	Al^{+3}	0	0	0	0	0
Molybdenum	Mo^{+6}	0	0	0	0	0
Argent	Ag^+	0	0	0	0	0
Cyanide	CN^-	0.012	0.003	0.002	0.004	0.004

(Zar, 1999). The correlation between the physico-chemical properties of the culture medium and the chemical contents of Spirulina was analysed by using Statgraphics Centurion XVI. Samples were taken each trial, and all analyzes were performed on six sampling. All means were presented with standard errors (±SE). Differences were considered significant at the 95% confidence interval.

3 Results

3.1 Optical density and biomass yield

The growth of the culture was evaluated according to the optical density. The biomass yield of the GW100 was significantly lowest among the other groups ($P < 0.05$),

Table 2. Biomass yield and biomass economic index of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	SM	GW25	GW50	GW75	GW100
Biomass yield (g/L)	1.14 ± 0.14^{b}	1.29 ± 0.13^{b}	1.32 ± 0.02^{b}	1.29 ± 0.01^{b}	0.62 ± 0.05^{a}
Medium cost (€/L)	0.0150	0.0114	0.0059	0.0019	0.0005
Biomass economic index (€/kg)	17.10 ± 2.10^{c}	14.70 ± 1.54^{c}	7.80 ± 0.14^{b}	2.40 ± 0.01^{a}	0.30 ± 0.02^{a}

The data was analyzed using one-way ANOVA. Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups.

Table 3. Proximate composition of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	SM	GW25	GW50	GW75	GW100
Protein (%)	53.15 ± 0.74^{a}	58.48 ± 1.10^{b}	52.90 ± 0.54^{a}	59.08 ± 0.20^{b}	54.15 ± 0.38^{a}
Lipid (%)	1.46 ± 0.02^{a}	1.89 ± 0.04^{b}	1.68 ± 0.03^{ab}	1.46 ± 0.09^{a}	2.80 ± 0.16^{c}
Ash (%)	9.66 ± 0.58^{a}	9.82 ± 1.13^{a}	11.27 ± 0.62^{ab}	11.74 ± 0.21^{ab}	13.36 ± 0.30^{b}

The data was analyzed using one-way ANOVA. Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups.

while SM, GW25, GW50 and GW75 were statistically similar (Tab. 2). The water hardness, sulfate, magnesium and manganese contents of the GW100 group were very high compared to other groups. This situation draws attention as a factor that negatively affects biomass yield, as seen in the correlation analysis (Tab. 3).

3.2 Economic analysis

The cost of the Schlösser medium used in the production of Spirulina was calculated as 0.0150 €/L (Tab. 2). The medium cost was reduced by geothermal water substitution instead of Schlösser medium. The biomass economic index (BEI) was calculated by considering the cost of the medium required to obtain 1 kg of dry Spirulina. The BEI decreases with the increase in geothermal water level in the experimental groups. The BEI of the SM and GW25 was statistically higher than the other groups ($P < 0.05$).

3.3 Biochemical analysis

The biochemical composition of all groups was presented in Table 3. The correlation between the physicochemical content of the culture medium and biochemical values of Spirulina was given in Table 4. The lowest protein content was determined in SM, GW50 and GW100 groups, while the GW25 and GW75 were the highest ($P < 0.05$). The phosphate content in GW25 and GW75 groups was higher than in the other groups ($P < 0.05$). Correlation results also indicated that protein content positively affected groups with high phosphate content. The highest lipid contents were determined in the GW100 and SM and GW75 were lower than GW25 ($P < 0.05$). The hardness, sulfate, magnesium, manganese and cadmium content of the GW100 group was a factor that increased the lipid level of Spirulina. An increase in the amount of geothermal in the nutrient medium caused the high-level magnesium in the culture media. Increasing the hardness of the culture water compared to the SM caused an increase in the lipid obtained. The ash content of Spirulina increased with the level of geothermal water, and the GW100 was significantly higher than that of the SM and GW25 groups ($P < 0.05$). An increase in magnesium and silicate acid was determined with the rise in the geothermal water component in the nutrient media. The amount of silicate acid and magnesium, together with cadmium, manganese and sulfate, were decisive factors for increasing the Spirulina ash content obtained at the end of the experiment.

3.4 Chlorophyll and phycocyanin analysis

Pigment amount and correlation between physicochemical content of the culture medium and pigments were given in Tables 5 and 6, respectively. The chlorophyll content of SM, GW25 and GW50 was higher than GW100 ($P < 0.05$). When the correlation analysis was examined, the increase in bromide and potassium increased chlorophyll-a level, as seen in the GW25 group, where the highest chlorophyll was obtained. In the GW100 group, high sulfate, calcium and magnesium decreased Spirulina's chlorophyll content. Increasing the amount of cadmium and manganese in the nutrient media is among the factors that adversely affect chlorophyll. The phycocyanin yield of Spirulina obtained from the experiment groups ranged between 80.19 mg/g and 93.69 mg/g, and no significant difference was found between the groups ($P > 0.05$). It has been determined that the phycocyanin purity rate taken from all groups is at a value that can be used in food grade. The purity ratio of phycocyanin selected from the GW75 group was the highest among the other groups ($P < 0.05$). When the correlation analyses were examined, nitrate in the culture medium had a first-degree effect on the purity ratio of phycocyanin.

Table 4. Correlation between nutrient salts in culture mediums and biochemical content of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	Biomass yield	Protein	Lipid	Ash
HCO_3^- (mg/L)	0.52	–0.15	–0.60	–0.96*
pH	0.67	–0.10	–0.68	–0.98*
EC (μS/cm)	0.56	–0.13	–0.64	–0.97*
Hardness (Fr°)	–0.95*	–0.21	0.94*	0.85*
NO_3^- (mg/L)	0.66	0.43	–0.66	0.01
SO_4^{-2} (mg/L)	–0.92*	–0.45	0.93*	0.80*
Cl^- (mg/L)	0.61	–0.08	–0.72	–0.98*
Br^- (mg/L)	0.84*	0.37	–0.65	–0.88*
I^- (mg/L)	0.03	–0.45	–0.41	–0.56
HPO_4^{-2} (mg/L)	0.44	0.99*	–0.27	–0.18
CO_3^{-2} (mg/L)	0.13	–0.38	–0.25	–0.78
Total Mineralization	0.52	–0.13	–0.60	–0.97*
H_2SiO_3	–0.70	0.11	0.63	0.95*
Na^+ (mg/L)	0.54	–0.11	–0.62	–0.97*
K^+ (mg/L)	0.86*	0.13	–0.83*	–0.96*
Mg^{+2} (mg/L)	–0.95*	–0.20	0.95*	0.85*
Ca^{+2} (mg/L)	–0.76	–0.34	0.42	0.69
Mn^{+2} (mg/L)	–0.97*	–0.24	0.95*	0.80*
Fe^{+2} (mg/L)	–0.01	–0.43	–0.33	–0.62
Cd (mg/L)	–0.95*	–0.23	0.96*	0.81*
Zn (mg/L)	0.23	0.40	–0.20	0.50
Cu (mg/L)	–0.64	0.39	0.72	0.30
Ni (mg/L)	–0.40	0.42	0.13	0.47
Cr (mg/L)	–0.78	–0.58	0.45	0.20

The data was analyzed using correlation analysis. The asterisks indicate strong positive (0.8 and above) or negative (−0.8 and below) correlations between the parameters.

Table 5. Pigments of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	SM	GW25	GW50	GW75	GW100
Chlorophyll (mg/g)	21.08 ± 0.12^b	25.09 ± 0.09^b	22.97 ± 0.14^b	17.24 ± 0.08^{ab}	9.17 ± 0.11^a
Phycocyanin yield (mg/g)	83.57 ± 0.27	93.70 ± 0.35	92.96 ± 0.29	80.20 ± 0.41	92.50 ± 0.48
Phycocyanin purity ratio ($A_{620/280}$)	1.33 ± 0.09^a	1.49 ± 0.19^b	1.55 ± 0.21^b	1.75 ± 0.08^c	1.31 ± 0.14^a

The data was analyzed using one-way ANOVA. Different letters in the same line indicate statistically significant differences ($P<0.05$) among the experimental groups.

3.5 Amino acids

The essential amino acid content of experimental groups was listed in Table 7. The correlation relationship between the physicochemical content of the culture medium and the amino acid profiles of Spirulina was given in Table 8. The isoleucine, leucine, methionine and threonine levels of Spirulina increased with the culture medium's nitrate and zinc content. Isoleucine, leucine, threonine and valine contents of Spirulina were found high in the GW50 and GW75 groups with the lowest carbonate value in the culture medium ($P < 0.05$). Arginine of the GW100 and phenylalanine of the SM and GW25 were significantly the lowest amongst the experimental groups ($P < 0.05$). The lysine of the GW75 and GW100 were higher than GW50 ($P < 0.05$). Methionine of the GW75 was significantly highest in all of the other groups ($P < 0.05$).

3.6 Minerals

The replacement of the Schlösser culture medium with different geothermal waters changed Spirulina's mineral content (Tab. 9). The sodium and potassium contents of the SM and GW100 groups, which have the lowest nitrate content in the culture medium, were significantly highest among all other groups ($P < 0.05$). The magnesium, calcium and manganese contents of Spirulina were the highest in the GW100 ($P < 0.05$). The phosphorus level of the GW50 group was significantly lower than GW100 ($P < 0.05$). The iron

Table 6. Correlation between nutrient salts in culture mediums and pigments of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	Chlorophyll	Phycocyanin yield	Phycocyanin purity
HCO_3^- (mg/L)	0.79	–0.07	–0.24
pH	0.90*	–0.03	–0.08
EC (µS/cm)	0.80*	–0.10	–0.19
Hardness (Fr°)	–0.90*	0.34	–0.47
NO_3- (mg/L)	0.23	–0.56	0.95*
SO_4-^2 (mg/L)	–0.81*	0.47	–0.58
Cl– (mg/L)	0.80*	–0.22	–0.10
Br– (mg/L)	0.96*	0.06	0.31
I– (mg/L)	0.18	–0.46	–0.47
HPO_4-^2 (mg/L)	0.25	–0.25	0.68
CO_3-^2 (mg/L)	0.51	0.10	–0.62
Total Mineralization	0.78	–0.10	–0.23
$H_2 SiO_3$	–0.94*	–0.12	0.06
Na^+ (mg/L)	0.79	–0.11	–0.20
K^+ (mg/L)	0.95*	–0.17	0.24
Mg^{+2} (mg/L)	–0.90*	0.34	–0.47
Ca^{+2} (mg/L)	–0.90*	–0.38	–0.31
Mn^{+2} (mg/L)	–0.88*	0.35	–0.54
Fe^{+2} (mg/L)	0.23	–0.32	–0.57
Cd (mg/L)	–0.87*	0.39	–0.53
Zn (mg/L)	–0.24	–0.41	0.84*
Cu (mg/L)	–0.49	0.26	–0.38
Ni (mg/L)	–0.69	–0.68	0.19
Cr (mg/L)	–0.58	–0.08	0.84*

The data was analyzed using correlation analysis. The asterisks indicate strong positive (0.8 and above) or negative (–0.8 and below) correlations between the parameters.

Table 7. Amino acid profile of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	SM	GW25	GW50	GW75	GW100
Arginine	3.65^b	3.07^b	3.93^b	3.97^b	1.28^a
Phenylalanine	3.99^a	4.15^a	5.36^b	5.56^b	5.57^b
Lysine	4.07^{ab}	3.93^{ab}	3.24^a	4.45^b	5.04^b
Isoleucine	5.06^a	5.27^a	6.66^b	7.39^b	5.50^a
Leucine	8.10^a	7.88^a	10.06^b	11.38^b	8.90^a
Methionine	0.13^a	0.07^a	0.21^a	0.91^b	0.21^a
Threonine	1.14^a	0.83^a	1.43^b	1.93^b	0.82^a
Tryptophan	2.55^a	2.56^a	3.65^{ab}	4.10^b	3.51^{ab}
Valine	6.22^a	6.70^a	9.07^b	7.98^b	5.98^a

The data was analyzed using one-way ANOVA. Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups.

levels of the GW50 and GW75, which have the highest nitrate content in the culture medium, were significantly lower than that of all other groups ($P < 0.05$). The zinc content of the GW100 was substantially higher than SM ($P < 0.05$). The copper level of the GW25 was significantly highest among the experimental groups ($P < 0.05$). There is no statistical difference in the selenium between the groups ($P > 0.05$). Strong correlations were found in different mineral contents with various nutrient salts, except copper (Tab. 10).

4 Discussion

The differences in nutrient source and culture medium level limit microalgae's intensive growth and change their pigment and biochemical composition (Mostert and Grobbelaar, 1987). The study results showed that Spirulina's production efficiency and biochemical composition were positively affected when the geothermal water was partially replaced instead of the Schlösser medium (Schlösser, 1982). This study obtained the lowest

Table 8. Correlation between nutrient salts in culture mediums and amino acid profile of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	Arginine	Phenylalanine	Lysine	Isoleucine	Leucine	Methionine	Threonine	Tryptophan	Valine
Bicarbonate (HCO_3^-)	–0.06	–0.93*	–0.57	–0.52	–0.60	–0.50	–0.21	–0.82*	–0.13
pH	0.62	–0.86*	–0.71	–0.37	–0.48	–0.44	–0.10	–0.73	0.06
EC (µS/cm)	0.54	–0.92*	–0.58	–0.48	–0.56	–0.47	–0.16	–0.80*	–0.10
Hardness (Fr°)	–0.93*	0.53	0.76	–0.18	–0.06	–0.08	–0.43	0.27	–0.46
NO_3^-	0.74	0.43	–0.30	0.93*	0.89*	0.83*	0.96*	0.68	0.79
SO_4^{-2}	–0.89*	0.49	0.57	–0.24	–0.13	–0.26	–0.49	0.21	–0.37
Cl^-	0.62	–0.89*	–0.57	–0.39	–0.46	–0.35	–0.05	–0.73	–0.06
Br^-	0.64	–0.68	–0.70	–0.10	–0.26	–0.21	0.03	–0.52	0.25
I^-	0.25	–0.66	–0.07	–0.50	–0.43	–0.28	–0.09	–0.58	–0.40
HPO_4^{-2}	0.25	–0.04	0.10	0.34	0.26	0.52	0.30	0.11	0.10
CO_3^{-2}	0.12	–0.93*	–0.33	–0.80*	–0.83*	–0.74	–0.53	–0.94*	–0.45
Total Mineralization	0.50	–0.94*	–0.54	–0.52	–0.59	–0.48	–0.20	–0.82*	–0.15
H_2SiO_3	–0.60	0.81*	0.80*	0.35	0.47	0.50	0.14	0.70	–0.15
Na^+	0.52	–0.93*	–0.56	–0.49	–0.57	–0.46	–0.18	–0.81*	–0.13
K^+	0.79	–0.73	–0.76	–0.01	–0.23	–0.18	0.16	–0.53	0.27
Mg^{+2}	–0.93*	0.53	0.76	–0.18	–0.06	–0.08	–0.43	0.27	–0.46
Ca^{+2}	–0.47	0.49	0.74	0.06	0.24	0.30	0.09	0.41	–0.35
Mn^{+2}	–0.95*	0.45	0.76	–0.27	–0.15	–0.15	–0.50	0.18	–0.53
Fe^{+3}	0.16	–0.77	–0.08	–0.65	–0.60	–0.43	–0.27	–0.72	–0.50
Cd^{+2}	–0.95*	0.47	0.73	–0.25	–0.13	–0.16	–0.50	0.20	–0.49
Zn^{+2}	0.31	0.80*	0.07	0.98*	0.98*	0.90*	0.85*	0.94*	0.66
Cu^{+2}	–0.81*	–0.07	0.77	–0.48	–0.43	–0.16	–0.62	–0.24	–0.78
Ni^{+2}	–0.24	0.37	0.84*	0.27	0.40	0.71	0.34	0.42	–0.35
Cr^{+3}	–0.58	–0.17	0.56	–0.63	–0.48	–0.36	–0.49	–0.32	–0.76

The data was analyzed using correlation analysis. The asterisks indicate strong positive (0.8 and above) or negative (–0.8 and below) correlations between the parameters.

Table 9. Mineral profile of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	SM	GW25	GW50	GW75	GW100
Na (Sodium)	1.62^{b}	1.05^{a}	1.00^{a}	0.78^{a}	1.44^{b}
K (Potassium)	1.50^{b}	1.39^{a}	0.92^{a}	0.98^{a}	1.54^{b}
Mg (Magnesium)	0.23^{a}	0.21^{a}	0.14^{a}	0.20^{a}	0.38^{b}
Ca (Calsium)	0.13^{a}	0.11^{a}	0.13^{a}	0.10^{a}	2.61^{b}
P (Phosphorus)	0.69^{ab}	0.60^{ab}	0.38^{a}	0.55^{ab}	0.91^{b}
Fe (Iron)	668.02^{b}	615.06^{b}	466.55^{a}	405.42^{a}	565.37^{b}
Zn (Zinc)	28.29^{a}	44.61^{ab}	34.80^{ab}	42.18^{ab}	86.40^{b}
Cu (Copper)	6.17^{a}	14.67^{b}	3.83^{a}	4.15^{a}	6.39^{a}
Se (Selenium)	27.92	27.24	27.52	27.69	28.13
Mn (Manganese)	31.00^{a}	34.21^{a}	24.70^{a}	27.64^{a}	63.22^{b}

The data was analyzed using one-way ANOVA. Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the experimental groups.

biomass yield in the GW100 group, with the highest hardness content and the most deficient potassium and bromide level. The GW50 group achieved a 1.32 g/L biomass yield and was 9.6 times more abundant in potassium than the GW100 group. Potassium is a vital nutrient mineral that contributes to photosynthesis and phytochemicals long-distance transport. The physiological and biochemical responses lead to decreased photosynthetic carbon assimilation when the potassium is insufficient in cells (Tränkner *et al.*, 2018). In the study, the biomass yield of Spirulina declined in the culture strains that had an inadequate amount of potassium. The sulfate, magnesium and manganese above the levels required for the effectiveness of the culture may have an effect that limits the full benefit of the minerals in the nutrient medium. As a remarkable point, the high sulfate content in the GW100 affects biomass yield negatively; however, the highest biomass yield in the GW50 group was obtained with the most elevated sulfate among the other geothermal groups. There is no statistical difference between the other groups regarding biomass yield, except for the GW100 group. While these results indicate the limiting effects of very

Table 10. Correlation between nutrient salts in culture mediums and mineral profile of Spirulina cultured in the Schlösser's medium and in the different ratios of geothermal water.

	Na	K	Mg	Ca	P	Fe	Zn	Cu	Se	Mn
Bicarbonate (HCO_3^-)	0.33	0.22	–0.49	–0.67	–0.28	0.65	–0.55	0.39	–0.39	–0.55
pH	0.14	0.03	–0.65	–0.78	–0.47	0.50	–0.68	0.37	–0.55	–0.68
EC (μS/cm)	0.29	0.18	–0.53	–0.70	–0.32	0.61	–0.57	0.37	–0.41	–0.59
Hardness (Fr°)	0.34	0.45	0.91*	0.99*	0.78	0.02	0.83*	–0.11	0.67	0.96*
NO_3^-	–0.82*	–0.90*	–0.65	–0.58	–0.71	–0.85*	–0.49	–0.48	–0.32	–0.69
SO^{-2}_4	0.44	0.42	0.83*	0.97*	0.68	0.08	0.66	–0.17	0.67	0.91*
Cl^-	0.23	0.11	–0.57	–0.76	–0.35	0.54	–0.60	0.31	–0.40	–0.65
Br^-	–0.34	–0.21	–0.75	–0.85*	–0.63	0.19	–0.60	0.58	–0.89*	–0.73
I^-	0.71	0.43	–0.04	–0.25	0.17	0.63	–0.23	–0.12	0.35	–0.20
HPO_4^{-2}	–0.71	–0.25	–0.23	–0.38	–0.21	–0.31	0.13	0.47	–0.58	–0.27
CO_3^{-2}	0.67	0.56	–0.12	–0.30	0.08	0.89*	–0.26	0.42	–0.09	–0.17
Total Mineralization	0.33	0.23	–0.48	–0.67	–0.27	0.65	–0.53	0.40	–0.38	–0.54
H_2SiO_3	–0.07	0.04	0.69	0.77	0.54	–0.46	0.72	–0.41	0.64	0.68
Na^+	0.30	0.20	–0.50	–0.69	–0.29	0.63	–0.54	0.39	–0.40	–0.57
K^+	–0.15	–0.22	–0.81*	–0.93*	–0.65	0.24	–0.76	0.31	–0.69	–0.85*
Mg^{+2}	0.34	0.45	0.91*	0.99*	0.77	0.02	0.83*	–0.10	0.67	0.96*
Ca^{+2}	0.44	0.28	0.70	0.69	0.66	–0.09	0.55	–0.63	0.97*	0.60
Mn^{+2}	0.42	0.53	0.94*	1.00*	0.82*	0.11	0.84*	–0.06	0.68	1.00*
Fe^{+3}	0.76	0.54	0.01	–0.22	0.23	0.77	–0.17	0.06	0.27	–0.13
Cd^{+2}	0.38	0.50	0.92*	1.00*	0.79	0.09	0.83*	–0.05	0.65	0.97*
Zn^{+2}	–0.78	–0.79	–0.24	–0.10	–0.38	–0.98*	–0.09	–0.58	–0.03	–0.25
Cu^{+2}	0.24	0.72	0.80*	0.64	0.80	0.38	0.92*	0.61	0.20	0.78
Ni^{+2}	–0.03	0.15	0.52	0.34	0.54	–0.25	0.64	–0.27	0.57	0.35
Cr^{+3}	0.94*	0.79	0.73	0.62	0.82	0.62	0.50	–0.16	0.86*	0.64

The data was analyzed using correlation analysis. The asterisks indicate strong positive (0.8 and above) or negative (–0.8 and below) correlations between the parameters.

high amounts of sulfate, the necessity of culture media containing sufficient quantities of sulfate emerges (Costa *et al.*, 2003).

The economic viability of microalgae production can be achieved if operating and maintenance costs are minimized, and the microalgae's biomass efficiency, bioactive components and chemical contents are maximized (Singh and Gu, 2010). One of the crucial strategies in reducing the operating cost in Spirulina production is to use natural waters or wastewaters containing carbonate and bicarbonate instead of expensive nutrient media, which can provide nutrients for Spirulina growth. In this study, the cost of the nutrient medium was reduced when the geothermal water, which is rich in mineral content, was partially replaced with the Schlösser medium.

Anions and cations in the culture medium play a decisive role in the carbon flux. Carbon flux has a decisive role in forming biochemical composition in Spirulina. Phosphorus deficiency affects energy-requiring processes such as protein synthesis, transcription and carbon cycling (Mühlroth *et al.*, 2017). Phosphate in the GW75 and GW25 media was higher than in the other groups, and the highest protein content was determined in the GW75 group. Spirulina's protein and lipid content mainly depend on the nitrogen source and levels in the culture medium (Rodrigues *et al.*, 2010). Nitrogen and phosphate are the essential nutrients for synthesising protein, nucleic acid, enzymes and energy-carrying molecules (ATP) (Juneja *et al.*, 2013). However, phosphate in the culture medium is one of the most critical compounds affecting Spirulina's protein content. Iron, phosphorus, potassium, magnesium, nitrogen, manganese and calcium are the leading elements in the cofactor and structural component features required in photosystems II and I in cyanobacteria. The chlorophyll molecule is formed in the presence of nitrogen and magnesium elements. During the kelvin cycle process in which organic matter is synthesized, the required ATP yield may vary depending on the adequate supply of the elements in the nutrient medium during photosystem II and I reactions (light-dependent reactions). Since ATP is formed with adenine, ribose and three phosphorus, the amount of phosphate in the nutrient medium in light-dependent reactions supported protein synthesis in the dark reactions (Calvin cycle) of the GW75 group. Iron is a minor but essential element found in the structure of ferredoxin and involved in the synthesis of chlorophyll. In the formulation with high protein yield (GW75), the amount of iron and nitrogen required for metabolic processes and other components is sufficient to achieve high efficiency. In our study, the phosphate content was not represented by a regular increase or decrease in the experimental mediums. It was observed that the protein content was significantly higher in the GW25 and GW75 groups, where the phosphate content was high compared to the other groups. This situation may be due to the use of polyethene materials in the analysis process. Also, there is no linear relationship between the increase in the culture medium's geothermal water level and the groups' protein and lipid values. This situation could be attributed to the irregularities or interactions in the molecular distribution of water when the geothermal water and the Schlösser medium are mixed. More detailed research should be done by preparing alternative culture media formulations that provide high protein, lipid and other bioactive component values.

An increase in lipid synthesis was observed in algae growing in the nitrogen deficiency in the culture medium, while a decrease in protein content was also noted. These results are similar to Juneja *et al.* (2013) and Minhas *et al.* (2016). Nitrogen deficiency in the culture medium affects the biochemical content of Spirulina. Nitrate deficiency in the culture medium is accepted as a triggering factor that increases the lipid level of microalgae (Uslu *et al.*, 2011). This study obtained the highest lipid content and the lowest biomass yield in the GW100 group, with the lowest nitrogen content in the culture medium. Bajwa *et al.* (2018) report that *Nannochloropsis oculata*, *Chlorella pyrenoidosa* and *Scenedesmus obliquus* grown in culture medium with sufficient nitrogen and phosphorus have high bioavailability and high lipid accumulation when there is insufficient nitrogen in the culture medium.

Microalgae generally have less than 10% of their dry weight as ash (Becker, 1986). The ash content of Spirulina in the study shows a decreasing trend as the mineralisation level of the culture medium increases. As the culture medium's geothermal water level increased, Spirulina's ash content increased. High sulfate, calcium, magnesium, silicate acid and cadmium content in the GW100 culture medium are reasons for the high ash content. Cardoso *et al.* (2021) declared low levels of the nutrients led to stress and decreased ash content of Spirulina.

As the culture medium's potassium and bromide contents decrease, Spirulina's chlorophyll content also tends to decrease. The GW100 culture medium determined the lowest chlorophyll content with the most deficient potassium and bromide content. The GW25 group, which has the optimum bromide and potassium level and positively affects photosynthesis, could be considered the ideal group in terms of pigment. El-Sheekh *et al.* (2021) declared that Kuhl's medium, which is rich in potassium nitrate (KNO_3), enhances the chlorophyll content of Spirulina. Michael *et al.* (2019) also noted the chlorophyll content of the high potassium contained medium was the highest. Conversely, in this study, the increase in sulfate in the culture medium reduced the chlorophyll content of Spirulina. Excess sulfate may have a reducing effect on chlorophyll (Datta and Sharma, 1990). Silicate has played a suppressive role in chlorophyll content, and it is especially noticeable as a factor that causes the chlorophyll content of the GW100 group to be significantly low. In the present study, the lowest amount of chlorophyll pigment was determined in the GW100 ($P < 0.05$) group, while the amount of chlorophyll tended to decrease with geothermal water substitution. However, this trend is not valid for phycocyanin yield. Because, as seen in Table 5, zinc is also effective in forming phycocyanin pigment, but this situation is not dominant in the chlorophyll pigment. However, it is understood that potassium, bromide, sodium and chlorine play a more active role in forming chlorophyll than phycocyanin pigment.

Spirulina contains two billiprotein known as C-phycocyanin and allophycocyanin. Phycocyanin is a water-soluble blue pigment that helps capture more light from the chlorophyll during photosynthesis (Abalde *et al.*, 1998; Tunail, 2009). Phycocyanobilin chromophore, responsible for the blue color of the phycocyanin molecule, provides the formation of the phycocyanin structure (Santiago-Santos *et al.*, 2004; Brosnan and Brosnan, 2006). Although the phycocyanin yield is not statistically different between groups, sulfate improves sprulina's phycocyanin yield. Higher phycocyanin yields were obtained in the GW100, GW50 and GW25 compared to the others. It is impossible to mention the same situation regarding the purity of phycocyanin. The highest phycocyanin purity was obtained in the GW75 group with the lowest sulfate content. A study also reported similar results at different ammonium sulfate levels in the culture medium of *A. maxima* (Mirhosseini *et al.*, 2021). The nitrate significantly affected the phycocyanin purity, although it did not considerably impact the phycocyanin yield. Zinc is an essential element in phycocyanin purity. Table 6 shows that the presence of zinc in the Spirulina nutrient medium supports high phycocyanin purity, and it can be mentioned that high carbonate and bicarbonate cause a decrease in phycocyanin purity. The structure of phycocyanin varies according to the type of microorganism and culture conditions (Contreras-Martel *et al.*, 2007). The most crucial phycocyanin producer among Cyanobacteria is *A. platensis*, a phototrophic cyanobacterium species. It has been found that phycocyanin pigment, which has a blue colour, has antioxidant and anticancer properties (Gault and Marler, 2009). In this study, the phycocyanin purity ratio obtained from all groups was classified as food-grade (Kuddus *et al.*, 2013) because its purity is more than 0.7.

The study determined the positive effects of nitrate and zinc in the culture mediums on the essential amino acid profile (Tabs. 6 and 7). The amount of isoleucine, leucine, threonine and tryptophan was positively affected by increased nitrate and zinc in the culture mediums. The high amount of these amino acids is associated with the low carbonate in the culture medium. Autotrophic algae use carbon dioxide > bicarbonate > carbonate to prioritise as the inorganic carbon source (Christenson and Sims, 2011). The other essential amino acids, except lysine, valine and phenylalanine of the GW75 group, which has the lowest amount of carbonate and the highest amount of nitrate and zinc in the culture medium, were higher compared to the others ($P > 0.05$). Methionine in Spirulina is the highest in the GW75 group ($P < 0.05$). Sulfur-containing compounds such as methionine and cysteine are potent antioxidants that prevent cell damage (Fanatico, 2021). Although methionine is from the sulfur-containing amino acid group, it indicates that the lowest sulfate-containing GW75 contains sufficient sulfate for methionine synthesis. Provided that adequate methionine is available, methionine can turn into cysteine. Although cysteine is not an essential amino acid, the presence of methionine, an essential amino acid, is necessary (Brosnan and Brosnan, 2006). There should be at least 250 mg/L nitrates in the culture medium to obtain Spirulina with high methionine content and phycocyanin purity. Different nutrients influence the yield and purity of phycocyanin under culture conditions. Phycocyanin is a pigment that forms a complex with cysteine. However, the relationship between phycocyanin purity and methionine appears to be a subject worth investigating. The increased sulfate levels, magnesium, manganese, copper and cadmium in the culture medium reduced Spirulina's arginine level ($P < 0.05$). The phenylalanine content of Spirulina was reduced by increased bicarbonate, chloride, sodium and carbonate in the culture medium. With the increase in geothermal water in the nutrient medium, a decrease in bicarbonate, carbonate, chloride and sodium was determined. The amount of phenylalanine decreased in the GW25 and SM due to the lower zinc and silicate concentrations ($P < 0.05$). Coca *et al.* (2015) stated protein productivity of beet vinasse supplemented Schlösser medium at low concentration (1 g/L) was higher than the control (Schlösser) medium.

Culture medium components for Spirulina production are essential in changing the mineral profile of Spirulina. For instance, the lack of nitrogen in the culture medium caused it to be rich in Spirulina's potassium and sodium (Michael *et al.*, 2019). In addition, the high chromium level in the GW100 and Schlösser nutrient media is among the factors that increase the sodium content of the harvested Spirulina. Our study concluded that the phosphorus amount of Spirulina was related to the amount of manganese and chromium in the culture medium. The mineral profile of Spirulina, harvested with the decrease of nitrate level in the culture media, was generally high, except for copper. The zinc level of Spirulina also increased with the copper level in the culture medium. With the increase in the amount of sulfate and the decrease in potassium in the culture medium, the calcium, magnesium and manganese content of Spirulina increased. The increased carbonate level in the culture medium has enabled Spirulina production with high iron content (Michael *et al.*, 2019). With the rise in the hardness of the culture medium water, the magnesium and calcium content of the harvested Spirulina also increased. In addition, the presence of bromide in the culture medium draws attention as a factor that reduces the calcium content of Spirulina.

Metals are natural soil and soil crust components, but groundwater like geothermal waters could accumulate these metals. Some metals are called micronutrients (Cu, Zn, Ni, Mn and Co, I, Fe) and are helpful for algal growth and vital functions (Wells *et al.*, 2017). However, some heavy metals such as Pb and Hg are toxic, and their excessive intake may affect metabolic processes and algae's physique (Mikulewicz *et al.*, 2017). For instance, there is no mercury in the geothermal water used in this research. Mandatory plant nutrients are elements that the plant must have to survive. These elements are defined as irreplaceable elements, and in their absence, there is no plant life (Rice, 2007; Fageria *et al.*, 2010).

This study investigated a novel, low-cost culture medium for Spirulina growth was formulated, using partially geothermal water instead of Schlösser medium to provide some of the nutrients required for this microalga. In this study, Spirulina growth was achieved in all experimental groups containing geothermal water, except GW100, and a significant amount of harvest was made. As a result, it is suggested that geothermal water could be used as the alternative medium for the optimum biomass and production of beneficial nutrients and some bioactive components in Spirulina. Also, it is recommended to conduct further studies on long-term preservation without losing its nutritional value in geothermal water.

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