Growing blue –green algae (spirulina platensis) to study some biochemical properties

Esraa Abdul-Adel [1] and Shifaa Ismael Jasim [2]

1 Dperment of Environment Pollution, environment science college, Iraq.

2 assist professor, Dperment of Environment Pollution, environment science college, Iraq

Email ID: shfaa819@gmail.com

Abstract:

The use of algae, especially spirulina, as a functional food has been proposed due to the fact that it is not only a protein-dense food source, but because its amino acid profile is considered to be a highly valuable protein of biological value. Spirulina also provides essential lipids (e.g., oleic acids (gamma-linolenic acids), which accompany For low content nucleic acids. It also contains an exceptionally high percentage of vitamin C, vitamin A and glutathione, as well as carbohydrates, fats and a wide range of photosynthetic pigments. Spirulina platensis algae was grown in the laboratory under controlled conditions in Zarrouk medium to examine its content of sugars, proteins, fats, chlorophyll, vitamin C, vitamin E, and glutathione. The results showed that spirulina contains 20.7% of sugars. And 28% of protein, 27 mg of fat, 3.79384 of chlorophyll, 1.850 mg.g of vitamin C, 2.10 mg of vitamin A, and 0.612 mg.g of glutathione.

ISSN 2072-3857

Introduction:

Arthrospira platensis (infomal name "*Spirulina*") is a filamentous, multicellular, blue-green photosynthetic microalgae belonging to the phylum blue-green algae[1]. It is an important source of nutrients such as proteins, minerals, carbohydrates, and several plant pigments that can be used as nutritional supplements. In addition, these microalgal cells have high digestibility due to the lack of cellulose which facilitates their use for human consumption [2]. The most important species used for consumption are *Spirulina maxima* and *Arthrospira platensis* [3], [4] . *Spirulina* is produced naturally in the alkaline waters of volcanic lakes and brackish waters. However, these algae can also be grown for increased biomass production, under controlled conditions in saline water (>30 g/L) at a pH in the range of 8.5-11.0 with high levels of solar radiation (2500 lux) and at temperatures in range 35–39°C [2], [5], [6]. The current production of *Spirulina* in the world is estimated at approximately 3000 metric tons and more than 70% of it is marketed for human consumption, mainly as a health food [6]. *Spirulina* is mostly consumed in the form of tablets, capsules and powder or added to various types of foods such as pasta, cakes and health drinks used as nutritional supplements or natural colorings[7]

2.Aim of the study

The aim of this research is to give a comprehensive overview of the nutritional properties of *Spirulina*. These properties are even more important because the production of these microorganisms is particularly suited to climatic and economic conditions. Hence, the work aimed to test the viability of local production of *Spirulina* and test its biochemical components.

3. Materials and methods

Algae isolation

The blue-green algae, *Spirulina platensis*, was purchased from HEALTHALGAE (Sweden) and it is necessary to transfer this algal isolate to a specific growth medium to promote its growth and enrichment.

1.Propagation of algal isolates

For cultivation, Zarrouk Medium [22] was used and its components are shown in Table1

ISSN 2072-3857

Ingredients	Concentration (g/l)
NaCl	**1.0**
$CaCl_2.2H_2O$	**0.04**
$NaNO_3$	**2.5**
$FeSO_4.7H_2O$	**0.01**
EDTA (Na)	**0.08**
K_2SO_4	**1.0**
$NaHCO_3$	**16.8**
K_2HPO_4	**0.5**
$MgSO_4.7H_2O$	**0.2**

Solutions with respective salts were sterilized separately by autoclave at 121°C and 15Pa pressure for 15min and subsequently mixed to achieve the final medium[8]. *S. platensis* cells were inoculated with 10% Zarrouk chemically defined medium (Vinoculation/Vmedia) in 500ml flasks and development was performed in three replicates at 32 ± 1°C, pH 9, and under irradiation of 135 µE/m2/s. Using cool white fluorescent lamps with a 12:12 h light/dark light cycle and daily continuous shaking by hand[9].

1. Estimating the total amount of sugars (carbohydrates).

50 µL (standard sugar or milk sample) was added to a clean and sterilized microplate, then 150µL of concentrated sulfuric acid was added slowly and gradually, then immediately 100µL of 5% phenol was added. The microplate was heated for 5 minutes at 90°C. It was then cooled to room temperature for 5 minutes, and then the absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 390nm[10]. The amount of total carbohydrates present was calculated using a standard chart and according to the equation below

Total sugar concentration = (A test/A standerd) * concentration of standerd

2. Determination of proteins

The Bradford method described by Bradford, (1976) used the protein in the crude and purified extract.

Solutions used:

a. Coomassie Blue G dye solution 250

Prepare the solution by dissolving 100mg of Coomassie blue dye G-250 in a mixture consisting of 100 ml of 85% phosphoric acid and 50 ml of ethyl alcohol. Then the volume was increased to one liter by adding cold distilled water and filtered using filter paper.

b. 1M sodium hydroxide solution: Dissolve 4g of sodium hydroxide in

50ml of distilled water, and bring the volume to 100ml.

c. Standard bovine serum albumin solution at a concentration of 1mg/ml: Dissolve 0.1g of bovine serum albumin in an amount of distilled water and bring the volume to 100ml.

How to work according to the following steps:

1. Gradient concentrations of bovine albumin solution were prepared at 2.5, 5, 7.5, 10, 12.5, 15, and 20 micrograms/ml.

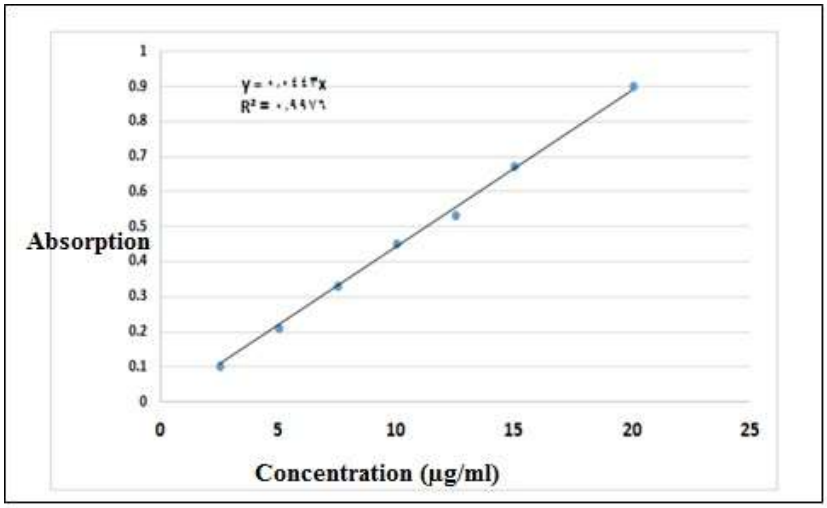

Figure 1: Standard growth curve for bovine serum albumin

2. Take 20 microliters of each concentration in the above step and add 50 microliters of sodium hydroxide solution with a concentration of 1molar to it.
3. Add 1ml of Coomassie blue dye solution G250-(A), mix the tubes well and leave for 5 minutes at room temperature, read the absorbance of each concentration in three replicates at a wavelength of 595nm (note: the uncharged form of the dye is blue).
4. Plot the standard curve of bovine serum albumin concentration (µg/ml) versus absorbance at a wavelength of 595nm.

3. Estimation of total fat

10ml of the sample was added to 1.5ml of ammonium hydroxide at the fat extraction flask, then 10ml of alcohol (95%) was added and the contents were mixed well again. Then add twenty-five ml of peroxide-free diethyl ether to the beaker, cap it, and mix vigorously for one minute. Then add 25ml of light petroleum ether and mix the contents vigorously for one minute. (Leave the tube to settle and then transfer to centrifuge at 600 rpm for 30 seconds.) The lipid solution was transferred to a suitable dish (previously dried in a hot air oven for 1 hour). To the lipid extraction flask, 5ml of ethanol is added and

ISSN 2072-3857

mixed well. The extraction is done with 15ml of ether and 15ml of light petroleum ether again. The final extraction is done with ether but without alcohol. The solvent is evaporated from the plate and the plate is dried to constant weight in a hot air oven at 102°C for 1h,then, it is cooled to room temperature in a desiccator (about 30 min) and weighed [11].

Fat % (W/W) = 100 (w1-w2) / w3

4. Estimation of total vitamin C (ascorbic acid).

The 2,4-dinitrophenyl hydrazine (DNP) method is widely used for the determination of total ascorbic acid in biological fluids. Ascorbic acid is oxidized by the cupric ion to form dehydroascorbic acid and then to 3-diketo-gulonic acid, which reacts with 2,4-dinitrophenylhydrazine to form red dihydrazone, which is measured at a wavelength of 520nm.

The method of work:

1. Plant samples were extracted and homogenized using 6% phosphoric acid, and centrifuged at 6000 rpm/min for 10 minutes at 4°C.
2. Using a volumetric vial prepare 25 ml of standard ascorbic acid solutions of each of the concentrations: 0.10, 0.40, 0.80, 1.20, 2.0, 3.0, and 4.0 mg/dl using 6% phosphoric acid.
3. take 1.2ml each of the sample filtrate (for the sample) or 1.2 ml of the standard solution. Place 1.2 ml of 6% phosphoric acid in two separate tubes to use as the buffer solution. (blank)
4. Add 0.4ml of dinitrophenyl hydrazine-thiourea-copper (DTC) reagent to all tubes (DTC reagent was prepared by 3g of DNP, 0.4g thiourea and 0.05g copper sulphate in 100ml of 9N H_2SO_4). Cover, mix the contents and incubate the tubes in a water bath at 37 °C for 3 hours.
5. Remove tubes from the water bath and cool it for 10 minutes in an ice bath. Add to all tubes 2.0 ml of cold sulfuric acid (12 mol/L), and mix the contents well (the temperature of the mixture should not exceed room temperature.)
6. Set the spectrometer at 520nm using the buffer solution, read the standard solutions and sample solutions. Plot the concentration of each standard solution against the absorbance values and extract the vitamin C concentrations from the measurement curve shown in the figure 2.

ISSN 2072-3857

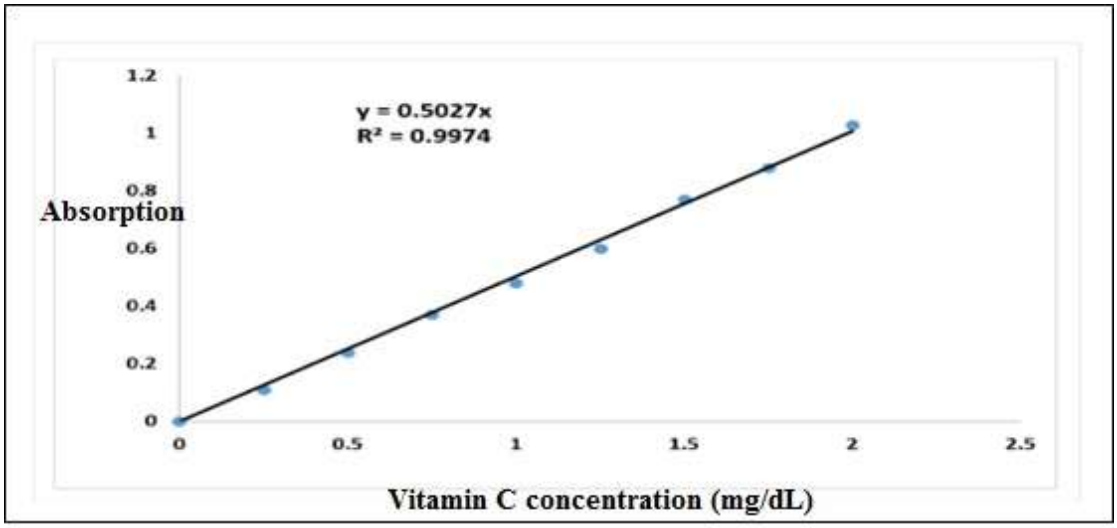

Figure 2: Standard curve for vitamin C

5. Determination of chlorophyll

Weigh 1g of the sample and mix well with 10ml of 80% acetone in a glass test tube and leave it in the refrigerator for twenty hours at a temperature of 4 °C in the dark, then shake it again and also leave it for a period ranging between 1-2 hours in the same conditions, after which it is quickly centrifuged. 3000 rpm for ten minutes, then re-acidify the filtrate by adding 1-2 drops of hydrochloric acid (0.1 N). The filtrate is taken to measure the absorbance using a spectrophotometer at wavelengths of 663 and 645nm. The chlorophyll concentration is calculated from the equation below:

Total chlorophyll mg/g^{-1} = (20.2A645 * 8.02A663/a * 1000* w) * v

4. Results and discussion

The composition of *Spirulina* may vary depending on culture conditions and analysis methods. Table 2 shows the results obtained in terms of measuring sugars, fats, vitamin C, protein, and chlorophyll in the *Spirulina* alga after growing it in Zarrouk medium and under laboratory conditions.

Table 2: Some biochemical values of *Spirulina platensis* powder

Component	Units	Concentration
Sugars	**%**	**20.7**
Fats	**mg. g^{-1}**	**27**
Vitamin C	**mg. g^{-1}**	**1.85**
protein	**%**	**28**
Chlorophyll	**Mg.g^{-1}**	**3.79384**

ISSN 2072-3857

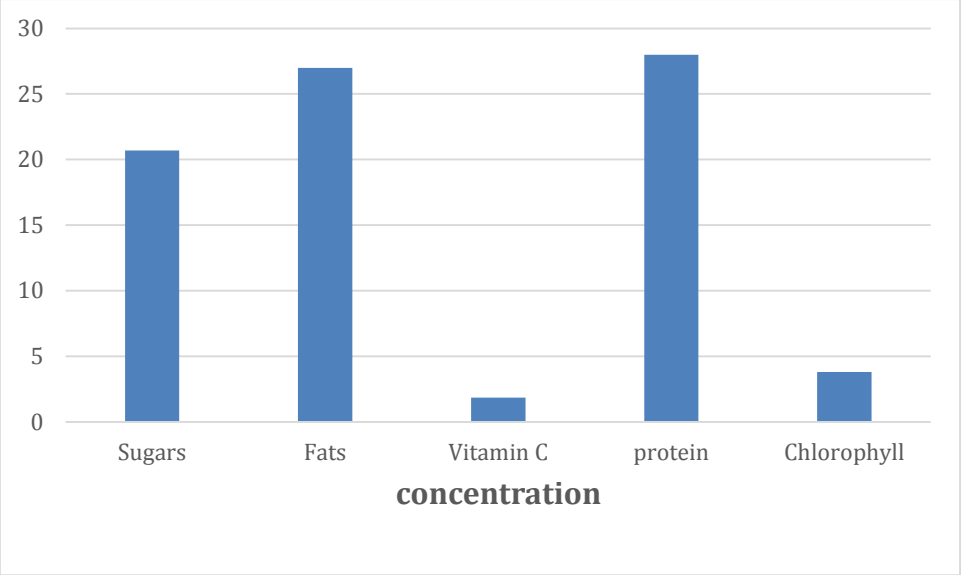

Figure 3: Some biochemical values of *Spirulina platensis* powder

As founded in the figure 3, the results mentioned in this study indicate that *Spirulina* contains 20.7% of sugars, 27mg.g-1 of fat, and 28% of protein. These results were similar to the results recorded by Madkour et al., 2012 and Salazar et al., 1998 where Madkour et al., 2012 found the protein and carbohydrate content of *S. platensis* grown in Zarrouk growth medium was 52.95% and 13.20%, respectively, while Salazar et al., 1998 obtained a carbohydrate content ranging from 20.0% to 60.0% and a protein content of 45.40% [12] [13]. Abd El-Baky et al., 2008 also recorded lipid values in *Spirulina* between 5.6 to 7%. El-Beltagi et al., 2020 and Chamorro et al., 1996 found the lipid content range from 6 to 13%[14] [15] [16]. A proximate analysis of freeze-dried *Spirulina* biomass by Milena *et al*., (2021) shows that the main component found in the *Spirulina* samples was proteins (47%) followed by lipids (36%) [17]. The protein content was similar to that recorded by Volkmann *et al*., (2008) which was 48% but slightly lower than the amounts found in S*pirulin*a strains (50-70%) obtained from different regions[18].

As for vitamins, they are food compounds, and they are required in small quantities, but they are very important for the human and animal body and their preservation. In this study, vitamin C was found at a concentration of 1.850 $mg.g^{-1}$, and this result was similar to the study conducted by Andrade *et al*., (2018) who found that these algae contain all vitamins, including vitamin A (carotene), vitamin D, vitamin E, vitamin K, vitamin C, and vitamin B, such as thiamine (vitamin B1) and riboflavin (vitamin B2). and niacin (vitamin B3) [19].

Measuring chlorophyll a is important in algae growth and monitoring water quality in natural

ISSN 2072-3857

waters. This study recorded a chlorophyll content of 3.79384 $mg.g^{-1}$. This was also found in the study conducted by Seyidoglu *et al*., 2017 [20], where it was found that *Spirulina* algae is one of the greatest Food sources rich in pigments, especially C-phycocyanin, which contains 14% of the iron element in it. In addition, it contains the highest value of chlorophyll (1%). Chlorophyll pigment is a type of phytonutrient that helps the body cleanse and detoxify itself**.** Haoujar *et al*., 2022 also found that *Spirulina* contains chlorophyll pigments and phycocyanin, both of which are powerful antioxidants[21]. The percentages of these pigments were 1.472% and 14.18%, respectively.

5. Conclusions and Recommendations

1. Conclusions

Spirulina can be classified as a superfood and food alternative due to its high nutrient content. *Spirulina* contains nutrients such as protein, vitamins, essential fatty acids, amino acids and photosynthetic pigments.

2. Recommendations

a. Establishing a special system for growing *Spirulina* algae for use in various fields of life.
b. Increasing research in this field with the aim of benefiting from these organisms, moving towards cultivating them in the field and searching for appropriate environmental conditions to reach the highest productivity.
c. Interest in studying the group of blue-green algae, especially the toxic species, because this topic poses risks to the general health of the population and aquatic organisms.
d. Conduct a diagnostic study of *Spirulina* at the molecular level under different treatments.
e. Study the effect of various alternative media used in growing *Spirulina* and record the extent of their impact on the biochemical composition of *Spirulina*.

6. References

[1] E. W. Becker and L. V Venkataraman, "Production and utilization of the blue-green alga Spirulina in India," *Biomass*, vol. 4, no. 2, pp. 105–125, 1984.

[2] J. Simpore *et al.*, "Nutrition rehabilitation of HIV-infected and HIV-negative undernourished children utilizing spirulina," *Ann. Nutr. Metab.*, vol. 49, no. 6, pp. 373–380, 2005.

[3] K. E. Apt and P. W. Behrens, "Commercial developments in microalgal biotechnology," *J. Phycol.*,

vol. 35, no. 2, pp. 215–226, 1999.

[4] M. Olaizola, "Commercial development of microalgal biotechnology: from the test tube to the marketplace," *Biomol. Eng.*, vol. 20, no. 4–6, pp. 459–466, 2003.

[5] A. Belay, T. Kato, and Y. Ota, "Spirulina (Arthrospira): potential application as an animal feed supplement," *J. Appl. Phycol.*, vol. 8, pp. 303–311, 1996.

[6] J.-H. Hwang *et al.*, "Spirulina prevents memory dysfunction, reduces oxidative stress damage and augments antioxidant activity in senescence-accelerated mice," *J. Nutr. Sci. Vitaminol. (Tokyo).*, vol. 57, no. 2, pp. 186–191, 2011.

[7] O. Ciferri, "Spirulina, the edible microorganism," *Microbiol. Rev.*, vol. 47, no. 4, pp. 551–578, 1983.

[8] A. Walter, J. C. de Carvalho, V. T. Soccol, A. B. B. de Faria, V. Ghiggi, and C. R. Soccol, "Study of phycocyanin production from Spirulina platensis under different light spectra," *Brazilian Arch. Biol. Technol.*, vol. 54, pp. 675–682, 2011.

[9] A. S. Sarpal *et al.*, "Investigation of biodiesel potential of biomasses of microalgaes chlorella, spirulina and tetraselmis by NMR and GC-MS techniques," *J Biotechnol Biomater*, vol. 6, no. 220, p. 2, 2016.

[10] M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. t Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances," *Anal. Chem.*, vol. 28, no. 3, pp. 350–356, 1956.

[11] D. Pearson, "Composition and Analysis of Foods. Dairy Products II. In 'Pearson's Composition and Analysis of Foods'. 9th edn.(Ed. RS Kirk, R Sawyer) pp. 530–680." Longman: London, 1991.

[12] M. Salazar, E. Martınez, E. Madrigal, L. E. Ruiz, and G. A. Chamorro, "Subchronic toxicity study in mice fed Spirulina maxima," *J. Ethnopharmacol.*, vol. 62, no. 3, pp. 235–241, 1998.

[13] F. F. Madkour, A. E.-W. Kamil, and H. S. Nasr, "Production and nutritive value of Spirulina platensis in reduced cost media," *Egypt. J. Aquat. Res.*, vol. 38, no. 1, pp. 51–57, 2012.

[14] H. S. El-Beltagi, F. Dhawi, I. S. Ashoush, and K. Ramadan, "Antioxidant, anti-cancer and ameliorative activities of Spirulina platensis and pomegranate juice against hepatic damage induced by CCl4," *Not. Bot. Horti Agrobot. Cluj-Napoca*, vol. 48, no. 4, pp. 1941–1956, 2020.

[15] G. Chamorro, M. Salazar, L. Favila, and H. Bourges, "Pharmacology and

ISSN 2072-3857

toxicology of Spirulina alga," *Rev. Invest. Clin.*, vol. 48, no. 5, pp. 389–399, 1996.

[16] H. H. Abd El-Baky, F. K. El Baz, and G. S. El-Baroty, "Characterization of nutraceutical compounds in blue green alga Spirulina maxima," *J. Med. Plants Res.*, vol. 2, no. 10, pp. 292–300, 2008.

[17] Hosseini, S.M.; Shahbazizadeh, S.; Khosravi-Darani, K.; Reza Mozafari, M. Spirulina paltensis: Food and function. Curr. Nutr. Food Sci. 2013, 9, 189–193.

[18] H. Volkmann, U. Imianovsky, J. L. B. Oliveira, and E. S. Sant'Anna, "Cultivation of Arthrospira (Spirulina) platensis in desalinator wastewater and salinated synthetic medium: protein content and amino-acid profile," *Brazilian J. Microbiol.*, vol. 39, pp. 98–101, 2008.

[19] L. M. Andrade, C. J. Andrade, M. Dias, C. Nascimento, and M. A. Mendes, "Chlorella and spirulina microalgae as sources of functional foods," *Nutraceuticals, Food Suppl.*, vol. 6, no. 1, pp. 45–58, 2018.

[20] N. Seyidoglu, S. Inan, and C. Aydin, "A prominent superfood: Spirulina platensis," *Superfood Funct. food Dev. superfoods their roles as Med.*, vol. 22, pp. 1–27, 2017.

[21] I. Haoujar, M. Haoujar, A. B. Altemimi, A. Essafi, and F. Cacciola, "Nutritional, sustainable source of aqua feed and food from microalgae: a mini review.," *Int. Aquat. Res.*, vol. 14, no. 3, 2022.

[22] Zarrouk, C. (1966). Contribution a letude d'une cyanophycee. Influence de divers facteurs physiques. et chimiques sur la croissance et la photosynthese do Spirulina maxima, Ph.D. Thesis, University of Paris, France.

ISSN 2072-3857