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Nutritional values of *Spirulina platensis* biomass cultivated in East Africa

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Abstract

Spirulina platensis is a biomass of cyanobacteria traditionally used for hundred years worldwide for its nutritional and pharmacological benefits. So far, this microscopic organism has not undergone many scientific studies in East Africa. Therefore, evaluating its nutritional properties could help to justify its potential and promote its utilization in the region. In this study, the determination of vitamins was performed by using a high-performance liquid chromatography (HPLC) method. Analysis of minerals and heavy metals was performed by using atomic absorption spectrophotometer (AAS). The protein content of *Spirulina platensis* was determined by using the Kjeldahl method. The spectrophotometry method was used for phytochemical analysis. The results showed that the vitamin A, vitamin B_9 and vitamin B_{12} content of *Spirulina platensis* was 27.4 µg/100g, 246.89 µg/100g, and 3.87 µg/100g respectively. Concentrations of iron, zinc, calcium, and phosphorous in *spirulina platensis* were found to be 82.2 mg/100g, 85.1 mg/100g, 1300 mg/100g, and 600 mg/100g respectively. The protein content of *spirulina platensis* was 46 g/100g. The concentrations of total phenolic and total flavonoid in *spirulina platensis* found to be 2.99 mg GAE/g and 1.92 mg QE/g respectively. These data indicated that the nutrient concentration of *spirulina platensis* is enough to meet the recommended daily allowance (RDA) of most essential nutrients. Mercury, lead, cadmium and arsenic contents of *spirulina platensis* was 0.000036 mg/kg, 0.0047 mg/kg, 0.00048 mg/kg and 0.0047 mg/kg respectively. All the analyzed heavy metals were within the recommended EU safety limits. Our findings confirmed the nutritional potential of *Spirulina platensis* cultivated in East Africa. Thus, *Spirulina platensis* could be considered as a potential alternative source of sustainable nutrition to address malnutrition in the region.

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Introduction

Spirulina is a genus of blue-green algae, which belongs to phylum cyanobacteria. *Spirulina* has different species however, *Spirulina platensis* and *Spirulina maxima* are the two commonly consumed species by humans and animals. Numerous studies reported that *spirulina* is the richest source of high-quality protein, vitamins, minerals, and other health-promoting bioactive compounds that are mostly inadequate in typical African diets (Kay and Barton, 2009; Bruna *et al.*, 2015; Saha and Murray, 2018). Besides, unlike other food crops, *spirulina* requires less space and inputs to grow (García *et al.*, 2017; Caporgno and Mathys, 2018; Khan *et al.*, 2018). *Spirulina* thrives in alkaline lakes where it is difficult or impossible for other microorganisms to survive. Lakes in East Africa along the Great Rift Valley including Lake Natron, Lake Tanganyika, Lake Nakuru, Lake Elementeita and also Lake Victoria have the special characteristic that makes them favorable for *spirulina* production (Habib *et al.*, 2011; Antonio, 2011).

Although East Africa has the most favorable environmental condition, production, as well as utilization of *spirulina*, is very limited in the region; besides, there is limited or no scientific information on the nutrient and phytochemical composition and other nutritional properties of *spirulina* grown in East Africa. Hence, we believed that the existence of limited scientific information restricted the wide production and utilization of *spirulina* in East Africa.

Therefore, this study aimed to profile the nutrient composition and other nutritional properties of locally cultivated *Spirulina platensis* biomass.

Materials and methods

Spirulina samples

The powder of *Spirulina platensis* used in this study was obtained from local producers from different areas of Kenya. *Spirulina platensis* was cultivated in a pond covered with a greenhouse. After harvesting, the biomass of *spirulina platensis* was dried and grounded to obtain powder products.

Determination of vitamins

The determination of vitamins was performed using HPLC following the methods described by Sami *et al.* (2014). For vitamin A, 1 g of pyrogallic acid, 70 mL ethanol, and 30 mL (50%) KOH were added into 10 g of powder *spirulina platensis*, stirred and refluxed for 40 minutes using a water bath at 50 °C. Extracts were obtained three times using 50 mL, 30 mL, and 20 mL ether concentrations. Distilled water was used to neutralize the extract. Further, the extract was concentrated to approximately 5 mL by using a water bath (50 °C), diluted to 10 mL by using methanol, filtered using a 0.45 μm membrane, and finally subjected to HPLC analysis.

To determine vitamin B_9 and B_{12}, about 2 g powder *spirulina platensis* was placed in 25 mL of sulfuric acid (0.1 N) solution and incubated for 30 minutes at 121°C. Then, the content was cooled and adjusted to pH 4.5 with 2.5 M sodium acetate, and 50 mg Takadiastase enzyme was added. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman No. 4 filter, and the filtrate was diluted with 50 mL of pure water and filtered again through a micropore filter (0.45 μm). Twenty microliters of the filtrate were injected into the HPLC system. Quantification of vitamin B_9 and B_{12} content was accomplished by comparison to standards.

Determination of minerals and heavy metals

Concentrations of minerals and heavy metals were determined using atomic absorption spectrophotometer (AAS) modifying a method described by Steponënienë and Tautkus (2006). Determination of minerals and heavy metals was carried out after the preparation of samples and standard solutions. Calibration solutions were used for the calibration of the atomic absorption spectrophotometer. The sample was prepared by adding 10 g powder *spirulina platensis* into a 250 mL volumetric flask and mixed with 10 mL of nitric acid. The mixture was heated for 10 minutes using Kjeldahl block digester. After cooling it down, 5 mL of nitric acid was added and heated again for 30 minutes.

After leaving the solution to cool for 10 minutes, 2 mL distilled water, 3 mL hydrogen peroxide, and 2 mL hydrochloric acid was added and heated again for another 10 minutes. The solution was filtered using Whatman filter paper 1 and diluted to 100 mL using distilled water. Readings by atomic absorption spectrophotometer were done at different wavelengths; 248.3 nm, 213.9 nm, 422.7 nm, 213.6 nm, 193.7 nm, 228.8 nm, 253.7 nm, 283.3 nm was used for iron, zinc, calcium, phosphorus, arsenic, cadmium, mercury and lead respectively.

Determination of phytate

Phytate was determined using an anion-exchange method following Ma *et al.* (2005). Samples were accurately weighed (1.0-2.0 g) and transferred into 100 mL conical flasks. A total of 40-50 mL of Na2SO4 (100g/L)-HCl (1.2%) was added. Flasks were then capped and shaken vigorously for 2 hours on a rotator at ambient laboratory temperature.

The supernatant was then filtered through qualitative filter paper no 4. A total of 10 mL of filtered extract was diluted to 30 mL with distilled water after mixing with 1 mL of 0.75M NaOH and then passed through an anion resin column (resin AG1-X4, ~ 100-200mesh, Biorad Laboratory Inc., column 0.8 x 10cm). The column was washed before use with 20 mL of 0.5 mol/L NaCl solution and deionized water. After sample application, the column was washed with 15 mL of distilled water and 20 mL of 0.05M NaCl solution to remove the inorganic phosphate. Then the retained phytic acid was eluated with 0.7M NaCl.

The post-column reagent was made up of a0.03% $FeCl_3$ solution containing 0.3% sulfosalicylic acid. A total of 4 mL of the reagent was added into 5 mL of collected eluate and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 500 nm using a spectrophotometer (LKB 4053, U.K.). A calibration curve for the colorimetric method was obtained by using phytate standards (P-8810 Sigma Co.). The phytate content of samples was calculated using the standard curve.

Determination of protein

The Kjeldahl method described by Mæhre *et al.* (2018) was used to determine *spirulina* protein content. Briefly, a powder sample of 1 g was measured and added to a digestion flask along with 25 mL sulfuric acid, 15 g potassium sulfate, and 0.3 g copper (II) oxide. The mixture was heated for 2 hours at 370 °C. After cooling it down, 300 mL distilled water was added. The digested sample was connected to the distillation apparatus and the distillate was collected into the collecting flask which contains 25 mL sulfuric acid (HCL), 150 mL distilled water, and a few drops of mixed indicator. The distilled solution was titrated with sodium hydroxide solution (0.1 mol/L) until the color of the solution is changed from violet to green. The volume was recorded at the point where the color is changed.

Determination of phytochemicals

The total phenolic (TPC) and flavonoid (TFC) contents were determined using a spectrophotometer following the method as described by Chandra *et al.* (2014) with slight modifications. In brief, powder *spirulina* (10 g) from each sample was extracted using 75 mL (95% v/v) methanol at 40°C for 10 minutes followed by centrifugation at 3500 rpm for 10 minutes. The clear supernatant was collected and stored in an amber bottle for analysis.

Preparation of gallic acid standards

Various concentrations of Gallic acid solutions in methanol (5-500 mg/L) were prepared. In a 20 ml test tube, 1 mL Gallic acid of each concentration was added and to that 5 mL of Folin-Ciocalteu's reagent (10%) and 4 mL of 7% sodium carbonate were added to get a total volume of 10 mL. The blue-colored mixture was shaken well and incubated for 30 minutes at 40 ºC in a water bath. Then the absorbance was measured at 765 nm against a blank (0.5 mL methanol, 2.5 mL 10% FolinCiocalteu's reagent, and 2.5 mL of 7% sodium carbonate). All the experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of Gallic acid were used to plot the calibration curve.

Total phenolic content

The total phenolic content in *spirulina* sample was determined by using the Folin and Ciocalteu reagent method. The test samples were prepared by mixing 0.5 mL of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent, and 2.5 mL 7% sodium carbonate. The samples were thereafter incubated at 45 °C for 45 minutes in a water bath. The phenolic content was calculated as Gallic acid equivalents GAE/g of dry sample based on a standard curve of Gallic acid (5-500 mg/L, $Y = 0.5129x + 0.0145$, $R^2 = 0.9985$).

Total flavonoids content

The total flavonoid content was estimated according to the aluminum chloride colorimetric method. For total flavonoid determination, quercetin was used to make the standard calibration curve. The stock quercetin solution was prepared by dissolving 50 mg quercetin in 10 mL methanol. Quercetin standard curve was built using a concentration of 50–250 µg/mL. An amount of 0.6 mL diluted standard quercetin solutions and the same amount of extract was separately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 minutes at room temperature. The absorbance of the reaction mixtures was measured at 420 nm. With a Varian UV-Vis spectrophotometer.

The concentration of total flavonoid content in the test samples was expressed as mg quercetin equivalent (QE)/g of dry sample based on a standard curve of quercetin (50-250 µg/mL, $Y = 0.0017x - 0.0089$, $R^2 = 0.9982$).

Statistical analysis

Data were analyzed using a statistical software IBM SPSS version 23. All the descriptive data were presented as mean values.

Results

Results showed that 100 g of *spirulina platensis* grown in East Africa contains a significant amount of essential nutrients and bioactive compounds that are important for human nutrition. As described in Tables 1 and 2, 100 g of *spirulina platensis* grown in East Africa contains minerals, vitamins, and protein in a concentration enough to meet the recommended daily allowance (RDA) for a young child.

Table 1. Vitamin A, vitamin B_9, vitamin B_{12}, and protein composition of *Spirulina platensis*.

Nutrient	Concentration
Vitamin A (µg/100g)	27.4
Vitamin B_9 (µg/100g)	246.89
Vitamin B_{12} (µg/100g)	3.87
Protein (g/100g)	46

The study further revealed that *spirulina platensis* has a very low concentration of heavy metals (Table 5). All the analyzed heavy metals were within the recommended EU safety limits. Also, as described in Table 3, the ratio of minerals to phytate are within the ranges that favor micronutrients bioavailability. As summarized in Table 4, *spirulina platensis* also contains a significant amount of well-known health-promoting antioxidants such as phenolic compounds and flavonoids.

Discussion

In this study, we profiled the nutrient and phytochemical composition of locally produced *spirulina platensis* to evaluate its potential to utilize it as an alternative food supplement to address undernutrition in East Africa. Our findings showed that *spirulina platensis* cultivated in East Africa are rich in essential nutrients. Moreover, a small amount of *spirulina platensis* is sufficient to meet the recommended dietary allowance (RDA) of most nutrients (Institute of Medicine, 2001). The analyzed 100 g *spirulina platensis* powder is sufficient to meet the recommended dietary allowance (RDA) of protein, iron, zinc, calcium, and vitamin B_{12} of individuals in all age groups.

Table 2. Concentrations of iron, zinc, calcium, phosphorus, and phytate in *Spirulina platensis* (mg/100g).

Nutrient	Concentration (mg/100g)
Iron	82.8
Zinc	85.1
Calcium	1300
Phosphorous	600
Phytate	1.86

The same amount of *spirulina platensis* could provide sufficient phosphorus to meet the recommended dietary allowance (RDA) of 0-8 years old children. Moreover, a 100 g *spirulina platensis* is enough to provide 48% and 86% phosphorous to meet the recommended dietary allowance (RDA) of 9-18 and 19-50 years old individuals respectively. The recommended dietary allowance (RDA) of vitamin B_9 for children aged 0-8 years can be sufficiently met by only 100 g *spirulina platensis*; about 100 g of *spirulina platensis* is enough to provide more than 50% of the recommended dietary allowance (RDA) of vitamin B_9 for individuals aged 9 to 50 years. On the other hand, a 100 g *spirulina platensis* can provide only 9% of the recommended dietary allowance (RDA) of vitamin A.

Table 3. Phytic acid to iron, phytic acid to zinc, and phytic acid to calcium molar ratios.

Sample	Phytate: Fe	Phytate: Zn	Phytate: Ca
Spirulina powder	0.0019	0.0021	0.000086
Suggested critical values [a]	> 1	> 15	> 0.24

[a] Norhaizan *et al.*, 2009.

Essential minerals especially iron and zinc are among the most deficient micronutrients in Africa and are associated with poor growth and development and impaired immune response (WHO, 2017).

The deficiency of essential minerals in the human body may be a result of inadequate intake or impaired absorption of minerals due to the interaction between essential nutrients or the presence of phytate and oxalate in the diet (Norhaizan and Faizadatul, 2009; Nguyen *et al.*, 2012).

It is often cited that a higher concentration of iron which is above 2:1 molar ratio can negatively affect zinc absorption especially when they are taken as a solution (Nguyen *et al.*, 2012; Rossander-Hult *et al.*, 2018). Furthermore, adequate intake of calcium, as well as phosphorus in the appropriate ratio (1-2:1), is vital for bone health and infant development (Loughrill *et al.*,2017).

Table 4. Concentrations of total phenolic and total flavonoid in *Spirulina platensis*.

Nutrient	Concentration
Total phenolic (mg GAE/g)	2.99
Total flavonoid (mg QE/g)	1.92

The computed molar ratios in this study demonstrated that there is no impairment of nutrient absorption due to interactions between minerals or between minerals and phytate. Gutiérrez-Salmeán *et al.* (2015) also reported no inhibition of *spirulina* nutrient absorption due to the presence of antinutritional factors. Furthermore, due to the lack of cellulose in *spirulina* cell wall, *spirulina* tends to be easily digested by the human digestive system (Dillon and Phucb, 1995; Karkos *et al.*, 2011). In this study, the safety of *spirulina* for human consumption was evaluated based on heavy metals concentration. Our

findings revealed that *Spirulina platensis* has a very little amount of mercury, lead, cadmium, and arsenic as compared with the recommended maximum level of heavy metals in foodstuff set by the European Commission (Europen Commission Regulation (EC), 2016). Moreover, studies reported that *spirulina* has neither an acute nor chronic toxicological effect on the human body (Naidu *et al.*, 2009; Gutiérrez-Salmeán *et al.*, 2015; Neumann *et al.*, 2018). Besides, *spirulina* is among the foods which are generally recognized as safe by the US Food and Drug Administration (FDA, 2011).

Table 5. The concentration of heavy metals in *Spirulina platensis* and the suggested maximum level of heavy metals in foodstuff (mg/kg).

Metal	Sample (mg/kg)	[a] Maximum level (mg/kg)
Mercury	0.000036	0.1
Lead	0.0047	0.1
Cadmium	0.00048	0.05
Arsenic	0.0047	0.1

[a] European Communities Commission Regulation (EC) No 1881/2006 of 19 December 2006. Setting maximum levels for certain contaminants in foodstuff.

Conclusion

In light of its nutritional potential, we thus concluded that *spirulina platensis* cultivated in East Africa is a safe source of essential nutrients. Therefore, its production, as well as utilization, should be highly promoted as a sustainable source of alternative food supplements to address under nutrition in the region.

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