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ORIGINAL ARTICLE

Biomass and nutritive value of Spirulina (*Arthrospira fusiformis*) cultivated in a cost-effective medium

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Abstract

Introduction Cultivation of spirulina at commercial-scales relies on analytical grade–based media, which are expensive and so are the product.

Purpose This study assessed the biomass, proximate composition, and other useful compounds in Spirulina (*Arthrospira fusiformis*) produced with a cost-effective culture medium (LCMA), and the results were compared with those from a standard Zarrouk medium–grown spirulina.

Methods The LCMA medium was formulated by using a commercial NPK10-20-20 fertilizer as a source of the three major nutrients for spirulina growth, and other three ingredients from Zarrouk medium. The experiment was conducted for 28 days in the glass aquaria under indoor conditions. Standard analytical methods were applied for the determination of proximate composition, chlorophyll, minerals, and vitamins in the spirulina biomass.

Result The LCMA medium showed the best growth conditions by accumulating higher chlorophyll content (0.99 ± 0.02%) and dry weight (0.75 ± 0.01 g/100 ml) as well as attaining higher optical density (2.06 at day 15) earlier than the Zarrouk medium. The results of the proximate analysis for spirulina cultured in the LCMA medium were of good quality, with the protein contributing more than 50% of its dry matter. It was further noticed that the LCMA was an ideal medium for optimization of vitamins and some minerals since it recorded a significant amount of most of the analyzed vitamins together with the minerals sodium and potassium compared with the Zarrouk medium.

Conclusion It is suggested that LCMA medium could be used as the alternative and cheap medium for maximization of biomass and production of useful biochemical compounds in spirulina species.

Keywords Spirulina · *Arthrospira fusiformis* · Biomass production · Biochemical composition · NPK10-20-20 fertilizer · LCMA medium

Introduction

Spirulina is the term used for the dry biomass of edible and toxic-free cyanobacteria of the genus *Arthrospira* (Sharoba 2014). The species are obligate alkaliphiles thereby surviving in warm, higher alkaline lakes of the tropical, and sub-tropical countries where other organisms rarely would survive (Belay 2008). The ability to flourish in extreme pH is a strategy of cyanobacterial species to avoid contamination by other micro-organisms (Touloupakis et al. 2016). For instance, in the soda lakes of East Africa, the *A. fusiformis* dominates other micro-flora and it almost forms a uni-algal bloom (Fužinato et al. 2010). In Tanzania, the *A. fusiformis* is abundant in the Lake Big Momela (Lugomela et al. 2006; Mulokozi 2016) and is the major food for Lesser Flamingo (Lugomela et al. 2006). Spirulina is nutritionally complete with a balanced amount of

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all beneficial nutrients. It contains high quality protein, which range between 50 and 70% of its dry weight (Falquet 1997; Hosseini et al. 2013), essential amino acids and fatty acids, vitamins, and dietary minerals (Belay 2008; Sharoba 2014; Gutiérrez-Salmeán et al. 2015). Furthermore, the biomass is very rich in antioxidants such as phenolics, flavonoids, vitamin E, and various light absorbing pigments (e.g., phycocyanin, chlorophylls and carotenoids), which are also essential in preventing the body against free radicals (Kumar et al. 2005; El-Baky et al. 2008; Chu et al. 2010; Michael et al. 2018). Due to the exceptional nutritive profile, spirulina has received much attention, and is cultivated massively in health-food industries to serve as food for human, animals, feed additive and pharmaceutical products (Kumar et al. 2005; Habib et al. 2008; Chu et al. 2010; Chen 2011).

The mass production worldwide is however constrained with the high cost of the growth medium, which mainly depends on the Zarrouk's medium (Belay 2008; Habib et al. 2008; Madkour et al. 2012; Tarko et al. 2012). The medium is expensive due to the analytical grade ingredients it composes. However, there are several efforts from different researches, which have been made to develop a convenient and a cost-effective culture media (Raoof et al. 2006; Chen 2011; Gami et al. 2011; Madkour et al. 2012; Kumari et al. 2015) that can produce spirulina biomass of comparable quality to the Zarrouk medium. For instance, Raoof et al. (2006) incorporated some nutrients of the Zarrouk's medium with other cost-effective chemicals to produce a less expensive culture medium known as RM6. At the end of the investigation, it was found that the new medium was less expensive in terms of cost of production but, in addition, it produced protein profile similar to that of Zarrouk (Raoof et al. 2006). Similarly, Kumari et al. (2015) formulated a cost-effective medium for mass production of spirulina by using NPK10-26-26 fertilizer of which the biomass and protein were superior over several standard media tested. Therefore, in joining effort to come up with a cost-effective medium for maximization of biomass while maintaining a good quality of spirulina, the present study was conducted to evaluate and compare the biomass and biochemical composition of spirulina (*Arthrospira fusiformis*) cultivated using the NPK-based medium versus Zarrouk medium.

Materials and Methods

Microalgae

The strain of *Arthrospira fusiformis* used in this study was obtained from the stock culture kept at the Institute of Marine Sciences, University of Dar es Salaam, Tanzania. The culture was previously isolated from the algal samples collected from Lake Big Momela, Tanzania (Mulokozi 2016). At the Institute, the culture was maintained in 2000-ml Erlenmeyer flasks in Zarrouk medium at the outdoor conditions. Prior to onset of this study, the culture was checked under the microscope for contamination detection and was purified by raising the pH and serial dilution techniques to obtain the uni-algal culture, although it did not reach axenic conditions.

Formulation of a low-cost medium with NPK fertilizer

The low-cost medium termed as LCMA was formulated by mixing four ingredients (Table 1). All the ingredients for the LCMA except the trace element solution, are of commercial grade and locally available. The major elements (nitrogen, phosphorus, and potassium) for spirulina growth in the LCMA medium were from NPK10-20-20 complex fertilizer, a common and well-known fertilizer for growing crops. NPK 10-20-20 is granular and water-soluble, composing of 10% of ammoniacal nitrogen (NH_3-N), 20% phosphorus pentaoxide (P_2O_5), and 20% potassium oxide (K_2O), with trace amount of sulfur. Sodium bicarbonate was added in spirulina medium as a source of carbon while sodium chloride offered the chloride for ideal salinity of the medium. The micronutrients are needed for proper growth of spirulina. NPK10-20-20 is cost-effective and easily accessed in the shops of agricultural inputs, whereas 1 kg costs only 2000 Tanzanian Shillings (≈ US $ 1).

Spirulina inoculation and cultivation

Table 1 shows the chemicals and composition of the culture media used in the experiment. The analytical grade ingredients for Zarrouk were purchased from a laboratory equipment

Table 1 Chemical compositions of the LCMA and Zarrouk media used in spirulina biomass production (Michael et al. 2018)

Component	Concentration (g/l)	
	Zarrouk	LCMA
$NaHCO_3$	18	10
NaCl	1	1
$MgSO_4{\cdot}7H_2O$	0.2	-
NaEDTA	0.08	-
$CaCl_2{\cdot}2H2O$	0.04	-
$NaNO_3$	2.5	-
K_2SO4	1	-
K_2HPO_4	0.5	-
$FeSO_4{\cdot}7H_2O$	0.01	-
NPK10-20-20 complex	-	0.5
Micronutrient	1 ml	1 ml
Distilled water	1 l	-
Boiled, cool tap water	-	1 l

Micronutrient composition (g/l): H_3BO_3, 2.86; $MnCl_2{\cdot}4H_2O$, 1.81; $ZnSO_4{\cdot}4H_2O$, 0.222; Na_2MoO_4, 0.0177; $CuSO_4{\cdot}5H_2O$, 0.08

and chemical supplier in Zanzibar (Zan-Lab Equip.) while the NPK10-20-20 fertilizer was obtained from the authorized dealer of agricultural inputs farmers in Dar es Salaam, Tanzania. Spirulina was cultivated in the formulated reduced-cost medium (LCMA) and standard medium known as Zarrouk, and the results of biomass and biochemical composition were compared among the two media. The experiment was carried out for 28 days in the growth chamber located at the Department of Botany, University of Dar es Salaam. Three aquarium tanks, each with 10 l carrying capacity were set for each Zarrouk and LCMA, inoculated with 100 ml of spirulina culture (0.038 g dry biomass) and 1900 ml of the medium. The culture was incubated at a temperature ranging from 28 to 30 °C under continuous illumination with white light emitting diodes (LEDs) supplying 4.5 klux light intensity at the surface of the vessels and a photoperiod of 12/12 h light/dark cycle. The pH of the culture before inoculation was recorded. The culture was continuously agitated using aerators fixed on the air pump in order to prevent clump formation. The light intensity was measured using a light meter (Testo 540 AG, Germany), while pH was measured using a pH meter (H196107 HANNA, Italy).

Spirulina productivity

Productivity was determined by measuring the dry weight, chlorophyll contents, and optical density (OD) at specific time intervals. The percentage performance in productivity (OD and dry weight combined) was calculated by multiplying the ratio of daily production and initial biomass by 100. The dry weight was determined on weekly basis by filtering a 100-ml culture sample through dried pre-weighed Whatman GF/C filter No. 1 paper (11 μm, 80 mm in diameter). The filtered biomass was washed with distilled water to remove adsorbed salts, oven dried at 60°C overnight and then left to cool. The filter paper containing dry spirulina was then weighed (SHIMADZU AVU 220), and the difference in weight between the first (fresh) and last (dry) was the dry weight, which was expressed as weight per volume (g/100 ml).

The optical density was determined in 3 days' intervals at the wavelength of 680 nm with UV-visible spectrophotometer (Jenway 6305) and 10-mm path length cuvette. The total chlorophyll content in spirulina biomass was determined spectrophotometrically after extraction with acetone and diethyl ether according to Quarmby and Allen (1989). Briefly, accurately weighed 0.1 g of the dry ground spirulina was dissolved in 30 ml aqueous acetone (85% v/v), frozen over night to allow cell membranes to rupture and extraction of pigments. The extracted sample was filtered, homogenized and an aliquot filtrate (25 ml) was transferred to a separating funnel. To the aliquot, 50 ml of diethyl ether was added for further extraction of the non-polar pigments, and water was added until the chlorophyll pigments passed into ether layer. The ether phase was transferred to a volumetric flask and anhydrous sodium sulfate (Na_2SO_4) added for drying out the water. The absorbance of ether containing chlorophyll was measured at 660 nm and 643 nm wavelengths using a UV-visible spectrophotometer (Jenway 6305) and a 10-mm path length cuvette. The composition of chlorophyll in the spirulina biomass was then calculated as per the following equations:

$$\text{Total chlorophyll (\%)} = \frac{\text{C (mg/l)} \times \text{ether aliquot (ml)} \times \text{acetone extraction (ml)}}{10^4 \times \text{acetone aliquot (ml)} \times \text{sample weight (g)}}$$

whereas C = chlorophylls in ether solution = 7.12 × OD660 + 16.8 × OD643: OD, optical density.

Proximate analysis

The proximate analysis was conducted to estimate the moisture, crude protein, crude lipids, fiber, soluble carbohydrate, ash, and digestible energy in the spirulina biomass. Analysis of moisture followed the procedure described by Quarmby and Allen (1989). The moisture content was determined by oven drying the fresh biomass of spirulina at 60°C overnight; the percentage loss in weight after drying was the moisture part of spirulina. For crude protein, a semi-micro Kjeldahl digestion followed by indophenol-blue colorimetric method (Emteryd 1989; Quarmby and Allen 1989) was used to determine the concentration of total nitrogen in the spirulina biomass. The percentage of crude protein was then calculated by multiplying the concentration of organic nitrogen by the factor of 6.25.

The crude lipid in spirulina dry biomass was analyzed according to Bligh and Dyer (1959). This involved the addition of the mixture of organic solvents containing chloroform, methanol, and water in the ratio of 1:2:0.8 to the polyethylene vial containing 5 g of spirulina powder. The mixture was homogenized under oxygen free-nitrogen and left to settle before the addition of chloroform and water to make the ratio of 2:2:1.8. After the extraction, the filtrate was transferred into a graduated separating funnel and a volume of chloroform layer which contains lipid was noted. The upper alcoholic layer (methanol/water) was discarded. The chloroform was evaporated under vacuum by using rotavapor (Heidolph, Germany) at a temperature of 40°C. The residue was oven dried and weighed, and the percentage of total lipids was calculated as per the following formula:

$$\text{Total lipids (\%)} = \frac{\text{residue weight (g)} \times \text{volume of chloroform layer (ml)} \times 100}{\text{Aliquot (ml)} \times \text{sample weight (g)}}$$

The total soluble carbohydrate (CHO) was extracted by hot water and calorimetric procedures described by Allen (1989). In brief, 1 g of spirulina powder was weighed into 100-ml

Erlenmeyer flask and 30 ml of distilled water was added. The flask was heated at boiling point of the water for 2 h; afterwards, the contents were filtered using Whatman filter No. 1 paper. The concentration of carbohydrate was determined by reading the absorbance at 625 nm in the spectrophotometer after adding an anthrone reagent to the filtrate. The crude fiber was analyzed by acid hydrolysis followed by alkali extraction method of which the weight of fiber was determined gravimetrically (Quarmby and Allen 1989; AOAC 1999). Briefly, 1 g of spirulina powder was weighed in a Pyrex beaker and added in boiling sulfuric acid (1.25% v/v). The mixture was washed with boiling water to remove the acid and then added to 1.25% sodium hydroxide for extraction. The alkali solution was removed from the fiber by a suction pump. The fiber was washed with boiled water and then oven dried at 105°C for 3 h. The weight of fiber in spirulina was then obtained gravimetrically. The total ash in spirulina biomass was determined by combusting the dry sample at 550°C in the muffle furnace for 2 hr.

Determination of digestible energy

The total digestible energy in spirulina samples was determined by adding the three sources of energy, i.e., crude protein, carbohydrate, and lipid according to Sharoba (2014), with the following formula:

$$\text{Total energy (kcal/100 g)} = [(\%\text{carbohydrate} \times 4) + (\%\text{protein} \times 4) + (\%\text{fat} \times 9)]$$

Determination of multi-minerals

The concentrations of macro-elements (calcium, potassium, sodium, and magnesium) and trace elements (manganese, zinc, and iron) in the spirulina biomass were determined by acid oxidation method as described by Emteryd (1989) and Girmshaw et al. (1989). The powdered sample of spirulina (0.1 g) was dissolved in the mixture of 1 ml per-chloric acid ($HClO_4$) and 5 ml of nitric acid (HNO_3) in the ratio of 1:5. The sample was digested at 120°C until it became colorless and the volume reduced to 1 ml. The volume was made up to 100 ml with distilled water and the concentration of individual minerals was determined using Atomic Absorption Spectrophotometer (AA240 Varian, USA).

Preparation of spirulina extract for analysis of vitamins

The extract was prepared by soaking 0.5 g of dry ground biomass of spirulina in a conical flask containing 100 ml of 95% ethanol. The sample was continuously stirred to ensure complete extraction. The extract was centrifuged for 10 min then filtered using Whatman No. 1 filter paper. Ethanol was evaporated from the supernatant in a rotary evaporator (Gmbh & Co.KG, Germany) under reduced pressure at 40°C. The obtained extract was kept in a refrigerator at 4 °C until further analyses.

Determination of vitamin content

Spirulina is a rich source of vitamin B groups and other potential vitamins, which are needed by the animal bodies for normal growth and health. The concentrations of vitamin B complex present in spirulina extracts for both LCMA and Zarrouk media were determined by the method of Rajput et al. (2011). In this method, the working solution for the standard vitamins and spirulina samples were prepared by dissolving a known weight of the standard vitamin and extracts in a known volume of distilled water (diluent) into a volumetric flask. Vitamins B1, B2, B3, B6, and B12 were determined from riboflavin, nicotinamide, pyridoxine hydrochloride, and cyanocabalamin stock solutions, respectively. The absorbance of the working solutions, spirulina samples, and blank were read at 430 nm for vitamin B1, 444 nm for vitamin B2, 450 nm for vitamin B3, 650 nm for vitamin B6, and 530 nm for vitamin B12 using a UV-visible spectrophotometer (Jenway 6305).

The concentration of vitamin E was determined from a calibration curve prepared using α-tocopherol solution according to the method of Rutkowski and Grzegorczyk (2007). In brief, 1 ml of spirulina ethanolic extract was poured into a test tube, centrifuged at 1500 rcf for 1 min, and 0.5 ml of the supernatant collected. To the supernatant, 0.25 ml of batophenanthroline (6.02 nM) was added, followed by 0.25 ml of iron chloride ($FeCl_3$) and 0.25 ml of phosphoric acid (H_3PO) then mixed by vortex. The absorbance of a resulting solution mixture was measured using the UV-visible spectrophotometer at 539 nm, and the concentration of vitamin E in spirulina extracts was expressed in IU.

The concentration of vitamin K in spirulina extract was determined spectrophotometrically at 635 nm from the calibration curve prepared using vitamin K standard. The procedures for preparation of the standard and sample solutions followed the method of Ashok and Kumar (2012).

Statistical analysis

All measurements were done in triplicate, and results expressed as mean ± standard deviation (SD). The statistical differences in the biomass and biochemical compositions of spirulina cultured in Zarrouk and LCMA media were tested using a two-sample *t* test of the Graph pad Instat program (version 3.06, 2003). Before the analysis, the data were subjected to the normality test using Kolmogorov–Smirnov, and

if the data behaved differently from the normal distribution, a non-parametric test such as Mann–Whitney test was used.

Results and discussion

Formulation of a low-cost medium with NPK10-20-20 complex fertilizer

Several trials were made before reaching the right proportion of NPK fertilizer in the LCMA medium. In the first attempt, the medium composing 0.8 g/l of NPK-10-20-20 was made for growing spirulina. The growth was very poor, and in 2 weeks the culture appeared yellowish-green indicating existence of too much nitrogen in the culture. Another medium made of 0.65 g/l of NPK10-20-20 was tested for spirulina growth; however, spirulina did not flourish well and so the experiment was terminated in 2 weeks. The last attempt by using 0.5 g/l of NPK10-20-20 provided a promising result, and the medium was considered an alternative to Zarrouk and used for further experiment of spirulina culture. The LCMA medium was found to be cost-effective in comparison with Zarrouk medium, in terms of quantity of chemical ingredients whereas the NPK complex fertilizer supplied the three major elements required for spirulina growth, and little amount of sodium bicarbonate was used to supply carbon in the LCMA medium.

Change in pH with spirulina growth

The pH of the culture increased progressively as shown in Fig. 1. The pH raised as the sodium bicarbonate, which is the source of carbon, dissociated to make hydroxyl ions (OH^-) available in the medium. Since spirulina is an alkaliphile, its growth was flourishing with increase in pH of the culture. However, the effect of pH on biomass production was not observed from day 21 when the pH dropped, the dry weight and optical density (OD) continued to increase (Figs. 1 and 2a, b). This is in contrast to the observation by Touloupakis et al. (2016) for the cyanobacterium *Synechocystis* PCC 6803, whereby the increase in pH above 7.5 caused a decrease in the production of dry weight. There was a lag phase of 3 to 4 days for spirulina cultured in the LCMA medium, this was a time for the organism to adapt to the culture conditions, and later the growth bloomed to even overshoot Zarrouk's medium (Fig. 2a, b). The LCMA was found to be the best growth medium where it attained higher OD (2.06) earlier, at day 15 compared with Zarrouk medium, of which the highest OD was 1.74 attained at day 21. It was noticed that LCMA medium had accumulated relatively higher dry weight, 0.75 ± 0.01 g/100 ml (equivalent to 0.254 mg/ml/day) against 0.69 g/100 ml (0.234 mg/ml/day) for Zarrouk medium. Similarly, the chlorophyll content (Fig. 2c) was higher for the LCMA (0.99 ± 0.02%) compared with the Zarrouk medium (0.91 ± 0.04%). In terms of percentage of productivity, LCMA performed better than Zarrouk, whereas it was 59% versus 54% for LCMA and Zarrouk media, respectively, though the difference in productivity was insignificant between the two media. The values of dry biomass obtained in the present study for the formulated medium (LCMA) are higher compared with previous studies, which used commercial fertilizer–based media for spirulina mass production, such as Raoof et al. (2006) using RM6 medium, Madkour et al. (2012) who substituted Zarrouk's ingredients with locally available chemicals, and Kumari et al. (2015) using NPK10-26-26. However, total chlorophylls are incomparable due to

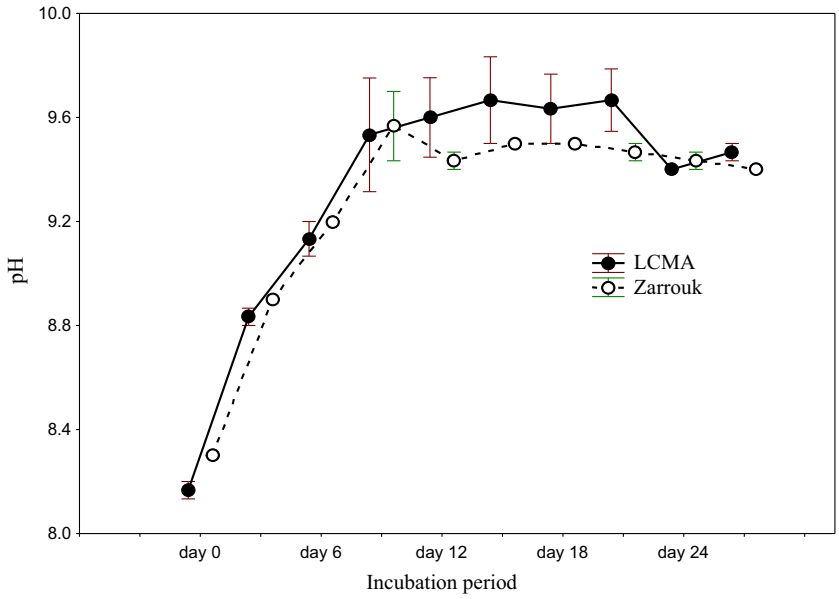
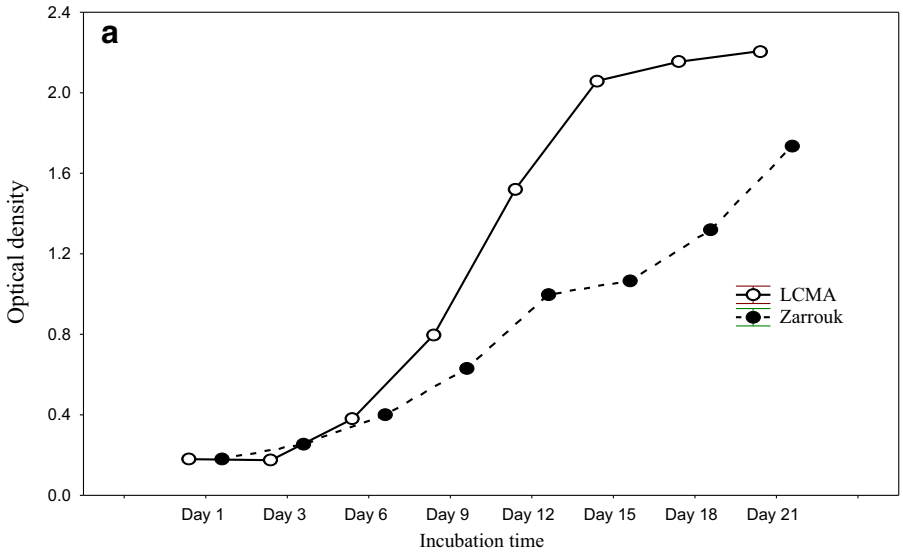
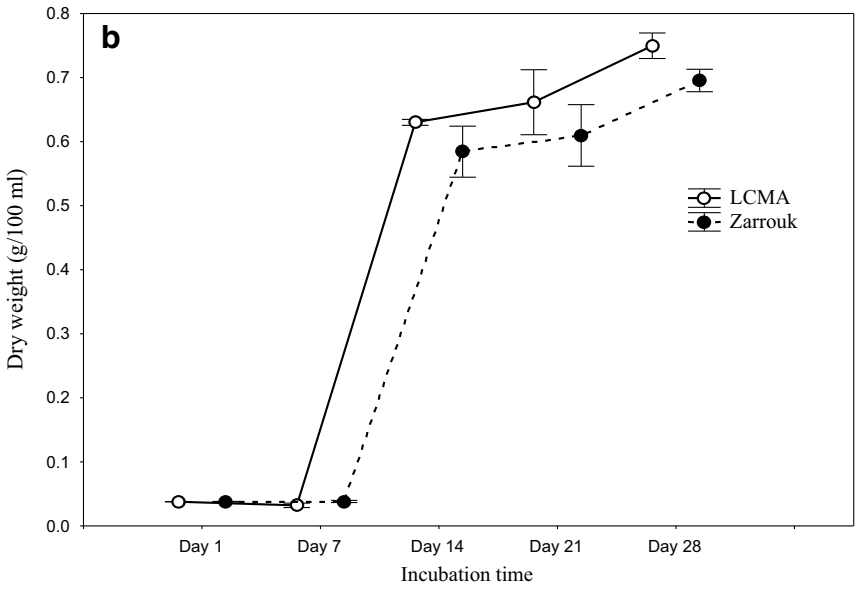


Fig. 1 Change in the pH during spirulina cultivation in the two media





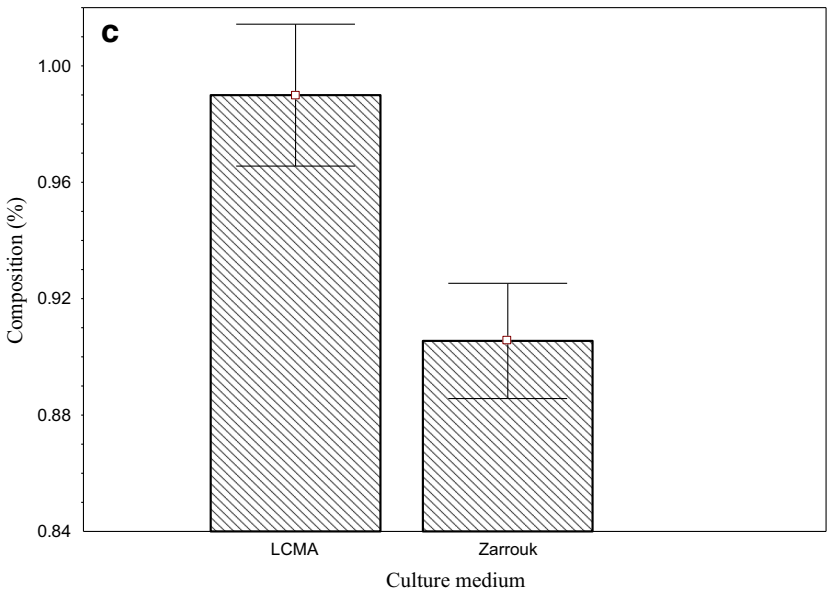


Fig. 2 Comparison of spirulina's growth and productivity between LCMA and Zarrouk media. **a** Change in OD with culture period; **b** change in dry weight with culture period; and **c** chlorophyll content at the end of the culture experiment

difference in analytical methods. Nevertheless, the differences in biomass and chlorophyll between the two media could possibly be due to the difference in the forms of nitrogen source. Studies show that ammonia nitrogen is easily assimilated than nitrate-nitrogen, which requires a reduction process to free the nitrogen (Madkour et al. 2012; Kumari et al. 2015).

Proximate composition

Proximate composition of spirulina biomass was significantly affected by the culture media except the ash content, which did not differ. The results for the proximate analysis are presented in Table 2. Among the analyzed components, the crude protein had a large percentage of spirulina's dry weight and it was higher for spirulina cultivated in the Zarrouk medium. Nevertheless, the level of protein was low in LCMA; it was still in the acceptable range (50–70%) reported by Falquet (1997) and Hosseini et al. (2013), and this justifies the use of a cost-effective medium for spirulina mass cultivation. The difference in protein content between the two media was possibly due to the difference in nitrogen composition, since Zarrouk medium contained more nitrogen from sodium nitrate (2.5 g/l) while LCMA had only 0.5 g/l from NPK10-20-20 complex fertilizer.

It was similarly found that spirulina from the Zarrouk medium contained significantly higher crude lipid ($p = 0.0003$, $t = 8.669$), crude fiber ($p = 0.0034$, $t = 5.217$), moisture ($p = 0.0011$, $U' = 36.000$), and digestible energy ($p < 0.0001$, $t = 36.187$), except for the soluble carbohydrate, which was high in the LCMA ($p = 0.0033$, $t = 17.440$). However, the ash content did not show significant variations ($p = 0.0986$, $t = 1.821$). Comparing the results of the present study with earlier findings for different species of *Arthrospira*/*Spirulina*, total lipids are higher than reported by Belay (2008), similar to those of Habib et al. (2008) but are lower than reported by Kumari et al. (2015) who also worked on production with NPK-based medium. For the crude fiber, the amount recorded in both Zarrouk and LCMA are higher than reported by Belay (2008), Habib et al. (2008), and Gutiérrez-Salmeán et al. (2015) but LCMA's values agree with the findings of Sharoba (2014). On the other hand, the higher carbohydrate content recorded in the LCMA was associated with a limitation of major elements, of which LCMA had lower composition compared to Zarrouk medium. Studies show that microalgae are able to alternate their metabolic strategies by accumulating higher carbohydrate or lipids when the environment is nutrient limiting (Markou et al. 2012). However, the values from both media were in a range previously reported by Aikawa et al. (2012), which is 8–17% for *Spirulina platensis*. The higher energy value in spirulina cultivated in the Zarrouk was mainly contributed by the higher contents of protein and lipid. A lower content of moisture observed in spirulina biomass cultivated in LCMA medium was potential for spirulina to accumulate more dry matter hence biomass maximization. However, the difference in moisture content between the two media could be due to early harvest of the biomass in Zarrouk medium, as LCMA reached a maximum growth before the standard medium, but both were harvested at the same time.

Table 3 Composition of minerals in spirulina cultured in the cost-effective medium and standard medium. Mean ± SD, $n = 3$

Component	Composition (mg/100 g)	
	LCMA medium	Zarrouk medium
Potassium (K)	1352.26 ± 0.90	1332.32 ± 1.78
Sodium (Na)	1185.36 ± 9.72	1150.36 ± 2.86
Calcium (Ca)	105.40 ± 0.73	115.19 ± 0.14
Magnesium (Mg)	142.82 ± 0.19	178.20 ± 0.34
Iron (Fe)	15.26 ± 0.02	23.80 ± 0.09
Manganese (Mn)	0.95 ± 0.01	1.46 ± 0.01
Zinc (Zn)	0.97 ± 0.01	2.07 ± 0.01

Mineral content in spirulina biomass

Minerals are essential nutrients, which are required by the body of animals and human to maintain the good health, though the amount needed is of minute quantity. The body of animals cannot make the minerals, but they solely depend on the food they take. It was found that *A. fusiformis* contains substantial amounts of both macro and micro-elements (Table 3). It was further noticed that potassium among the macronutrient is an abundant element in spirulina biomass. There were significant variations among the mineral compositions whereas spirulina biomass from LCMA exhibited

Table 2 Proximate compositions of spirulina biomass cultivated in the LCMA and Zarrouk media. Mean ± SD, $n = 3$

Culture media	Composition (%)						kJ/100 g
	CP	CL	CHO	CF	Ash	MO	Energy
LCMA	52.85 ± .39	6.61 ± 0.06	15.29 ± 0.41	9.79 ± 0.35	9.55 ± 0.24	5.45 ± 0.17	329.89 ± 5.32
Zarrouk	65.00 ± 0.26	6.84 ± 0.05	13.62 ± 0.64	11.37 ± 0.39	9.93 ± 0.44	9.92 ± 0.08	379.58 ± 8.68

CP crude protein, *CL* crude lipid, *CF* crude fiber, *CHO* carbohydrate, *MO* moisture

higher amount of sodium ($p = 0.04$, $t = 4.821$) and potassium ($p = 0.0006$, $t = 39.387$). On the other hand, higher amounts of calcium ($p = 0.0014$, $t = 26.834$), magnesium ($p < 0.0001$, $t = 120.05$), manganese ($p = 0.0003$, $t = 57.451$), iron ($p < 0.0001$, $t = 147.61$), and zinc ($p < 0.0001$, $t = 125.11$) were recorded in spirulina from Zarrouk medium. The study relates higher amount of sodium and potassium in spirulina cultured in LCMA medium with the good uptake of minerals from the medium and tap water. In similar context, the minerals recorded in spirulina cultivated in Zarrouk are those among the medium ingredients, which are not present in the LCMA medium. In comparison to other studies, the amounts of almost all minerals for both LCMA and Zarrouk media were lower than reported by Gutiérrez-Salmeán et al. (2015) except sodium and zinc from Zarrouk medium.

Vitamins

Vitamins are nutritional compounds, which are required in small amount but are very important for the wellbeing and health maintenance of the body of animals and humans. In this study, it was found that LCMA medium produced a vitamin-rich spirulina compared with Zarrouk (Table 4). The statistical analysis showed that LCMA medium had significant higher amount of vitamin E ($p = 0.003$, $t = 56.714$), vitamin K ($p < 0.0001$, $t = 177.57$), vitamin B2 ($p < 0.001$, $t = 353.34$), vitamin B3 ($p = 0.0004$, $t = 50.575$), and vitamin B12 ($p = 0.0439$, $t = 4.612$) with the exception of vitamin B1 ($p = 0.0056$, $t = 13.288$) and B6 ($p < 0.0001$, $t = 884.00$) that were high in Zarrouk medium. The compositions of vitamins recorded in this study are relatively higher than for other *Arthrospira* species (Belay 2008; Gutiérrez-Salmeán et al. 2015). Surprisingly, spirulina biomass recorded the highest composition of vitamin B3 than all other vitamins in both media, and this was contrary to earlier reports, which show the vitamin B12 to be higher among the B-group vitamins (Madkour et al. 2012; Hosseini et al. 2013; Sharoba 2014).

Table 4 The composition of vitamins in spirulina cultivated in the NPK-based medium (LCMA) and analytical grade–based medium (Zarrouk). Mean ± SD, $n = 3$

Component	Composition (/100 mg)	
	LCMA	Zarrouk
Vitamin E	19.78 ± 0.02 IU	18.45 ± 0.04 IU
Vitamin K	28.38 ± 0.03 mg	24.24 ± 0.02 mg
Vitamin B1	41.62 ± 0.43 mg	44.59 ± 0.06 mg
Vitamin B2	49.57 ± 0.04 mg	39.37 ± 0.08 mg
Vitamin B3	183.05 ± 0.60 mg	165.92 ± 0.02 mg
Vitamin B6	3.32 ± 0.02 mg	9.21 ± 0.03 mg
Vitamin B12	4.73 ± 0.02 mg	4.29 ± 0.17 mg

However, the variation in vitamins and other nutritional constituents among researches could be due to several factors including the culture medium, place where the experiment was conducted, species, culture condition, and analytical method used (Gutiérrez-Salmeán et al. 2015).

Conclusions and recommendation

The LCMA medium has only four chemical ingredients from which the NPK10-20-20 complex fertilizer supplied the major macronutrients for spirulina growth. This significantly cuts the cost of purchasing individual chemical ingredients. In the production of biomass and accumulation of chlorophyll pigments, the LCMA gave better results and attained a maximum optical density earlier than Zarrouk medium. The observed statistical differences in proximate composition, minerals, and vitamins would not affect the quality of spirulina cultivated in LCMA as the compositions recorded in the present study are reliable and conform to earlier findings. The study indicates that LCMA would be an ideal culture medium for maximization of vitamins and some minerals such as sodium and potassium, because the compositions of many of the vitamins were high in spirulina produced in LCMA medium. The study therefore suggests the use of LCMA medium for mass cultivation of spirulina species as it is cheap and produces reliable quality. But in order to establish the base of the quality of spirulina produced in LCMA medium, we recommend further studies to quantify the amino acids and essential fatty acids as they are also affected by culture medium among other factors.

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Data analysis and interpretation: AM MSK CVL
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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Involvement of human and animals in the study N/A

Informed consent N/A

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