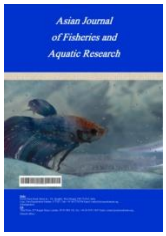

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Proximate Composition Analysis of *Spirulina platensis* in Lab Scale Cultivation: Prospects of Digested Rotten Guava as a Culture Media

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

An experiment was conducted to evaluate the proximate composition of *Spirulina platensis* and growth performance in supernatant of different concentrations (supernatant of 20, 40 and 60 rotten guavas) of digested rotten guava medium (DRGM). The growth rates in terms of optical density, dry cell weight and chlorophyll a of spirulina were varied from each other. The cell weight of Spirulina was attained a maximum of 0.818 ± 0.003 mg/L in 60% DRGM followed by 0.815 ± 0.0015 and 0.809± 0.0012 mg/L in supernatant of 20 and 40%, respectively on the 10th day of culture. Similar trend was also observed in the case of optical density of the media contained Spirulina, chlorophyll a content (mg/L), total biomass (mg/L), specific growth rates (on the basis of cell weight and chlorophyll a) and total biomass of Spirulina. Cell weight of Spirulina grown in these media had highly significant ($P < 0.01$) correlation with the chlorophyll a content ($r = 0.746$) and total biomass ($r = 0.742$) of Spirulina. The growth performance of Spirulina in supernatant of 60% DRGM was significantly ($P < 0.01$) higher than that of grown in 20% and 40% DRGM. The percentage of crude

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protein was found 53.35 ± 0.32% in supernatant of 40% DRGM. The crude lipid was attained of 10.15 ± 0.14% in supernatant of 60% DRGM which was significantly (P < 0.05) higher than that of grown in 20% and 40% of DGRM.

Keywords: Spirulina platensis; proximate composition; growth performance; digested rotten guava medium (DRGM).

1. INTRODUCTION

"With the aim of increased aquaculture production through applying adequate feed, there are a large numbers of feed industries are developed in Bangladesh. Due to increased aquaculture practice, demand of good quality feed is increasing day by day. Prime quality feed is essential for fish growth. Maintain feed conversion ratio (FCR) close to 1 is highly depends upon good feed. Feed should have adequate protein content which facilitates high growth. Net protein utilization should be around 27%" [1]. But fish meal and bone meal are not available in Bangladesh. So, we can find to alternative sources. We can use to alternative fish meal to spirulina. Spirulina is a "superfood" which is the most nutritious, rich in protein and concentrated whole food known to humankind [2]. "It has a vibrant history occupied an intriguing biological and ecological niche in the plant kingdom. Spirulina is a spiral-shaped, blue-green microalgae that grows naturally in the wild in freshwater alkaline lakes, natural springs, and saltwater. Its deep blue green color is what gives the water greenish hue" [3]. "Spirulina is also cultivated and harvested in man-made reservoirs around the world. For centuries, civilizations the world over have cultivated and cherished spirulina for its health-improving benefits. The Aztecs harvested the spirulina from Lake Texcoco in Mexico. It grows well in supernatant of different digested agro-industrial wastes available in Bangladesh" [4].

Spirulina may be grown in agro-industrial wastes [5], rotten fruits [6], chicken wastes [7]. Among fruits huge quantity of guava spoils (rotten) in different markets in the country. Therefore, market is allowed to digest (aerobic & anaerobic) and supernatant may be used for the growth of spirulina. This inexpensive low-cost medium may be used to produce *Spirulina platensis* culture which can contribute significantly for the development of fisheries and fish production. It takes inorganic nutrients for the supernatant and grows. "Many factors are important for the production of spirulina at large scale, of which most important factors are nutrient availability, temperature and light. The filamentous cyanobacteria such as spirulina are found to be most compatible microorganisms for the utilization of waste and wastewaters as they are able to produce large quantity of biomass and their harvesting is also relatively easy because of their structure. Also, these wastes reduce the cost of nutrient medium and act as a source of cheap nutrient medium for cultivation of spirulina" [8]. "The commercial production of spirulina can be made cost effective by reducing the input cost with cheap and readily available materials without sacrificing the production efficiency. They are very small a microscopic and 300-500 micrometer in length. Spirulina contains 50-70% protein, 10-12% carbohydrate, 6% fat, 7% minerals and a lot of vitamins" [8]. "However, according to the researchers, one kg of *Spirulina spp* is similar to 1000 kg of other vegetables" [9].

Spirulina has been studied for single cell protein (SPC), vitamins, minerals, proteins and polyunsaturated fatty acids (γ-linolenic acid), therapeutic properties, antioxidant activity. Several cultivation methods like open ponds, tubular photo bioreactors, inclined glass panels have been tried. Cost and composition of cultivation media along with growth rate of the algae we challenging factors for commercially viable production. The most convincing trials are of course those conducted among populations which traditionally eat spirulina. Culture and growth performance of *Spirulina platensis* in supernatant of digested rotten guava to evaluate the proximate composition of spirulina; to analyze growth parameters 30 in supernatant digested rotten guava; and to find out the suitable concentration of the medium for maximum growth of *Spirulina platensis.*

2. MATERIALS AND METHODS

2.1 Study Area

The study carried out in Live Food Aquaculture Laboratory, Department Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, and Bangladesh.

2.2 Collection of Rotten Guava

The rotten guava was selected as medium for *Spirulina platensis* culture. It was collected from Kamal Ronjit market (K.R.) of Bangladesh Agricultural University, Mymensingh-2202, and Bangladesh. It was thought that the proximate composition of this media might be suitable for the growth of culture species.

2.3 Analysis of Proximate Composition of Rotten Guava (RG)

Before media preparation, the proximate composition of rotten guava was analyzed to know its nutritional status. The analysis was performed in Fish Nutrition Laboratory, Department Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, and Bangladesh, following standard methods [10].

2.4 Moisture

The sample was weighed in a previously pre-weighed small crucible in triplicates. The samples contained in the crucible were dried to moisture from it 105^{o}C for 24 hrs. After drying, the samples with crucible were cooled with the help in a desiccator. Then, cooling at room temperature and weighed in a sensitive balance. The percentage of moisture of the sample was calculated using the following equation:

$$\%\ \text{moisture} = \frac{X - Y}{X} \times 100$$

Where,

X = Weight of sample before drying; and
Y= Weight of sample after drying.

2.5 Crude Protein

Kjeldhal Auto 1030 Analyzer was used for determination of crude protein content of samples. A sample of 0.5g and a blank were digested in the digestion tube. 10 ml of concentrated sulphuric acid (H_2SO_4), 2.0 ml of H_2O_2 and one Kjeldhal tablet were added in the tubes and mixed gently by electric mixer. Then, the digestion tubes were set in digestion chamber fixing at 420°C for 45 minutes. The digestion the tubes were allowed to cool and 75 ml of distilled water was added in each tube. 50 ml of 40% NaOH was added before titration. After titration with 1% boric acid and 0.2 N HCl the reading for the samples and blank were recorded. The readings were calculated the following formula:

Percentage of nitrogen

$$= \frac{\text{Milliequivalent wt. of N} \times \text{ml of titrant} \times \text{strength of HCl}}{\text{Samples wt. (g)}} \times 100$$

For animal, % Protein = % Nitrogen x 6.25; and
For plant, % Protein = % Nitrogen x 5.85

2.6 Crude Lipid

Lipid content was estimated by solvent extraction of lipid using Soxlet apparatus. 2.0 g dried samples were taken into extraction thimbles to place into the extraction unit along with the weighed extraction cups having 50 ml of solvent as acetone. Extraction of first 15 minutes was in the boiling position and the cups were released and dried in the oven for 30 minutes. The percentage of crude lipid was determined using the following equation:

$$\%\ \text{Lipid} = \frac{\text{Weight of cup with lipid} - \text{initial weight of cup}}{\text{weight of sample (g)}} \times 100$$

Ash: The pre-weighed crucible containing dried sample from the moisture determination was pre-ashed. The samples were kept into a muffle furnace at 550°C for 6.0 hrs. The crucible containing ash was cooled in a desiccator. The percentage of ash was determined by using the following:

$$\%\ \text{Ash} = \frac{\text{Weight of ash with preweighed crucible} - \text{weight of crucible}}{\text{weight of dried sample}} \times 100$$

NFE: NFE were calculated the following formula:

Nitrogen Free Extract = 100 - (Moisture + Crude protein + Crude lipids + Ash).

2.7 Estimation of Cell Weight (Dry Weight) of Spirulina (Clesceri et al., 1989)

Sample containing 15 ml spirulina suspension was filtered through a Sartorius filter paper of mesh size 0.45 µm and diameter 47 mm. The filter papers were dried in an oven for 24 hrs. overnight at 70°C and weighed prior to filtration. The filtered samples were washed three times to remove insoluble salts. After that the filter papers were put in a glass petri dish and kept in the oven at 70°C overnight. For cooling, petri dish

was put into desiccator for 20 minutes and then filter papers were weighed. The dry weight of algae on the filter paper was measured using the following equation:

Dry weight (mg/L),

$$W = \frac{FFW - IFW}{Amount\ of\ sample\ taken\ for\ filtration\ (ml)} \times 100$$

Where,

W = Cell dry weight in mg/L;
FFW = Final filter paper weight in g; and
IFW = Initial filter paper weight in g.

2.8 Estimation of Chlorophyll a of Spirulina (Clesceri et al. 1989)

The samples of *Spirulina platensis* were collected in different times and chlorophyll a content of *S. platensis* was estimated. Ten ml of *S. platensis* sample were filtered with an electric filtration unit using filter papers (Sartorius filter paper of 0.45 µm mesh size and 47 mm). These filtered samples together with filter paper was taken into test tubes, ground with glass rod and finally mixed with 10 ml of 100% redistilled acetone. Each of the test tubes was wrapped with foil papers to inhibit the contact of light. The wrapped test tubes were kept into a refrigerator (LMS Laboratory Refrigerator) over night. Then the refrigerated samples were homogenized for 2 minutes followed by centrifugation at 4000 rpm for 10 minutes. After centrifugation, the supernatant was isolated and taken for chlorophyll a determination. Optical densities of the samples were determined at 664 nm, 647 nm and 630 nm by using UV spectrophotometer (Milton Roy, Spectronic 1001 plus) [Clesceri et al. 1989]. A blank with 100% acetone was run simultaneously.

Chlorophyll a content was calculated by the following formula:

Chlorophyll a (mg/L) = 11.85 (OD 664) – 1.54 (OD 647) – 0.08 (OD 630)

2.9 Total Biomass of Spirulina (*S. platensis*)

Total biomass was calculated using the following formula given by Vonshak and Richmond [11]:
Total biomass = Chlorophyll a x 67.

Specific growth rate (SGR) on the basis of dry weight, chlorophyll a content and total biomass of spirulina (Clesceri et al. 1989): Specific growth rate (µ/day) of cultured spirulina on the basis of dry weight.

SGR (µ/day) = In $(X_1-X_2)/t_1-t_2$

Where,

X_1 = Dry weight of biomass concentration of the end of selected time interval;
X_2 = Dry weight biomass concentration at beginning of selected time interval;
And t_1-t_2 =Elapsed time between selected time in the day.

Specific growth rate (µ/day) of cultured Spirulina on the basis of chlorophyll a

SGR (µ/day) = In $(X_1-X_2)/t_1-t_2$

Where,

X_1 = Chlorophyll a at the end of selected time interval;
X_2 = Chlorophyll a at the beginning of selected time interval;
And t_1-t_2 = Elapsed time between selected time in the day.

Specific growth rate (µ/day) of cultured Spirulina on the basis of total biomass
SGR (µ/day) = In $(X_1-X_2)/t_1-t_2$

Where,

X_1 = Total biomass at the end of selected time interval;
X_2 = Total biomass at the beginning of selected time interval; and
t_1-t_2 = Elapsed time between selected time in the day

2.10 Culture and Collection of *Spirulina platensis*

Spirulina platensis was collected from the stock in the live food culture laboratory, Department of aquaculture, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202, and Bangladesh. Nine conical flasks (2L capacity) were used for the culture of spirulina.

2.11 Maintenance of Pure Stock Culture of *Spirulina platensis*

Pure stock culture of *Spirulina platensis* was maintained in the laboratory [12]. “Growth of *Spirulina platensis* were observed at every alternative day and was checked under microscope to confirm its purity following some keys given” by Bold and Wynne [13], Vymazal [14] and Phang and Chu [15].

2.12 Preparation of Digested Rotten Guava Media (DRGM)

The compositions of Rotten Guava Medium (RGM) were prepared for culture of *Spirulina platensis*. To decompose in 5.0 L glass bottle 50 g/L rotten guava was allowed for 34 days under aerobic condition in the Live Food Culture laboratory, Department of Aquaculture, BAU, Mymensingh. Then a Light reddish white colored supernatant from bottle was diluted and made three concentrations at the rate of 20%, 40% and 60% digested rotten guava. Then the supernatant of three different concentrations were taken in 1.0 L flask with three replications. For the preparation of rotten guava medium, digested and continuous aeration 5 litter volumetric flask was filtered with plankton net after (10.10.18 to 14.11.2018) 34 days left. Then, using distilled water in three replications, the filtered rotting guava was diluted and added 0.8 g (0.2 g/L) urea in accordance with the aforementioned instructions. The medium was then thoroughly mixed before being sterilised in a high pressure, bumping water autoclave for 15 minutes at 115°C. Before growing microalgae, the media were left in the autoclave for three days to ensure that there were no contaminants. The method used during the creation of digested rotten guava media was mixed, autoclaved, and cooled.

2.13 Experimental Design of *Spirulina platensis* Culture

Three types media viz., Rotten guava (RG) were used to culture *Spirulina platensis.* Inoculum of *Spirulina platensis* was collected from the pure stock culture. Experimental design is shown in (Table 1).

2.14 Analysis of Proximate Composition Spirulina

Best optical density of spirulina (*S. platensis*) was found on 10th day of culture. In that day, spirulina was filtered, collected in petridish and kept in an oven at 40°C for overnight for drying. Then the proximate composition of cultured *S. platensis* was analyzed in the Fish nutrition Laboratory, Faculty of Fisheries, BAU, Mymensingh by standard methods [10]. All the procedures for determination of proximate composition, the procedures as described in the Chapter 3.2.2 were followed.

2.15 Statistical Analysis

"Analysis of variance (ANOVA) of mean cell weight and chlorophyll a of *S. platensis* cultured in different media (treatments) were done and to find whether any significant among treatment mean was done by Ducan's Multiple Range Test (DMRT) at 5% level of probability" [16].

3. RESULTS

3.1 Proximate Composition (%) of Spirulina *(Spirulina platensis)*

There was no significant variation among the crude protein of *Spirulina* grown in the supernatant of three different DRG (Table 2). The percentage of crude protein of *Spirulina* was 53.25 ± 0.32, 53.35 ± 0.34 and 53.28 ± 0.32% when grown in the supernatant of 20, 40 and 60% DRG media, respectively. There was no significant ($P > 0.05$) variation among the crude protein percent of spirulina cultured in three different media of DRG. There was no significant ($P > 0.05$) difference of crude lipids of spirulina when cultured in the supernatant of 20, 40 and 60% digested rotten guava. Ash (%) of *Spirulina* grown in supernatant of 20% (10.22 ± 0.13%) 40% (10.16 ± 0.17%) and 60% (10.33 ± 0.21%) digested rotten guava. There was no significant ($p> 0.05$) difference among the ash of s*pirulina* grown in supernatant of 20, 40 and 60% DRG. There was no significant variation among the NFEs of *spirulina* grown in supernatant of 20, 40 and 60% DRG as the values found for 20% (16.28 ± 0.32%), 40% (16.10 ± 0.18%) and 60% (15.99 ± 0.22%), respectively.

Very small amount of crude fibre (%) was found in *spirulina* grown in the supernatant of three different digested rotten guava (DRG). However, it was varied from 0.69 ± 0.0% when *Spirulina* grown in the supernatant of 40% DRG to 0.71 ± 0.04% and 0.71 ± 0.03% when cultured in the same of 60% DRG, respectively (Table 2).

3.2 Optical Density of Media Contained Spirulina

Optical density (OD) of media contained spirulina was found to increased up to 10th day of culture in all the media of digested rotten guava (DRG), and Kosaric medium and then decreased up to 14th day of experiment (Fig. 1). However, highest OD of 20% DRG culture contained spirulina was 0.631 ± 0.0023, where highest OD of 40% DRG culture contained spirulina was found 0.704 ± 0.0015. The OD of supernatant of 60% DRG contained spirulina was 0.725 ± 0.0012.

3.3 Chlorophyll a of Spirulina

Chlorophyll a of spirulina was found also higher on 10th day of culture than other days of culture in supernatant of all the media (Fig. 2). Chlorophyll a of spirulina increased from first day (0.00158 ± 0 g/L) up to 10th day (0.770 ± 0.0012 g/L) of culture in 20% digested rotten guava media (DRTM) and then decreased up to 14th day (0.710 ± 0.0012 g/L) of experiment. However, chlorophyll a of spirulina cultured in supernatant of 40% DRG was 0.768 ± 0.0012 g/L on 10^{th} day and then decreased up to 14th day (last day) of culture. Chlorophyll a of spirulina grown in supernatant of 60% DRG was 7.365 ± 0.20 g/L on 10th day from first day (0.0016 ± 0) and then decreased up to 14th day (last day) of experiment (Fig. 2).

3.4 Total Biomass of Spirulina

Total biomass of spirulina was increased from first day (0.106 ± 0.003) mg/L up to 10th day (67.77 ± 0.44 mg/L) in the culture of 20% digested rotten guava media (DRGM) and then decreased up to 14th day (47.57 ± 0.42 mg/L) of experiment. The highest total biomass of spirulina grown in the culture of 40% DRGM was recorded 51.46 ± 0.28 mg/L on 10th day of culture and then decreased up to 14th day (42.35 ± 0.19 mg/L) during the experiment. Total biomass of spirulina cultured in the culture of 60% DRGM was increased from first day (0.107 ± 0.04 mg/L) up to 10th day (57.75 ± 0.20 g/L) and then decreased up to 14th day (41.88 ± 0.14 mg/L) of experiment (Fig. 3).

3.5 Comparison of Growth Parameters of Spirulina

Optical density of 20% DRG (0.631 ± 0.002) ,40% DRG (0.704 ± 0.0015) and 60% DRG (0.725 ± 0.0012). There was no significant ($P > 0.05$) difference among optical densities of 20, 40 and 60% DRG during the study. There was no significant ($P > 0.05$) difference of cell weight of spirulina grown in 20, 40 and 60% DRG. Chlorophyll a of spirulina grown in 60% DRG (0.862 ± 0.0012 mg/L) was significantly ($p < 0.05$) higher than that of spirulina cultured in 40% (0.768 ± 0.0012 mg/L) and 20% DRG (0.770 ± 0.14 mg/L). There was no significant difference among the Chlorophyll a of spirulina grown in supernatant of 20, 40 and 60% DRG during the study (Table 3).

3.6 Correlation among the Growth Parameters of Spirulina

Cell weight of spirulina (*Spirulina platensis*) had highly significant ($p< 0.05$) direct correlation with chlorophyll a ($r = 0.746$) of spirulina grown in the supernatant of different digested rotten guava. Similarly, total biomass of *S. platensis* was highly ($p< 0.05$)and directly correlated with chlorophyll a ($r = 0.795$) of spirulina cultured in the supernatant of various digested rotten guava. Again, total biomass of spirulina was found to be highly ($p< 0.05$) and directly correlated with the cell weight ($r = 0.742$) of spirulina grown in the supernatant of different digested rotten guava.

3.7 Specific Growth Rate (SGR) in Respect to Total Biomass of Spirulina

The SGR in respect to total biomass of spirulina was significantly ($P < 0.05$) varied from that of spirulina grown in the supernatant of 20, 40 and 60% DRG. There was no significant ($P < 0.05$) difference recorded among the SGRs on the basis of total biomass of *S. platensis* grown in the supernatant of 20, 40 and 60% DRG (Table 4).

Table 1. Experimental design for *Spirulina platensis* culture using supernatant of three different concentrations of digested rotten guava (DRG)

Types of medium	Treatments	Replications	Amounts rotten guava (%)	Duration of culture (days)
Supernatant of DRGM	1	3 (101, 102 and 103)	20	14
	2	3 (201, 202 and 203)	40	
	3	3 (301, 302 and 303)	60	

Table 2. Proximate composition (% in dry matter basis) of *Spirulina platensis*

Treatments	T1 (20% DRG)	T2 (40% DRG)	T3 (60% DRG)
Moisture	9.44 ± 0.05	9.55 ± 0.04	9.53 ± 0.05
Crude Protein	53.25 ± 0.32[b]	53.35 ± 0.34[b]	53.28 ± 0.32[b]
Crude Lipids	10.10 ± 0.16[a]	10.14 ± 0.17[a]	10.15 ± 0.14[a]
Ash	10.22 ± 0.13[b]	10.16 ± 0.17[b]	10.33 ± 0.21[b]
NFE*	16.28 ± 0.32[a]	16.10 ± 0.18[a]	15.99 ± 0.22[a]
Crude Fiber	0.70 ± 0.04	0.69 ± 0.03	0.71 ± 0.04

**NFE (Nitrogen Free Extract) = 100 - (Moisture + Crude protein + Crude lipids + Ash). Figures in common letters in the same row do not differ significantly at 5% level of probability*

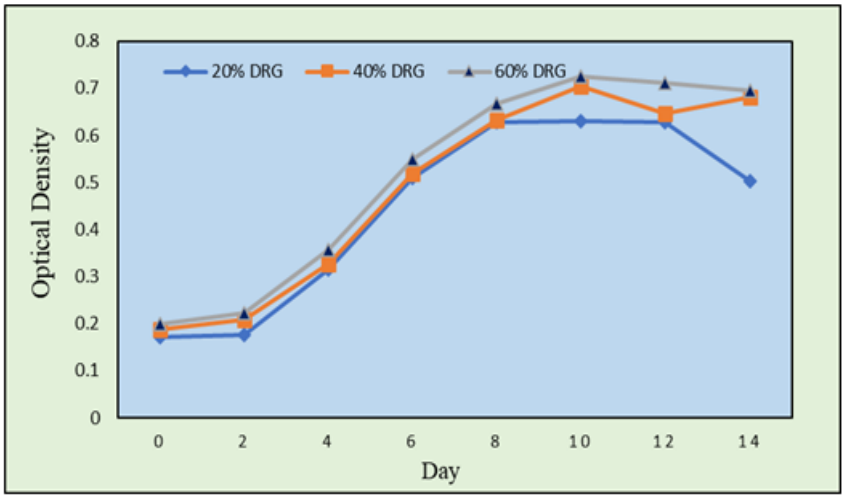

Fig. 1. Mean values of optical density of media contained *Spirulina platensis*

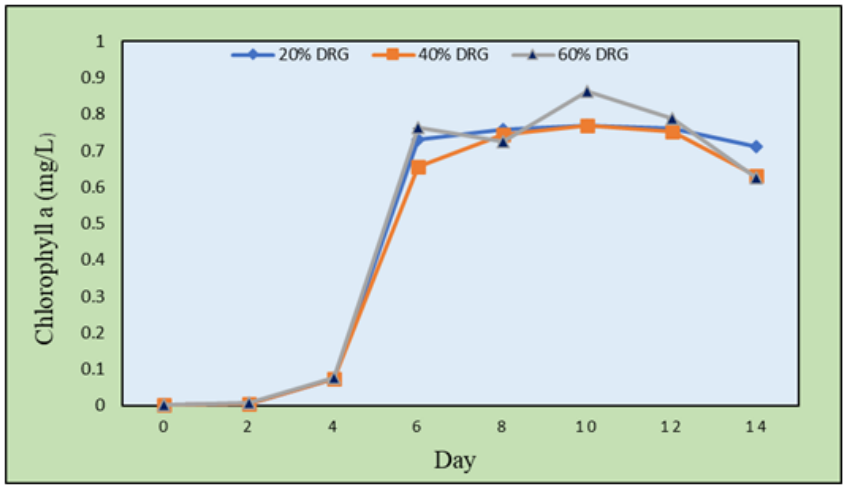

Fig. 2. Mean values of chlorophyll a (mg/L) of *Spirulina platensis*

4. DISCUSSION

"The cell weight of *Spirulina platensis* in supernatant of digested rotten guava were found 0.0023 to 0.815 mg/L in 20% digested rotten guava media (DRGM), 0.0024 to 0.809 mg/L in 40% DRGM, 0.0023 to 0.818 mg/L in 60% DRGM. The growth performance of *Spirulina platensis* in supernatant of 60% DRGM was found better than 20% and 40% DRGM. This variation might be due to the differences in nutrient concentrations and composition of varied

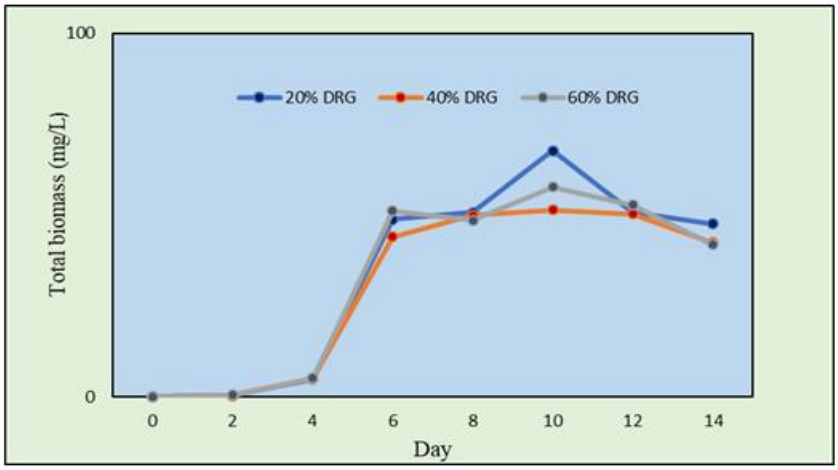

Fig. 3. Mean values of total biomass (mg/L) of *Spirulina platensis* where is distribution total biomass (0 to 100)

Table 3. Comparison of cell weight, chlorophyll a and total biomass of *Spirulina platensis*

Parameters	T1 (20% DRG)	T2 (40% DRG)	T3 (60% DRG)
Optical density	0.631 ± 0.002^{b}	0.704 ± 0.0015^{b}	0.725 ± 0.0012^{b}
Cell weight (mg/L)	0.815 ± 0.0015^{b}	0.809 ± 0.0012^{b}	0.818 ± 0.0013^{b}
Chlorophyll a (mg/L)	0.770 ± 0.14^{b}	0.768 ± 0.0012^{b}	0.862 ± 0.0012^{b}
Total biomass(mg/L) *	67.77 ± 0.43^{b}	51.46 ± 0.28^{c}	57.75 ± 0.20^{bc}

**Total biomass = Chlorophyll a x 67 [11]. Figures in common letters do not differ significantly at 5% level of probability*

Table 4. Specific growth rates (SGRs) on the basis of cell weight, chlorophyll a and total biomass of *Spirulina platensis*

Parameters	T1 (20% DRG)	T2 (40% DRG)	T3 (60% DRG)
SGR of cell weight	0.21 ± 0.010^{b}	0.22 ± 0.009^{b}	0.21 ± 0.011^{b}
SGR of Chlorophyll a	0.22 ± 0.004^{b}	0.21 ± 0.003^{b}	0.23 ± 0.004^{b}
SGR of total biomass	0.54 ± 0.012^{b}	0.52 ± 0.012^{b}	0.53 ± 0.013^{b}

Figures in common letters in the same row do not differ significantly at 5% level of probability

media" [17]. "On the other hand 40% DRGM showed lower growth performance of *Spirulina platensis* in relation to 20 and 60% DRGM. This might be due to lower nitrogen and phosphate concentration of the nutrients in the media" [18]. During the present study, digested organic medium like rotten guava which has the similarity with the findings of Dineshkumar et al. [19] and Sukumaran et al. [20]. During culture of *Spirulina platensis*, the exponential phase was found up to 10th day from the beginning and then the cell weight declined i.e., stationary phase started. Satter [4] recorded "the cell weight and chlorophyll a content of *S. platensis* was significant (P<0.05) higher in 4.0 g/L digested poultry waste than other media where light intensity, aeration and temperature played significant role to the culture system".

Similarly, Sharker [21] conducted "an experiment on the culture of *Spirulina platensis* in various concentrations viz., 0.3, 0.4 and 0.5 g/L of papaya skin powder medium (PSPM) and Kosaric medium (KM) in the laboratory for three months carried out for a period of 12 days". In the present study, the chlorophyll a content of inoculated *Spirulina platensis* was 0.0015 mg/L which attained a high content of 0.862 mg/L in 60% DRGM at the 10th day of culture. These findings are not more or less similar with the findings of Phang et al. [22], Habib et al. [23] and Satter [4]. This might be due to lower nitrogen and phosphate concentration of the nutrients in the media. Dineshkumar et al. [19] studied that *Spirulina platensis* grew well in natural medium such as Conway medium, Zoarrouic medium and kosaric medium.

In the present study, supernatant of digested rotten guava was used as a media of three concentrations for the culture of *Spirulina platensis.* The supernatant of 60% digested rotten guava showed maximum optical density on the 10th day of culture comparing with 20% and 40 % supernatant of DRGM which has the similarity with the findings of Habib et al*.* [23,24], Satter [4]. From the above discussion, the growth performance of *Spirulina platensis* in supernatant of 60% DRGM was found better than 20% and 40% DRGM. It can be said that the physical properties, chemical parameters and technical facilities used in the present study were more or less similar to those used by other researchers. This study finds out a suitable concentration of digested rotten guava medium (DRGM) as an organic nutrient medium for culture and growth of *Spirulina platensis*.

The experiment revealed the proximate composition, growth performance of *Spirulina platensis* where the initial cell weight was 0.0023 mg/L which attained a maximum cell weight of 0.818 mg /L in 60% DRGM, 0.809mg/L in 40% DRGM and 0.815 mg/L in 20% DRGM on the 10th day of the culture period. Similarly, the chlorophyll a content of inoculated *S. platensis* was 0.0015 mg/L which attained the highest content of 0.862 mg/L in 60% DRGM, 0.768 mg/L in 40% DRGM, 0.770 mg/L in 20% DRGM on the 10th day of culture period.

5. CONCLUSION

The percentage of crude lipids (10.15 ± 0.14%) of spirulina cultured in supernatant of 60% DRGM was significantly ($P < 0.05$) and almost two times higher than that of spirulina grown in 20% and 40%, respectively. The percentage of crude protein of *Spirulina* was 53.25 ± 0.32, 53.35 ± 0.34 and 53.28 ± 0.32% when grown in the supernatant of 20, 40 and 60% DRG media, respectively. There was no significant ($P > 0.05$) variation among the crude protein percent of spirulina cultured in three different media of DRG. There was no significant ($P > 0.05$) difference of crude lipids of spirulina when cultured in the supernatant of 20, 40 and 60% digested rotten guava. Ash (%) of *Spirulina* grown in supernatant of 20% (10.22 ± 0.13%), 40% (10.16 ± 0.17%) and 60% (10.33 ± 0.21%) digested rotten guava. There was no significant ($p>0.05$) difference among the ash of s*pirulina* grown in supernatant of 20, 40 and 60% DRGM. There was no significant variation among the NFEs of Spirulina grown in supernatant of 20, 40 and 60% DRG as the values found for 20% (16.28 ± 0.32%), 40% (16.10 ± 0.18%) and 60% (15.99 ± 0.22%), respectively. Specific growth rate (SGR) in respect to cell weight of spirulina grown in 60% digested rotten guava (DRG) medium was significantly ($P <0.05$) higher than that of spirulina cultured in the supernatant of 20, 40% DRGM. This medium may be used commercially as the collection and preparation of these organic media require little cost, less labour and is available throughout Bangladesh. However, it might be suggested that more research and cost-benefit analysis have to be performed to evaluate the grow-out potential of spirulina in lab-based cultivation. The culture techniques may dwindle the cost of production and might be considered as a media for *Spirulina platensis* cultivation.

COMPETING INTERESTS

Authors have declared that no competing interests exis.

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