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Evaluation of Chemical Composition for *Spirulina platensis* in Different Culture Media.

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ABSTRACT

Spirulina platensis was cultivated in laboratory under controlled conditions in four different culture media, BG-11, modified BG-11, Zarrouk´s (ZM) and synthetic human urine (SHU). The effect of culturing media on chemical composition, amino acids content, fatty acids profile and minerals content were determined. The highest amount of protein (59.8%) was recorded when grown in BG-11. Whereas, modified BG-11 was the best medium in regard to both amino acids contents and maximum total lipid (8.13%). The most important unsaturated fatty acid γ-linolenic, was found at maximum percentage (4.7%) when grown in SHU medium. Whereas ZM was the best medium to obtain the highest percentage of arachidoic acid (17.63%).The highest percentage of ash in *S. platensis* was recorded when grown in ZM. Regarding to the minerals content, the maximumP, Ca, Mg, Zn and Cu (182.7, 155.8, 8.4, 5.1 and 5.5 mg/100g DW, respectively) were recorded in BG-11, while growing in ZM displayed the highest amount of K, Na and Fe (593.4, 766.7 and 39.9 mg/100g, respectively).
Keywords: *Spirulina platensis*, chemical composition, amino acids, unsaturated fatty acids, minerals.

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INTRODUCTION

Spirulina is a photosynthetic, filamentous, multicellular blue green microalgae which grows in wide range fresh, marine and brackish water. It grows well in a highly alkaline environment of pH 10-12 (Soundarandian and Vasanthi, 2008). Due to the easy of *Spirulina* culturing, harvesting and drying process, it becomes the most common popular species in microalgal biotechnology studies (Belay, 2008 and Mani *et al*., 2008). Chemical analysis of *Spirulina* showed that it is an excellent source of proteins, lipids, vitamins, minerals, carbohydrates and pigments. The biochemical composition depends upon the *Spirulina* source, culture conditions and season of production (Phang *et al*., 2000, Habib *et al*., 2008 and Parages *et al*., 2012).

The protein content of *Spirulina* varies between 50 and 70% of its dry weight, exceeds that of meat, dried milk, eggs, soybeans or grains. *Spirulina* proteins are complete, since all the essential amino acids are present, forming 47% of total protein weight. The highest values for the essential amino acids are those for leucine, valine and isoleucine and the most poorly represented are the sulfur-containing amino acids methionine and cysteine (Becker, 2007 and Belay, 2008).

Lipid content in both *Spirulina platensis* and *Spirulina maxima* varies between 5.6 and 7% (Kay, 1991 and Habib *et al*., 2008). While, other authors reported that lipid content may reach 11% by using better extraction system (Hudson and Karis, 1974; Yoshida and Hoshi, 1980). Half of the total lipids in *Spirulina* are fatty acids (Cohen, 1997). Analysis of *Spirulina* fatty acids showed that polyunsaturated fatty acids (PUFAs) represented high percentage of total lipid reaching 30%. *Spirulina* is rich in γ-linolenic acid *i.e*. 36% of total PUFAs and also provides linoleic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid (Habib *et al*., 2008).

On the other hand, carbohydrates constitute 15-25% of *Spirulina* dry weight. Simple carbohydrates, glucose, fructose and sucrose are present only in very small quantities (Ciferri and Tiboni, 1985). The major polymeric component in *Spirulina platensis* is a branched polysaccharide, structurally similar to glycogen. Also high molecular weight anionic polysaccharides with antiviral and immunomodulating activities have been isolated from *Spirulina* (Parages *et al*., 2012 and Theodore and Georgios, 2013).

All the essential minerals are found in *Spirulina* with range 2.7 and 3.0% of dry weight under laboratory conditions. However, in commercial *Spirulina* production, minerals may reach 7% of dry weight (Theodore and Georgios, 2013). Meanwhile, *Spirulina* mineral content varies depending on culture medium and environmental conditions. Also, it is reported that *Spirulina* is a rich source of potassium and contains considerable amounts of calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium and zinc (Johnson and Shubert, 1986 and Habib *et al*., 2008).

Concerning the differences in chemical composition of *Spirulina platensis* in different culturing media, few reports are available in this respect. Therefore, and parallel to previous work regarding the impact of culturing media on biomass production and pigments content of *S. platensis* (Marrez *et al*., 2013), the objective of this study is to evaluate the influence of four culture media BG-11, modified BG-11, Zarrouk's and SHU media under controlled conditions on chemical composition of *Spirulina platensis*.

Materials and Methods

Microorganism

Spirulina platensis strain was isolated from Al-Khadra Lake, Wadi Al-Natroon, El-Baheira governorate, Egypt. Isolation and purification of *S. platensis* was performed by streaking plate method of Stein (1973) using BG-11 agar medium (Allen, 1973). Morphological identification of *S. platensis* was carried out using a phase contrast microscope (Carl Zeiss, Jena, Germany) according to Desikachary (1959), Prescott (1978) and Hindak (1988 and 1990).

Culture media

Four culture media were investigated for the cultivation of *S. platensis*: BG-11 medium (Rippka *et al*., 1979), modified BG-11 medium (El-Sayed, 2004), Zarrouk’s medium, ZM (Zarrouk, 1966) and synthetic human urine medium, SHU (Gordon, 1982).

The optimum growth conditions (30±2°C and light intensity of 4.5 Klux m^{-2}) provided by fluorescent lamps were applied for this study according to Rafiqul *et al*. (2005), Soundarandian and Vasanthi (2008), Hemlata and Fatma (2009) and Chauhan and Pathak (2010). Suspension of *S. platensis* containing 1.2 x 10^7 filament ml^{-1} was prepared to inoculate the tested media. *S. platensis* was cultivated in 500 ml Erlenmeyer flasks containing 250 ml of representative media using shaking incubator (MP-7552, cv-cc power supply, hsiHefer, San Francisco). Experiments were initiated with 10% (v/v) of inoculum. After 30 days of inoculation *S. platensis* biomass was harvested from each medium by centrifugation at 4500 rpm for 15 min and biomass was overnight dried in oven at 50°C (Marrez *et al*., 2013).

Chemical composition of *Spirulina platensis*

The moisture content, total ash, minerals (K, Na, Ca, Mg, P, Mn, Zn, and Cu) and crude fiber were determined according to AOAC (2000).Total carbohydrates were determined according to the Renol-Reaction method of Gerhardt *et al*. (1981).

Total protein was determined by the conventional Micro-Kjeldahl method (AOAC, 2000).

Amino acid profile was carried out according to Bailey (1967) using Eppendrof-Germany LC3000 amino acid analyzer. The flow rate was 0.2 ml/min, while pressure of buffer and reagent were from 0.0 to 50 bars and 0.0 to 105 bar, respectively, while reaction temperature was 123°C.

Total lipids were extracted from *S. platensis* dry weight according to the AOAC (2000) method using Soxhelet apparatus.

Fatty acid methyl esters (FAMEs) of the total lipid were prepared by transesterification using 2% sulphuric acid in methanol (Christie, 1993).The fatty acid analysis was done by gas chromatography (Perkin Elmer Auto System XL) equipped with flame ionization detector and a DB5silica capillary column (60 m ×0.32mm i.d.). The oven temperature was maintained initially at 45°C and programmed to 60°C at a rate 1°C/min, then it programmed from 60°C to 240°C at a rate of 3°C/min. Helium was used as the carrier gas at flow rate 1 ml min^{-1}. The injector and the detector temperatures were set at 230°C and 250°C, respectively.

Statistical Analysis

Statistical significance was measured using Statistica Version 9 (State Soft, Tulsa, Okla., USA).

RESULTS AND DISCUSSION

Effect of culture media on the chemical composition of *S. platensis*

The chemical composition of *S. platensis* grown in different culture media were determined (Table 1). In all cases, the moisture content in *Spirulina* was between 5 and 10%. The moisture content was significantly different ($p< 0.05$) between the tested media. The best medium exhibited highest *Spirulina* dry matter was BG-11, as it had the lowest moisture content (6.33%). Similar findings were reported by French (1979) and Flamant (1988). In general, the dry mater content was greater than 90% in all media. Interestingly, these results were much higher than those reported in nature from different sites in Chad (ITRAD, 2009) being 70% dry matter.

The protein content in *S. platensis* used during this study confined between the ranges of 49.47 and 59.79% of dry weight. Alvarenga *et al.* (2011) found that *Spirulina* protein content represented 58.2%, however, higher protein content (69.2%) were obtained by Mbaïguinan *et al.* (2006) in *Spirulina* from Kanem Lake Chad. The high protein content in *Spirulina* grown in BG-11 and modified BG-11 was probably due to the occurrence of high level of nitrogen in the medium. In this respect, Piorreck*et al.* (1984) reported that increasing the nitrogen level in the nutrient medium leads to an increase in the biomass and protein content in *Spirulina*.

The lipid content was significantly ($p< 0.05$) influenced by changing the culturing media and varied from 6.57% to 8.13%. Several authors had reported that *Spirulina* lipid values ranged from 5.6 to 7% (Earthrise, 1986 and Fox, 1996) or from 6 to 13% (Cohen, 1997 and Xue, 2002). The lipid content in *Spirulina* might be influenced by the extraction method or the type of used solvent. According to Danesi *et al.* (2002) blue green algae do not show any significant changes in the percentage and composition of lipids and fatty acids when grown at different concentrations of nitrogen. Ungsethaphand *et al.* (2009) pointed out that lipid level of *S. platensis* decreased when sodium bicarbonate added in nutritional medium. This finding supports the result in this study as Zarrouk medium showed the lowest lipid amount. Fidalgo *et al.* (1998) indicated that the use of urea for marine microalgae *Isochrysis galbana* resulted in higher lipids and fatty acids contents than the use of nitrate or nitrite. Also, Hsieh and Wu (2009) found that the growth rate and total lipid content of *Chlorella* sp.

also varied with the level of urea concentration during the cultivation. These results reinforce why total lipids content in modified BG-11 was higher than other culture media.

The ash content in *Spirulina* significantly differed ($p < 0.05$) from culture medium to another, with values of 13.0, 10.55, 8.57 and 8.40% for ZM, modified BG-11, SHU and BG-11 media were obtained, respectively. Habib *et al.* (2008) reported that many factors may affect the bioaccumulation of minerals in *Spirulina* and the most important were culturing on different media, at different temperatures, pH and salinity.

The crude fiber content of *S. platensis* differed significantly ($p < 0.05$) from medium to another ranged from 2.44% in BG-11 to 5.23 % in ZM medium. Albert *et al*. (2012) revealed that the fiber content in *Spirulina* varied from 4 to 7 %, which is close to the results of the present study. However, Moreiro *et al*. (2013) reported that crude fiber content in *S. platensis* was 0.5%. While, Habib *et al*. (2008) reported *S. platensis* fiber content ranged from 8 to 10%.

The carbohydrates content in *Spirulina* varied from 12.42% in modified BG-11 to 22.80% in SHU medium. Similar results obtained by Madkour *et al*. (2012) who reported that carbohydrates represented 23.2 - 24.5% of *Spirulina* dry weight.

Table 1: Chemical composition of *Spirulina platensis* grown in different culture media.

Chemical composition (%)	Growth medium			
	BG-11	Modified BG-11	Z.M	SHU
Moisture	6.33±0.04^{D}	7.12± 0.02^{C}	8.16±0.03^{B}	8.81±0.06^{A}
Crude protein	59.79±4.05^{A}	57.67± 1.79^{AB}	53.92±1.27^{B}	49.47±0.98^{C}
Ash	8.40±0.52^{C}	10.55±0.32^{A}	13.00±0.43^{A}	8.57 ±0.53^{C}
Total lipids	8.03±0.14^{A}	8.13±0.18^{A}	6.57±0.22^{B}	6.63±0.19^{B}
Crude fiber	2.44±0.09^{D}	4.11±0.18^{B}	5.23±0.22^{A}	3.72±0.21^{C}
Carbohydrates	15.01±0.43^{B}	12.42±0.27^{C}	13.12±0.69^{C}	22.80±0.73^{A}

(n=3), different letters are significantly different ($p < 0.05$)

Effect of culture media on amino acids contents

As shown in Table 2, the amino acids composition of *S. platensis* demonstrates considerable variations in response to the applied culture media. The amino acids profile in dried *S. platensis* biomass harvested from modified BG-11 medium contained the highest amount of essential amino acids as well as non-essential amino acids, except proline. The highest proline contents 30.54 mg g^{-1} was recorded in BG-11 medium. In general, aspartic, glutamic, alanine, leucine and proline represented the highest percentage in this respect.

Choi *et al* (2003) studied the effect of different nitrogen sources like ammonium, nitrite, nitrate and urea on growth and amino acids contents of *S. platensis*. They found that the amino acid content after 30 days of cultivation was the highest in urea group. Similar findings were obtained during this study, as all essential and non-essential amino acids recorded the highest amounts in modified BG-11 medium which contains urea instead of sodium nitrate as a source of nitrogen.

Table 2: Amino acids composition of *Spirulina platensis* in different growth media after 30 days of cultivation.

Amino acid	Amino acids concentration mg g^{-1} dry matter in different culture media (Mean±S.E)			
	BG-11	Modified BG-11	Z.M	SHU
Essential amino acids				
Isoleucine	11.23±0.91^{B}	14.16±0.98^{A}	11.32±0.87^{B}	7.43±0.84^{C}
Leucine	26.99±0.87^{C}	29.42±1.27^{A}	27.25±1.04^{B}	18.05±0.77^{D}
Lysine	11.70±0.58^{D}	19.10±1.01^{A}	18.24±0.94^{B}	13.98±0.77^{C}
Methionine	2.94±0.24^{C}	5.31±0.81^{A}	4.82±0.27^{B}	1.61±0.21^{D}
Phenylalanine	22.35±0.89^{C}	23.78±1.21^{A}	22.64±1.11^{B}	15.88±0.83^{D}
Threonine	9.42±0.66^{C}	13.59±0.87^{A}	13.00±0.71^{B}	9.68±0.68^{C}
Valine	14.49±0.77^{C}	18.40±1.41^{A}	15.51±1.14^{B}	11.87±0.72^{D}
Histidine	12.25±1.07^{B}	13.46±1.19^{A}	11.74±0.97^{C}	7.39±0.81^{D}
Non-essential amino acids				
Cystin	1.27±0.23^{C}	3.30±0.41^{A}	1.62±0.21^{B}	0.77±0.11^{D}
Alanine	25.47±0.62^{C}	33.81±1.21^{A}	26.41±0.77^{B}	21.04±1.01^{D}
Aspartic	23.19±0.77^{C}	36.69±1.09^{A}	33.12±0.83^{B}	18.82±0.51^{D}
Serine	10.87±0.88^{C}	18.43±1.14^{A}	13.84±0.98^{B}	10.00±0.73^{D}
Glycine	9.06±0.72^{C}	15.06±1.04^{A}	11.53±0.98^{B}	8.66±0.63^{D}
Glutamic acid	34.42±0.89^{C}	47.03±1.12^{A}	38.57±1.09^{B}	26.29±0.87^{D}
Arginine	22.68±0.81^{C}	44.91±1.02^{A}	38.36±0.92^{B}	21.79±0.88^{D}
Tyrosine	13.70±0.74^{D}	19.74±1.03^{A}	16.56±0.94^{B}	14.64±0.77^{C}
Proline	30.54±0.94^{A}	14.88±0.34^{D}	20.33±0.77^{C}	28.31±0.83^{B}
Ammonia	54.35±1.07^{B}	54.91±1.12^{A}	43.61±0.99^{C}	38.25±0.87^{D}

(n=3), different letters are significantly different ($p < 0.001$)

Effect of culture media on fatty acids profile

The percentages of fatty acids in lipids of *S. platensis* grown in different media are listed in Figure 1. In the present study, the percentage of unsaturated fatty acids ranged from 15.86 to 32.07%, while the saturated fatty acids ranged from 67.93 to 84.14%. The major fatty acids were palmitic acid (C16:0) ranging from 11.11 to 45.33% of total fatty acids, myristic acid (C14:0) ranging from 8.78 to 22.68 while haptadecanoic acid (C17:0) ranged from 2.3 to 18.8%.

The main concern in this study was the occurrence of polyunsaturated fatty acids (PUFA) in *S. platensis* which have a significant nutritional importance. Results in Figure (3) revealed that the PUFA ranged from 12.06 to 26.26% of total fatty acids, where the highest percentage of linoleic acid was recorded in SHU medium (8.29%) followed by modified BG-11(2.31%), Z.M (2.03%) and BG-11 medium (1.43%). The percentage of γ- Linolenic acid (GLA) were 4.7, 2.37, 1.43 and 1.01% when grown in SHU, modified BG-11, BG-11 and Z.M media, respectively. GLA, which is a commercially important PUFA, is effective in lowering plasma cholesterol and in stimulating prostaglandins and has been used as a dietary supplement for the treatment of various chronic health problems (Kay, 1991).The dry matter of *S. platensis* cultivated in ZM contained the highest percentage (17.63%) of arachidonic acid when compared with cultivation in other media.

Yilmaz *et al*. (2010) reported that values of the fatty acids oleic, linolenic and γ-Linolenic acid were increased by increasing of salinity from 10% to 30% in *Spirulina* medium.

This result explains the reason of increase these fatty acids in SHU medium, which contains high amount of sodium chloride than any other tested media.

Effect of culture media on minerals contents in *S. platensis*.

The minerals content (mg100g^{-1} dry matter) in *S. platensis* grown in different media is represented in Table 3. The phosphorous content was significantly influenced by the culture media (p< 0.01) and ranged from 89.7-182.7 mg/100g. These results are different from those of Cornet (1992) who reported that P content ranged from 670-900 mg100g^{-1}. Also, Koru *et al*. (2008) determined P in *Spirulina* which valued by 916 mg100g^{-1}. Likewise, Albert *et al*. (2012) reported that P values ranged from 207 - 357.5 mg100g^{-1} in *Spirulina* from different sites in Chad.

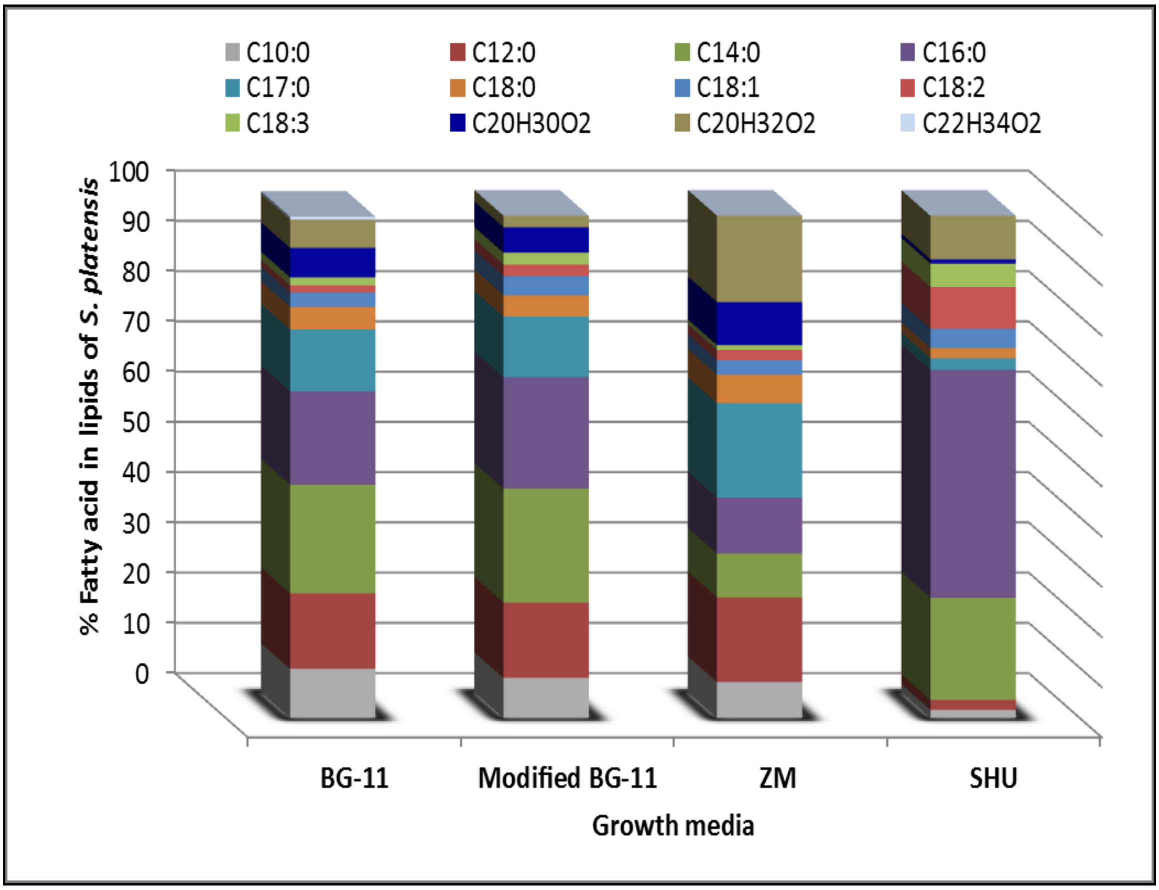

Figure 1: Fatty acids profile of *Spirulina platensis* lipid in different growth media after 30 days of cultivation.

Common name of C10:0, Capric acid; C12:0, Lauric acid; C14:0, Myristic acid; C16:0, Palmitic acid; C17:0, Margaric acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; C18:3, γ- Linolenic acid; $C_{20}H_{30}O_2$, Abietic acid; $C_{20}H_{32}O_2$, Arachidonic acid; $C_{22}H_{34}O_2$, Docospapentaenic acid.

The potassium contents in *Spirulina* were low (130.7 -593.4 mg100g^{-1}) in different media when compared with those reported by Koru *et al*. (2008) with average of 1800 mg100g^{-1}, while these values were high when compared with the range of 128 to 188 mg100g^{-1} that reported by Albert *et al*. (2012).

In addition, the highest sodium content 766.7 mg 100g^{-1} was recorded in Z.M, followed by 608.3, 216.7 and 105.7 mg100g^{-1} in SHU, modified BG-11 and BG-11 media, respectively. These values were lower than 1090 mg100g^{-1} that reported by Koru (2009). In this respect, Albert *et al*. (2012) found that there were positive correlation between potassium and sodium contents in *Spirulina* and its content in water. This may explain why potassium and sodium contents in *Spirulina* grown in ZM and SHU media were higher than that found when cultured in BG-11 and modified BG-11 media.

At the same time, the calcium content in *Spirulina* varied ($p< 0.01$) when grown in various evaluated media to another and ranged from 21.7 to 155.8 mg100g^{-1}. These values are higher than the range of 3.1 to 3.2 mg100g^{-1} that reported by Albert *et al*. (2012), but were lower than the value168 mg100g^{-1} that reported by Koru *et al*. (2008) and 468 mg100g^{-1} that reported by Belay (2008).

The magnesium contents ranged from 3.3mg/100g in SHU medium and 8.4 mg100g^{-1} in BG-11 medium. These values were high when compared with 1.8 and 7.85 mg/100g that reported by Albert *et al*. (2012), but lower than 900 mg100g^{-1} which reported by Babadzhanov *et al*. (2004).

The analysis of variance indicated significant differences ($p< 0.01$) in iron content in *Spirulina* cultures in different media. The highest content of iron (39.9 mg100g^{-1}) was recorded in ZM where the lowest content (10.1mg100g^{-1}) was scored in SHU medium. These values were low when compared with the values 53.6 mg100g^{-1} obtained by Koru *et al*. (2008) and 87.4 mg100g^{-1} obtained by Belay (2008).

The manganese contents were lower than 3.3 mg100g^{-1} in all tested media. These results are lower than that reported by Avino *et al*. (2000) and Koru *et al*. (2008).

Table 3: Minerals concentration (mg100g^{-1} DW) of *Spirulina platensis* in different growth media.

Mineral	Mineral concentration (Mean±S.E)			
	BG-11	Modified BG-11	Z.M	SHU
P	182.7 ±4.24^{A}	123.1±1.46^{B}	89.7±2.72^{D}	101.2±0.73^{C}
K	130.7±2.96^{D}	170.0 ±2.89^{C}	593.4±15.52^{A}	505.9±7.2^{B}
Ca	155.8±0.51^{A}	63.7 ±0.73^{B}	33.5±0.87^{C}	21.7±0.44^{D}
Na	105.7 ±0.67^{D}	216.7±4.41^{C}	766.7±45.30^{A}	608.3±22.05^{B}
Mg	8.4±0.09^{A}	6.2 ±0.06^{B}	4.9±0.09^{C}	3.3±0.08^{D}
Fe	28.4±1.08^{B}	12.4 ±0.16^{C}	39.9±0.25^{A}	10.53±0.17^{D}
Mn	1.3±0.09^{B}	0.72±0.04^{D}	0.79±0.05^{C}	3.2±0.061^{A}
Zn	5.1±0.71^{A}	2.6±0.21^{B}	1.9±0.07^{C}	0.52±0.01^{D}
Cu	5.5 ±0.19^{A}	5.1±0.66^{B}	2.4±1.84^{C}	1.4±0.11^{D}

n=3, different letters are significantly different ($p< 0.01$)

The zinc contents in *Spirulina* were 5.1, 2.6, 1.9 and 0.5 mg100g^{-1} in BG-11, modified BG-11, ZM and SHU media, respectively. These values were higher than that of 1 mg100g^{-1} obtained by Johnson and Schubert (1986) and 1.2 mg100g^{-1} reported by Babadzhanov *et al*. (2004)

The copper contents in *Spirulina* were 5.5, 5.1, 2.4 and 1.4 mg100g^{-1} in BG-11, modified BG-11, ZM and SHU media,respectively.These values were lower than 216mg/100g that reported by Albert *et al*. (2012), but higher than that obtained by Belay (2008) and Cornet (1992) who found that copper contents was 1.45 and 2.1 to 4 mg100g^{-1} in *Spirulina*, respectively.

CONCLUSION

The type of cultivation media plays an important role in the chemical composition of *S. platensis*. High yield of dry matter, protein and lipids were observed when grown in both BG-11 and modified BG-11. However, cultivation in SHU medium encourages production of carbohydrates when compared with other media. *S. platensis* from modified BG-11 was characterized by its high content of either essential or non-essential amino acids. Growing in ZM and SHU can gave good opportunity to yield *S. platensis* dry matter rich in unsaturated fatty acids arachidonic and γ- Linolenic acid, respectively. Also, high content of K and Na (> 0.5%) can be obtained when grown in ZM and SHU. Finally, the growth media could be selected regarding the nutritional value of the required product.

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