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Cover Page Footnote

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Effect of Mg^{2+} and Fe^{2+} Concentrations in Culture Medium on CGF Formation from Microalgae *Chlorella pyrenoidosa* Ink and Analysis of Amino Acids by Liquid Chromatography-Mass Spectrometry

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Abstract

Chlorella pyrenoidosa (*C. pyrenoidosa*) contains Chlorella Growth Factor (CGF), which consists of protein and polysaccharides. CGF is located inside the nucleus of cells and is beneficial to humans as a food supplement, an immunity booster, and an antioxidant. CGF formation of *C. pyrenoidosa* is influenced by medium composition. *C. pyrenoidosa* INK was cultured in a modified basal medium (MBM) with various concentrations of Mg^{2+} (0.5, 1.0, and 1.5 g/L) and Fe^{2+} (3.5×10^{-4} and 5.0×10^{-4} g/L). The experiments were run and analyzed under a completely randomized design using a 2-L bottle with three replications. The results showed that MBM with 1.0 g/L of Mg^{2+} and 3.5×10^{-4} g/L of Fe^{2+} yielded the optimal growth curve for *C. pyrenoidosa*. Analysis of protein content was carried out using the Lowry method with a spectrophotometer at λ=750 nm, and the obtained results were 0.0974 mg/mL (extract) and 6.4097 mg/ml (supernatant). Furthermore, analysis of glucose content was carried out using the phenol sulfate method (λ = 490 nm), and the obtained results were 49.331 ppm (extract) and 1566.911 ppm (supernatant). Analysis of amino acids in CGF using liquid chromatography-mass spectrometry (LC-MS) indicated the presence of tyrosine, proline, glutamate, alanine, valine, tryptopan, phenylalanine, methionine, and leucine-isoleucine.

Abstrak

Pengaruh Konsentrasi Mg^{2+} dan Fe^{2+} dalam Media Kultur terhadap Pembentukan CGF oleh Mikroalga *Chlorella pyrenoidosa* INK dan Analisis Asam Amino dengan Kromatografi Cair-Spektrofotometri Massa. *Chlorella pyrenoidosa* (*C. pyrenoidosa*) mengandung Chlorella Growth Factor (CGF), yang terdiri dari protein dan polisakarida. CGF terletak di dalam inti sel dan bermanfaat bagi manusia sebagai suplemen makanan, booster imunitas, dan antioksidan. Pembentukan CGF oleh *C. pyrenoidosa* dipengaruhi oleh komposisi medium. *C. pyrenoidosa* INK dikultur dalam media basal dimodifikasi (MBM) dengan berbagai konsentrasi Mg^{2+} (0,5, 1,0, dan 1,5 g/L) dan Fe^{2+} ($3{,}5\times10^{-4}$ dan $5{,}0\times10^{-4}$ g/L). Percobaan dilakukan menggunakan rancangan acak lengkap dalam botol 2L dengan tiga kali pengulangan. Hasil penelitian menunjukkan bahwa MBM mengandung Mg^{2+} 1.0 g/L dan Fe^{2+} 3.5×10^{-4} g/L menghasilkan kurva pertumbuhan *C. pyrenoidosa* yang optimal. Analisis kandungan protein dilakukan dengan metode Lowry menggunakan spektrofotometer pada λ=750 nm, menghasilkan 0,0974 mg/mL (ekstrak) dan 6,4097 mg/mL (supernatan). Selanjutnya, analisis kadar glukosa dilakukan dengan metode fenol sulfat (λ=490 nm), hasil yang diperoleh 49,331 ppm (ekstrak) dan 1566,911 ppm (supernatan). Analisis asam amino dalam CGF menggunakan spektrometri massa kromatografi cair (KC-SM) menunjukkan adanya tirosin, prolin, asam glutamat, alanin, valin, triptopan, fenilalanin, metionin, dan leusin-isoleusin.

Keywords: chlorella growth factor, C. pyrenoidosa INK, *Mg^{2+}, Fe^{2+}, LC-MS*

Introduction

Microalgae have emerged as an attractive natural source for extensive screening of novel compounds with interesting biological and functional activities, which may lead to the discovery of therapeutically useful agents or new food ingredients [1]. One type of microalgae that has attracted considerable research interest owing to its potential health benefits is the genus Chlorella. Because Chlorella contains almost 60% protein and is able to produce protein 50 times more efficiently than other protein crops, this unassuming alga could well serve as a valuable protein source in developing countries as well as in other areas of the world where the population is outstripping available land.

Chlorella pyrenoidosa (*C. pyrenoidosa*) is a unicellular green alga that grows in fresh water. It has the highest chlorophyll content of any known plant and also contains high concentrations of certain vitamins, minerals, dietary fiber, nucleic acids, amino acids, enzymes, and other substances [2]. This alga has a strong cell wall that prevents its native form from being adequately digested; thus, chlorella can be digested by humans only after its cell wall is broken.

C. pyrenoidosa contains a wide range of potent antioxidants including CGF, beta-carotene, vitamin E, vitamin C, and polyphenolic compounds. CGF is a water-soluble extract and contains a variety of substances including amino acids, peptides, proteins, vitamins, sugars, and nucleic acids. The estimated CGF content of raw *C. pyrenoidosa* is approximately 5%. In particular, CGF is known to be effective in growth and recovery post illness [3, 4].

The growth of microalgae is dependent on an adequate supply of essential macronutrients (carbon, nitrogen, and phosphorus), metal ions (sodium, potassium, and calcium), and micronutrients such as traces of other metal ions (iron, zinc, and copper) Metals ions, such as iron and magnesium, play an important role in photosynthesis. In particular, magnesium occupies a strategic position as the central element of the chlorophyll molecule, and all microalgal species have an absolute need for this element [5]. Magnesium can regulate transport through the Ca-channels of the cell membrane by improving their permeability [6]. Magnesium is also involved in the aggregation of ribosomes in functional units and in the formation of catalase. Iron is the most important limiting nutrient among all the micronutrient metals present in the oceans [7], and it is important in many metabolic functions of phytoplankton, such as electron transport in the Calvin cycle, the respiratory electron transport processes, nitrate and nitrite reductions, nitrogen fixation, chlorophyll synthesis, and detoxification/degradation of reactive oxygen species (such as superoxide radicals and hydrogen peroxide). It is also implicated in a number of other biosynthetic pathways and degradation reactions [8]. Apart from nutrient availability, various factors, including light, pH, temperature, and salinity, affect not only photosynthesis and biomass productivity but also activity and mechanisms of cellular metabolism.

In order to improve CGF productivity from microalgae, we investigated the optimal culture condition of *C. pyrenoidosa*. Various concentrations of Mg^{2+} and Fe^{2+} in the medium affect Chlorella growth and CGF yield. Some studies have reported on the effects of trace elements on microalgae growth. Magnesium has been included as a macronutrient in all formulations for freshwater microalgae [9], but it has not been considered for marine microalgae [10] owing to the high Mg content in seawater. The main purpose of this study is to obtain high productivity of *C. pyrenoidosa* (Chlorophyta) in a modified basal medium (MBM) by adding 0.5 to 1.5 g/L of magnesium (Mg^{2+}) and 3.5×10^{-4} to 5.0×10^{-4} g/L of iron (Fe^{2+}).

Materials and Methods

Microalgal culture. Microalgal strain of *C. pyrenoidosa* was obtained from the Fresh Water Microalgal Laboratory, Center for Biotechnology-LIPI, Indonesia. The strain was routinely subcultured as follows. In brief, every two weeks, the culture was transferred into a fresh MBM, which was composed of the following salts (g/L): KNO_3, 1.25; K_2HPO_4, 1.25; $MgSO_4\cdot7H_2O$, 1; $CaCl_2\cdot2H_2O$, 0.11; H_3BO_3, 0.0011; $MnCl_4\cdot4H_2O$, 0.00015; $ZnSO_4\cdot7H2O$, 0.0009; $CuSO_5\cdot6H_2O$, 0.00016; $Co(NO_3)_2\cdot6H_2O$, 0.00005; $FeSO_4\cdot7H_2O$, 0.0005; and Na_2EDTA, 0.005 [11]. In order to prevent contamination by other microorganisms, all the laboratorial material and glassware (test tubes and glass flasks) were washed with water and detergent, rinsed with distilled water, and autoclaved at 121 °C for 20 min. Stock culture of *C. pyrenoidosa* was grown photoautotrophically in MBM for the preparation of inoculum. The pH of the medium was adjusted to 7.0; the temperature was maintained at room temperature (25 °C ± 2 °C); light/dark (L/D) photoperiod, 12/12 h; and light intensity, 2,500 Lux, with bubbling of aseptic air continuously. The growth experiment was conducted at different concentrations of Mg^{2+} and Fe^{2+} in a 2-L bottle. Thus, *C. pyrenoidosa* cells with the same inoculation density (~ 0.70; OD λ = 680 nm) were grown. The different concentrations of metals ions in MBM are listed in Table 1.

Determination of microalgae growth density. The growth of all the cultures was evaluated by obtaining daily readings of the absorbance with a UV-Vis spectrophotometer at 680 nm. Thus, the biomass concentrations were obtained on the basis of the calibration curve of absorbance versus weight biomass concentration. The logarithmic phase was reached when the absorbance values increased, followed by harvesting of the biomass.

Table 1. Different Concentrations of Mg^{2+} and Fe^{2+} in *Modified Basal Medium* (MBM)

Metal Ions	Concentration (g/L)					
	A	B	C	D	E	F
$MgSO_4\cdot7H_2O$	0.5	1.0	1.5	0.5	1.0	1.5
$FeSO_4\cdot7H_2O$	0.00035	0.00035	0.00035	0.0005	0.0005	0.0005

Harvest of chlorella biomass. After the culture reached the logarithmic phase, the microalgae biomass was harvested by centrifugation at 3,000 revolutions per minute (rpm) for 15 min using a centrifuge (Biofuge). The biomass sample thus obtained was weighed in a pre-weighed centrifuge glass tube. Then, the centrifuge tube containing the biomass sample with co-solvents was subjected to ultrasound treatment for 30 min.

CGF extraction. Wet biomass of *C. pyrenoidosa* INK in each culture of MBM with different concentrations of Mg and Fe was washed with distilled water and sonicated at a resonance of 40 kHz for 30 min. Cell disruption was observed under a microscope with 40×magnification. Perfect cell extraction with warm water was carried out at a temperature of 40 °C; the sample was left to stand for 24 h so that the CGF could be extracted.

Analysis of CGF. Samples of CGF for protein and carbohydrate analysis were centrifuged at 3000 rpm for 10 min. The supernatant was maintained at 4 °C until analysis. Intracellular carbohydrates were determined using the modified phenol-sulfuric acid method [12] with glucose as a standard. Total intracellular protein was determined according to the procedure of Lowry [13] using bovine serum albumin (BSA) as a standard.

Determination of protein (Lowry method). Standard BSA solution (1000 ppm) was diluted with distilled water to obtain the following concentrations: 0, 50, 100, 200, 300, 400, and 500 ppm. A total of 0.5 mL of each solution was added to 0.5 mL of 1N NaOH, boiled for 20 min, and cooled. Then, 0.5 mL of Cu-alkali reagent was added and the mixture was shaken until it became homogeneous. Next, the Folin-Ciocalteu reagent was added into each tube, and the mixture was shaken until it became homogeneous; then, it was allowed to stand for 30 min. Absorption was measured with a UV-Vis spectrophotometer at a wavelength of 750 nm.

A total of 200 µL of each sample was pipetted, diluted with distilled water to a volume of 0.5 mL, and subjected to the same treatment as the BSA standard. Distilled water was used as the blank. The results obtained were plotted into the regression equation of the BSA standard to obtain the protein content of CGF.

Determination of carbohydrates (phenol–sulfuric acid method) [12]. The phenol-sulfuric acid colorimetric method was used. This method allows for the differentiation of various types of sugars present in a sample and is more sensitive than methods such as the 3,5-dinitrosalicylic acid (DNS) [14].

A standard solution of glucose (1000 ppm) was diluted with distilled water to obtain the following concentrations: 0, 20, 40, 60, 80, and 100 ppm. A total of 1.0 mL of each solution was added to 0.5 mL phenol (5%) and 2.5 mL H_2SO_4 p.a., and the mixture was shaken until it became homogeneous; then, it was allowed to stand for 10 min. Next, the mixture was boiled for 15 min. Absorption was measured with a UV-Vis spectrophotometer at a wavelength of 490 nm.
A total of 200 µL of each sample was pipetted, diluted with distilled water to a volume of 1.0 mL, and subjected to the same treatment as the standard solution of glucose. Distilled water was used as the blank. The spectrophotometric absorbance was converted into carbohydrate concentration using a calibration curve established for glucose.

Amino acid analysis. CGF extracts were prepared by crushing 0.5 g of the powdered material using a mortar and pestle with 2 mL water. The extracts were then filtered, and the filtrates were used in liquid chromatography-mass spectrometry (LC-MS) injection for amino acid detection. For LC-MS, UPLC-QToF-MS/MS system (Waters) was used with a UV detector. Data analysis was conducted using MassLynx software (version 4.1) and UPLC Acquity SDS (Waters; column, Acquity UPLC BEH C18 1.7 µm, 2.1×50 mm). The conditions of the analysis were as follows: temperature, 40°C; mobile phase used, A - H_2O + 0.1% formic acid and B-acetonitrile + 0.1% formic acid; injected volume, 5.0 µL; flow rate, 0.30 mL/min (Table 2).

In the first part of the experiment, standard amino acids were injected for LC-MS. The spectra were obtained for each amino acid with varying retention times. In the second part of the experiment, the CGF extracts were injected for LC-MS under similar conditions as those described above. The spectra were compared with the standard amino acid spectra, and the presence of amino acids was evaluated. Thus, the presence of amino acids was confirmed by their retention times and [M+1] + peaks, and compare to standards [15].

Statistical analysis. Statistical analysis of the data was conducted using one-way analysis of variance (ANOVA). The significant data were analyzed using Duncan's new multiple range test with a 95% confidence

Table 2. Gradient Program

Time (min)	%A	%B
0	95	5
1	95	5
6	100	0
7	100	0
7,5	95	5
9	95	5

MS: XEVO - G2QTOF (Waters)
ESI positive (resolution mode); Capillary voltage, 3 kV; Sample cone voltage, 38 V; Desolvation T, 300°C; Source T, 110°C; Desolvation gas, 500 L/h; Cone gas, 16 L/h

interval, and statistical evaluation was carried out using SPSS software (version 15).

Results and Discussion

Figure 1 shows a microalgal strain of *C. pyrenoidosa* INK cells observed under a microscope.

Growth of *Chlorella pyrenoidosa* INK. Variations in the concentrations of Mg^{2+} and Fe^{2+} in MBM affect the growth of *C. pyrenoidosa* microalgae. Their influence can be seen from the calculation of the number of cells *C. pyrenoidosa* using a hemocytometer during cultivation. The population growth in each medium is shown in Figure 2. The number of living cells increased exponentially on the fifth day after inoculation, and it approached around 96.4×10^6 cells/mL in the optimum medium B.

Estimation of *C. pyrenoidosa* growth (cell count) in different media showed different growth patterns, and the modified medium B showed maximum growth, followed by medium C, A, E, and D; minimum growth was observed in medium F. The cell count clearly indicated that the best growth of *C. pyrenoidosa* was obtained in modified medium B (MBM with 1 g/L of Mg^{2+}; 3.5×10^{-4} g/L of Fe^{2+}). The total cell count increased 4.11 times from medium B to medium E (initial MBM with 1 g/L of Mg^{2+} and 5.0×10^{-4} g/L of Fe^{2+}).

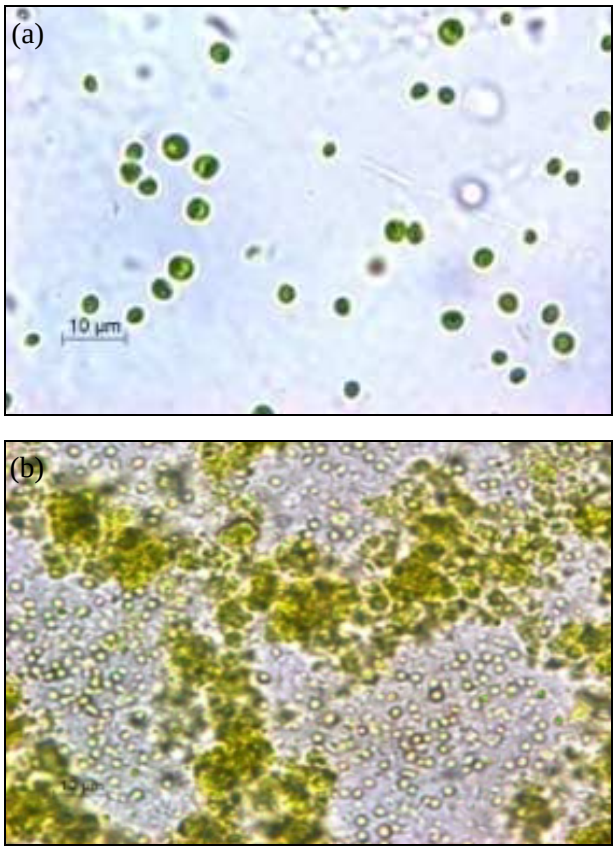

Figure 1. Difference between whole Cells and Crushed ones. The Intact Cells are Individually Visible (a) Whereas Sonication Causes Cell Disruption (b) The Photograph was taken with a Negative Phase-contrast Microscope (Leica) at 400×

Biomass of *C. pyrenoidosa* INK. Figure 3 shows the effect of different concentrations Mg^{2+} and Fe^{2+} on the biomass weight of *C. pyrenoidosa* INK. It can be seen that the metal ions in MBM affected the biomass weight of *C. pyrenoidosa* to a certain extent. Moreover, the biomass weight of *C. pyrenoidosa* increased gradually and varied with the concentrations of Fe^{2+} and Mg^{2+}; it reached the maximum value at 1 g/L of Mg^{2+} and 5×10^{-4} g/L of Fe^{2+} in MBM (medium E), probably because Fe^{2+} is the contributing factor in many types of enzymes and is essential for the formation of cytochrome, ferredoxin, and Mo-Fe protein. The photosynthesis of algae depends on Fe, and at the same time, Fe is an important component of nitrate and nitrite reductase [16].

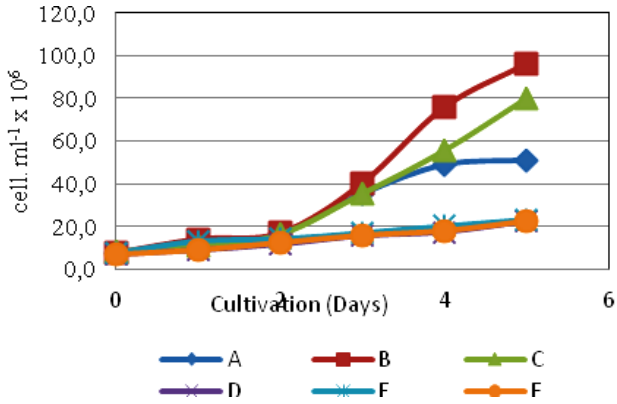

Figure 2. Effect of Different Concentrations of Mg^{2+} and Fe^{2+} in MBMon Total Cell Count *of C. pyrenoidosa* INK

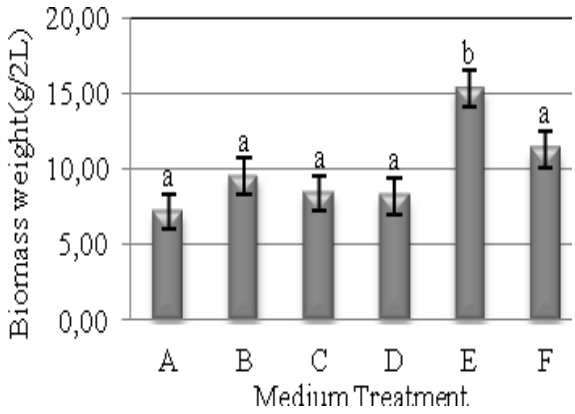

Figure 3. Effect of Different Concentrations of Mg^{2+} and Fe^{2+} in MBMon *C. pyrenoidosa* INK Biomass

Protein content. The Lowry method is one of the most accurate methods for quantifying proteins. In this method, the spectrophotometric absorbance of the unknown sample at a wavelength of 750 nm is compared with a calibration curve obtained using standard solutions of a protein. BSA is the most commonly used standard. The calibration curve was linear in the absorbance range of 0–0.7 corresponding to a protein concentration range of 0–500 mg/L. Within this range, the correlation coefficient was 0.9951 (Figure 4). Intracellular protein can be determined accurately only if all of it is accessible to the reagents used in the Lowry method. Therefore, the cells must be pretreated so that they release all the cellular protein.

Analysis of protein content was carried out for both the extract and the supernatant. Protein content in the supernatant was higher than that in the extract. According to Table 3, the highest protein content was obtained as 6.35 ± 0.108 mg/mL in medium C (1.5 g/L of Mg^{2+} and 0.00035 g/L of Fe^{2+}), The results showed an increase of up to 110.96% as compared to the MBM standard (Group E contained 1 g/L of Mg^{2+} and 0.0005 g/L of Fe^{2+}). According to the statistical analysis, medium C shows significant differences from the other media. Mg^{2+} and Fe^{2+} play an important role in the activation of enzymes that allow for biochemical reactions inside the compartment of the cytoplasm and cell organelles.

Glucose content. Analysis of total sugar was carried out using the phenol-sulfuric acid method with glucose as a standard. The calibration curve was linear in the absorbance range of 0–1.5, corresponding to glucose concentrations, and the correlation coefficient was 0.9997 (Figure 5).

The highest total sugar content of CGF (water soluble, supernatant) in the present condition was obtained in medium C (containing 1.5 g/L of Mg^{2+} and 0.00035 g/L of Fe^{2+}) as 2.21 ± 0.156 mg/mL (Table 4). The results showed an increase of up to 137.63% as compared to the MBM standard (Group E contained 1 g/L of Mg^{2+} and 0.0005 g/L of Fe^{2+}). According to the statistical results, medium C shows significant differences from the other media. Mg^{2+} and Fe^{2+} play an important role in photosynthesis by chlorophyll, which produces sugar.

Analysis of amino acid composition. CGF is a unique group of substances present only in the nucleus of Chlorella, accounting for up to 18% of its total weight. It is very rich in nucleic acids (RNA and DNA) as well as other substances such as amino acids, peptides, vitamins, minerals, and polysaccharides.

Table 3. Total Protein Content of CGF from *C. pyrenoidosa* Culture under Different Concentrations of Mg^{2+} and Fe^{2+} in MBM

Medium	Protein Concentration (mg/mL)	
	Precipitate	CGF solution
A	0.05 ± 0.017^{a}	5.43 ± 0.311^{c}
B	0.10 ± 0.010^{b}	5.36 ± 0.478^{c}
C	0.11 ± 0.007^{b}	6.35 ± 0.108^{d}
D	0.25 ± 0.010^{c}	3.97 ± 0.290^{b}
E	0.23 ± 0.007^{c}	3.01 ± 0.053^{a}
F	0.23 ± 0.018^{c}	3.98 ± 0.377^{b}

Each value is Mean ± SD (n = 3)

Table 4. Total glucose content of CGF from *C. pyrenoidosa* culture under different concentrations of Mg^{2+} and Fe^{2+} in MBM

Medium	Total Glucose Concentration (mg/mL)	
	Precipitate	CGF solution
A	0.03 ± 0.009^{a}	1.04 ± 0.067^{ab}
B	0.05 ± 0.003^{b}	1.22 ± 0.049^{c}
C	0.06 ± 0.010^{b}	2.21 ± 0.156^{d}
D	0.02 ± 0.003^{a}	1.16 ± 0.013^{bc}
E	0.03 ± 0.016^{a}	0.93 ± 0.055^{a}
F	0.02 ± 0.003^{a}	1.14 ± 0.116^{bc}

Each value is Mean ± SD (n = 3)

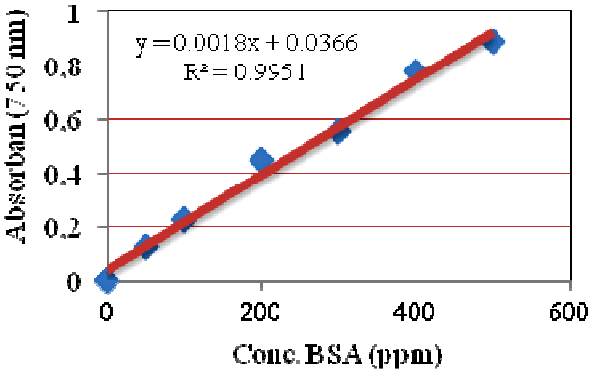

Figure 4. BSA calibration curve with spectrophotometer at λ = 750 nm (r = 0.9951, Vxo = 9.14%)

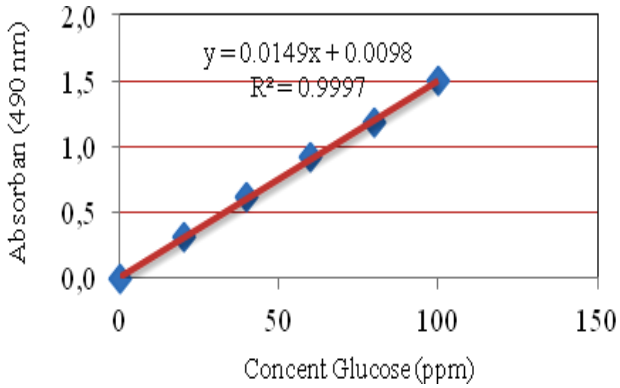

Figure 5. Glucose Calibration Curve with Spectrophotometer at λ = 490 nm (r = 9997; Vxo = 1.81%)

In the present study, efforts were made to establish the presence of amino acids in CGF of *C. pyrenoidosa* using LC-MS (Figure 6). Thus, this work involves a qualitative analysis of amino acids present in CGF. The amino acid study shows that CGF of *C. pyrenoidosa* is a source of some important amino acids, namely, tyrosine, proline, glutamate, alanine, valine, tryptopan, phenylalanine, methionine and leucine-isoleucine, their observed $[M+H]^+$ almost identical to data of Chemspider (Table 5 - Table 13). Figure 7a – 7i showed that the mass spectra of detected amino acids in samples; these mass spectra.

Table 5. Amino Acids Contained in the CGF and, Their Exact Molecular Weight $[M+H]^+$ Compared to Chemspider

	MW http://www.chemspider.com/	M +H+
Tyrosine	181.0739	182.0817
Proline	115.0633	116.0712
Glutamate	147.0532	148.0610
Alanine	89.0477	90.0555
Valine	117.0790	118.0869
Tryptophan	204.0899	205.0977
Phenylalanine	165.1900	166.087
Methionine	149.0510	150.0589
Leucine/ Isoleucine	131.0946	132.1025

Table 6. Test of Variances Homogeneity of Biomass Weight

Levene Statistic	df1	Df2	Sig.
1.600	5	12	.234

Table 7. Anova Biomass Weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	130.851	5	26.170	5.422	.008
Within Groups	57.922	12	4.827		
Total	188.773	17			

Table 8. Test of Variances Homogeneity of Protein in Biomass

Levene Statistic	df1	df2	Sig.
1.801	5	12	.187

Table 9. Anova Protein levels in Biomass

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.114	5	.023	146.329	.000
Within Groups	.002	12	.000		
Total	.116	17			

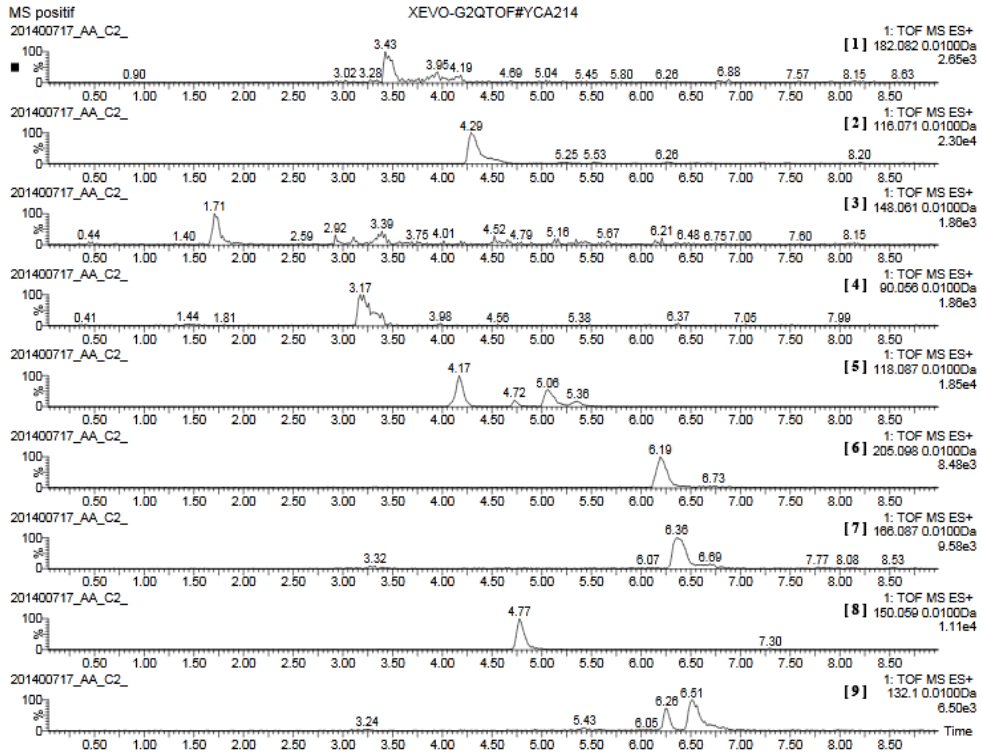

Figure 6. Selected ion Chromatogram of Amino Acids in Samples using Waters; Column, Acquity UPLC BEH C18 with a Runtime of 9 min, (1) Tyrosine, (2) Proline, (3) Glutamate, (4) Alanine, (5) Valine, (6) Tryptophan, (7) Phenylalanine, (8) Methionine, (9) Leucine/isoleucine

Table 10. Test of Variances Homogeneity of Protein in Supernatant

Levene Statistic	df1	df2	Sig.
4.006	5	12	.023

Table 11. Anova Protein Levels in Supernatant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22.867	5	4.573	48.468	.000
Within Groups	1.132	12	.094		
Total	24.000	17			

Table 12. Test of Variances Homogeneity of Glucose in Biomass

Levene Statistic	df1	Df2	Sig.
2.314	5	12	.109

Table 13. Anova Glucose Levels in Biomass

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	5	.001	8.208	.000
Within Groups	.001	12	.000		
Total	.004	17			

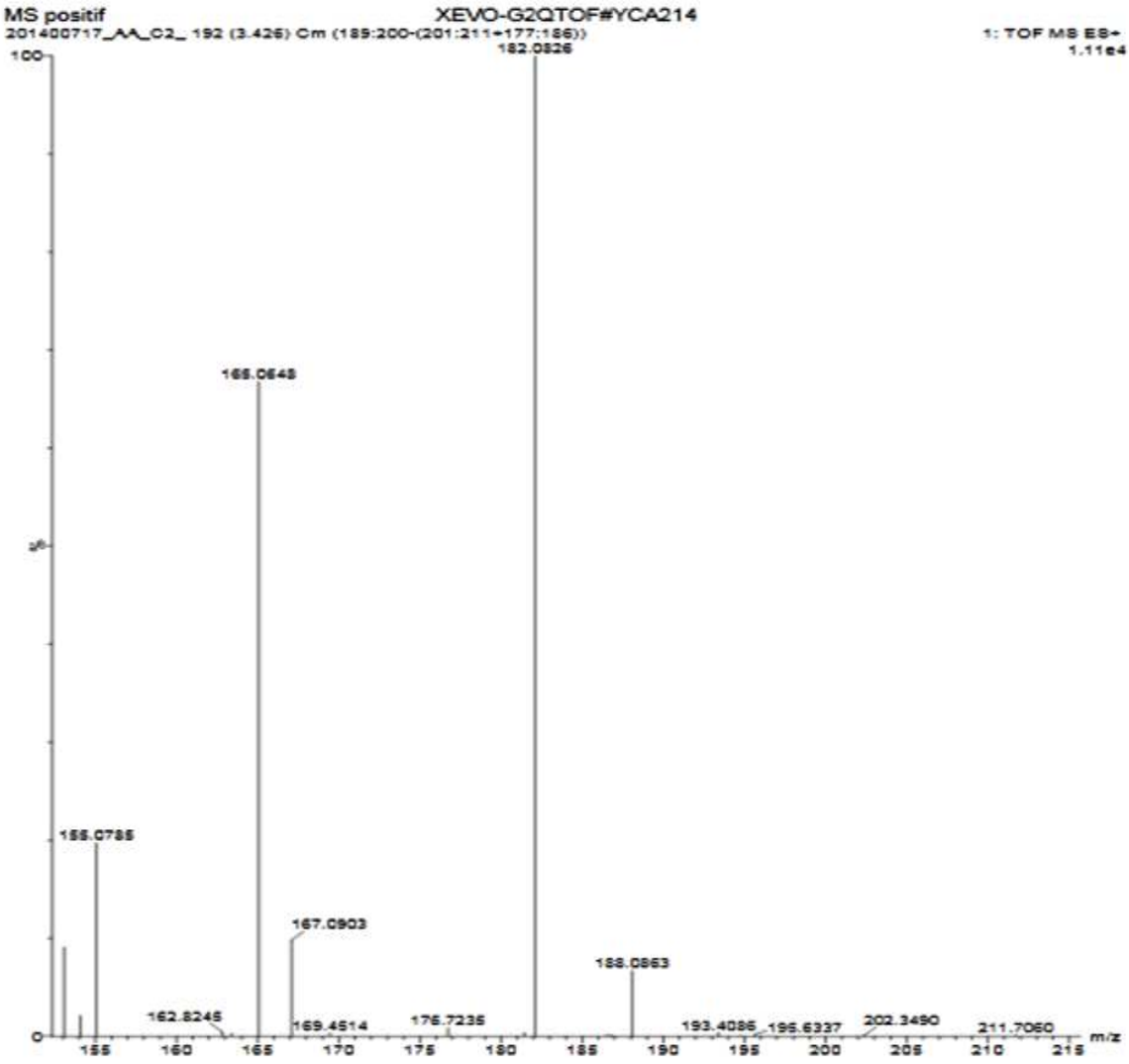

Figure 7(a). Mass Spectra of Amino Acid-Tyrosine

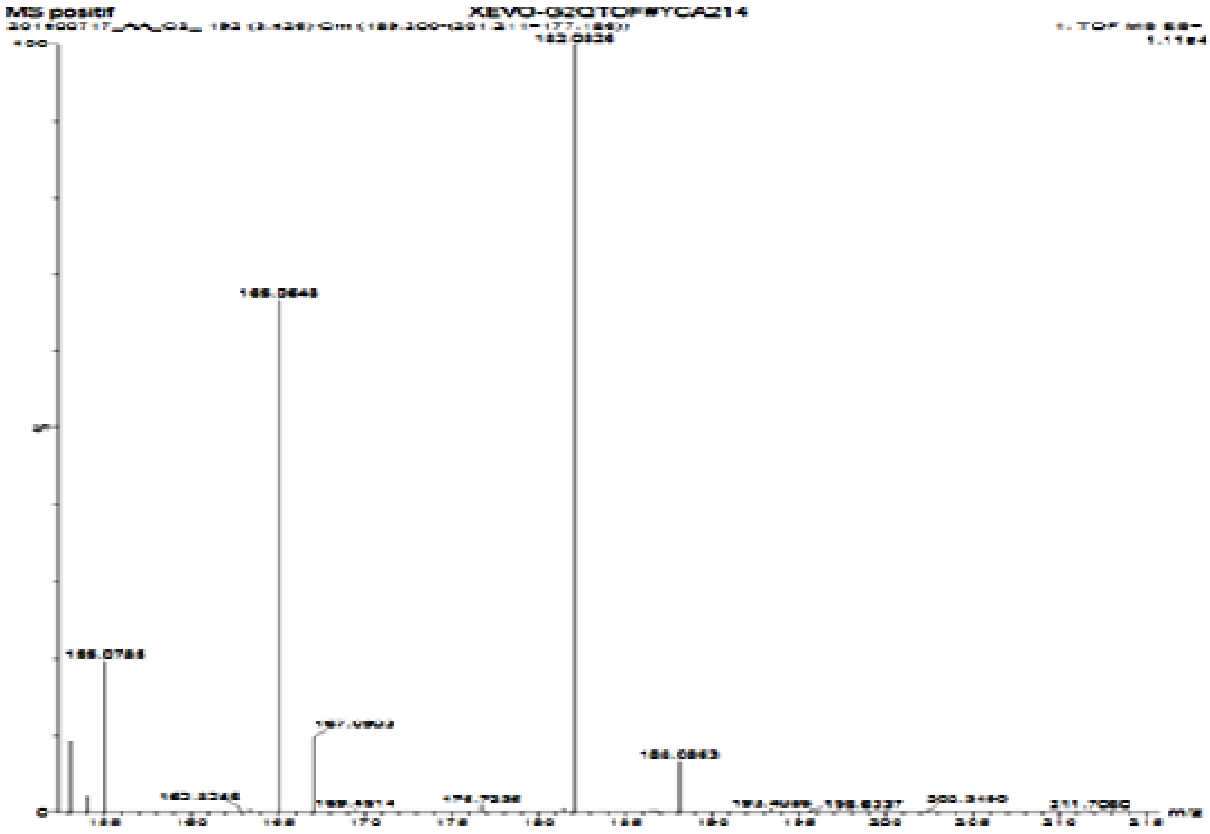

Figure 7(b). Mass Spectra of Amino Acid – Proline

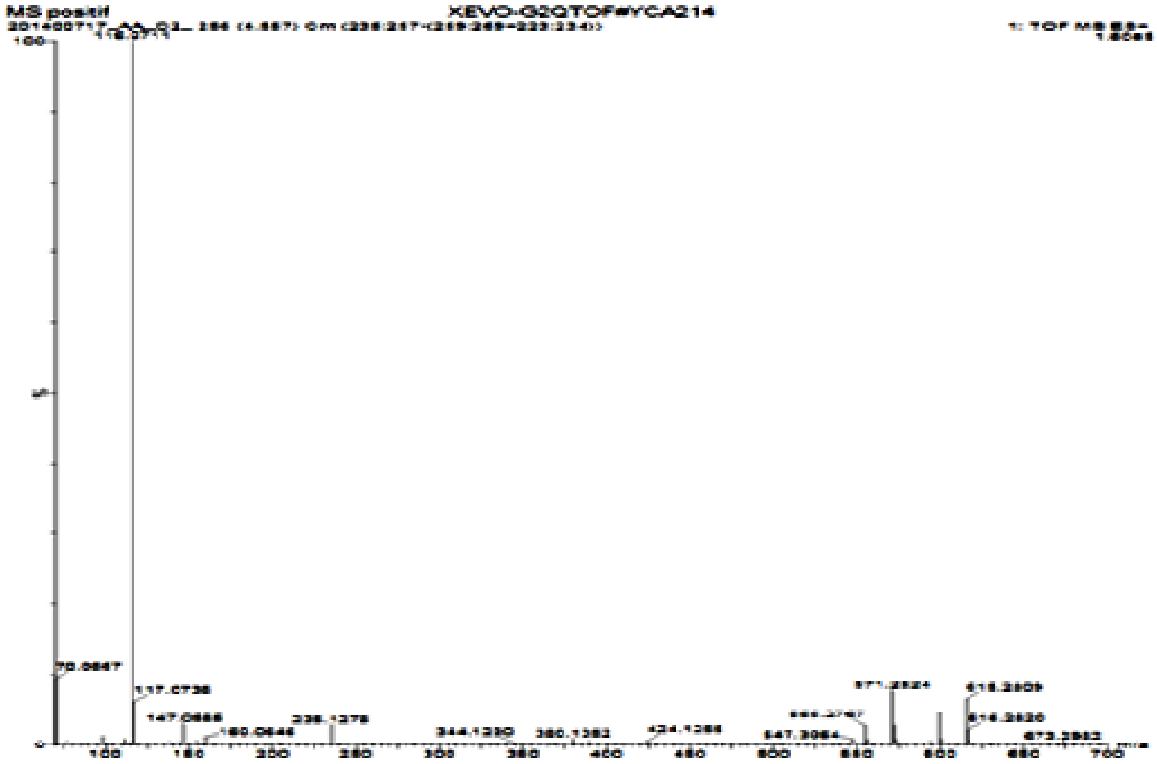

Figure 7(c). Mass Spectra of Amino- Glutamate

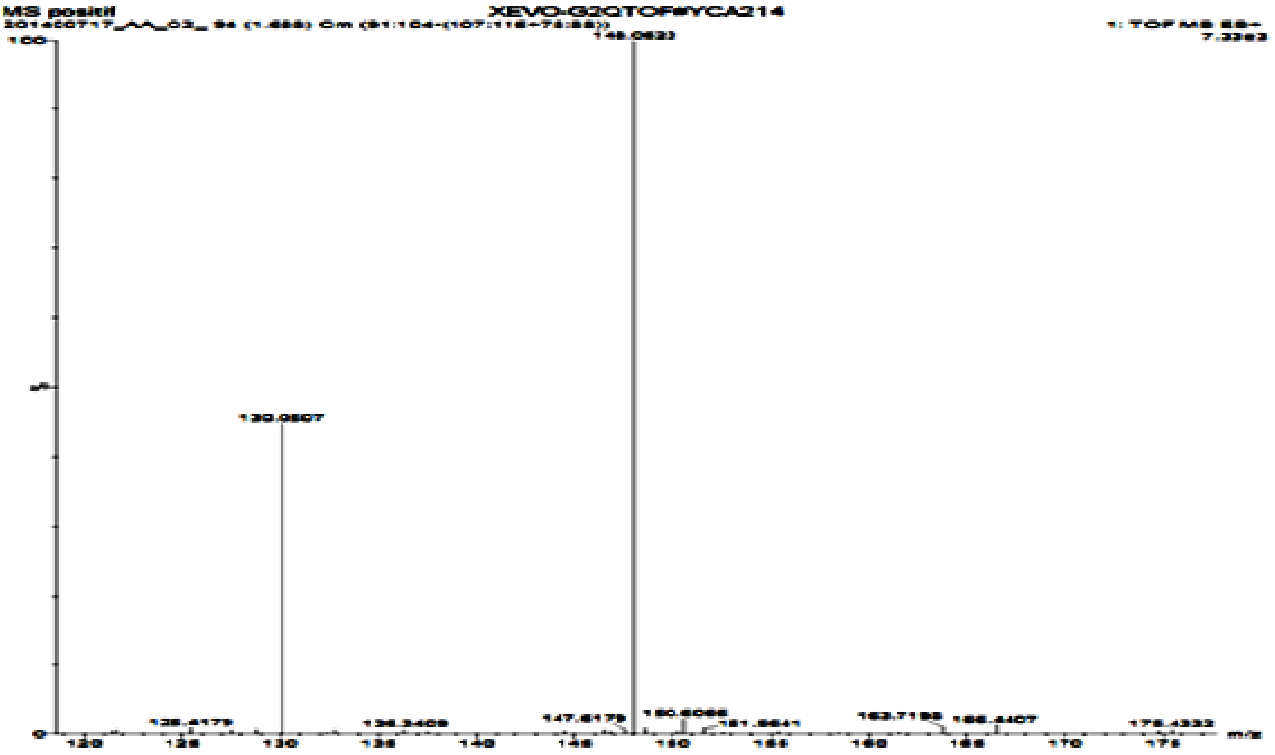

Figure 7(d). Mass Spectra of Amino Acid-Alanine

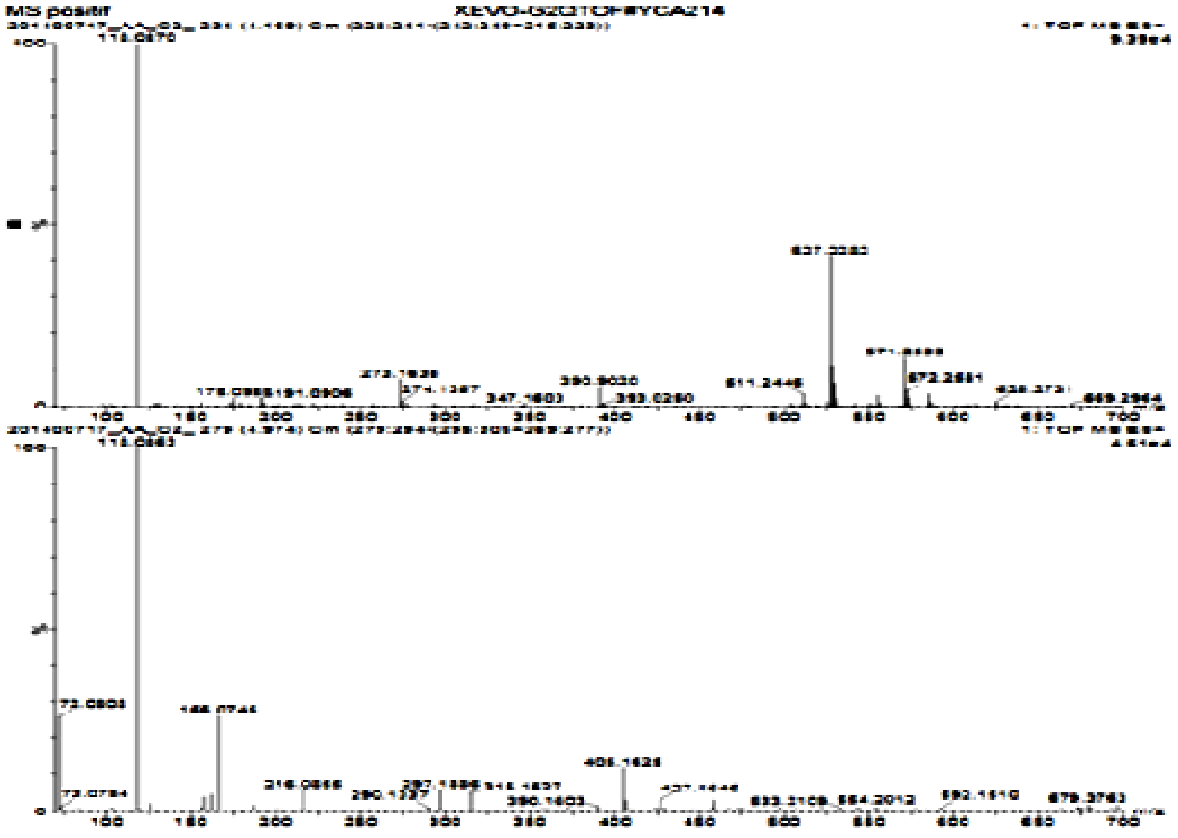

Figure 7(e). Mass Spectra of Amino Acid- Valine

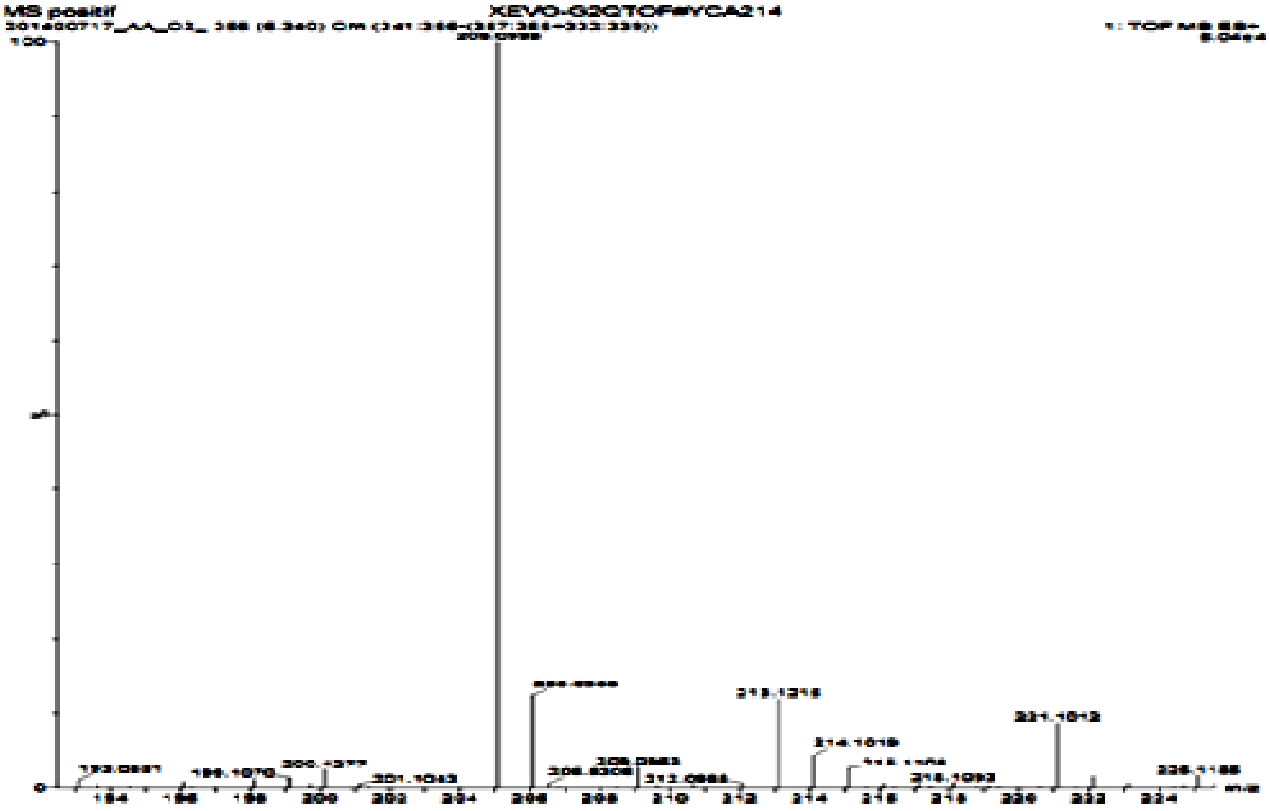

Figure 7(f). Mass Spectra of Amino Acid- Tryptophan

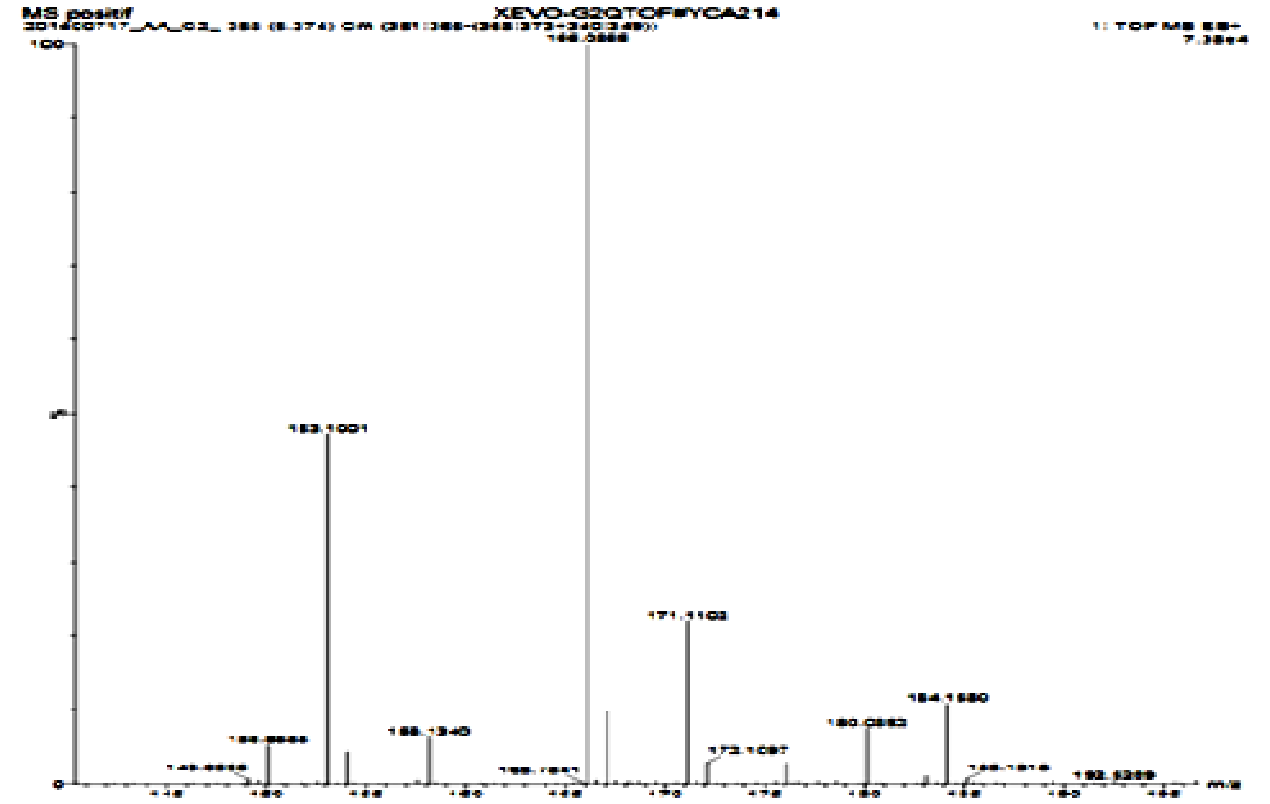

Figure 7(g). Mass Spectra of Amino Acid- Phenylalanine

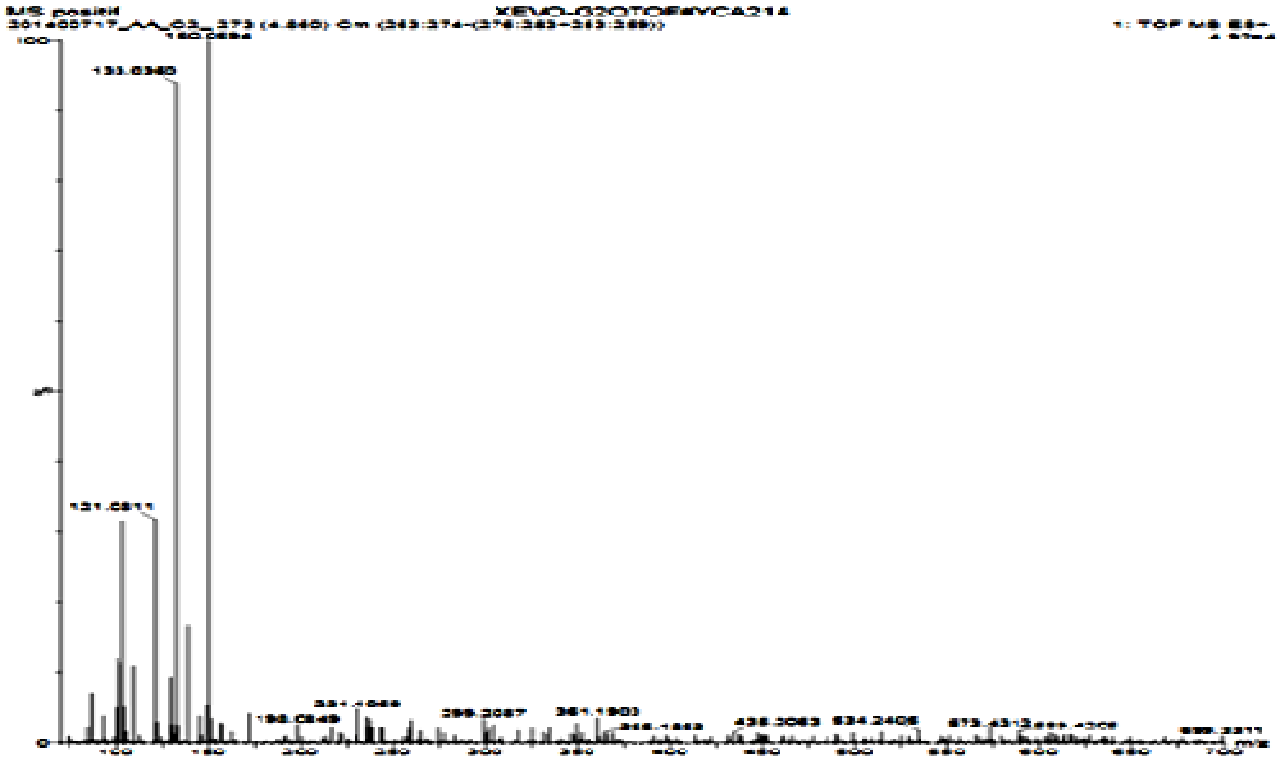

Figure 7(h). Mass Spectra of Amino Acid- Methionine

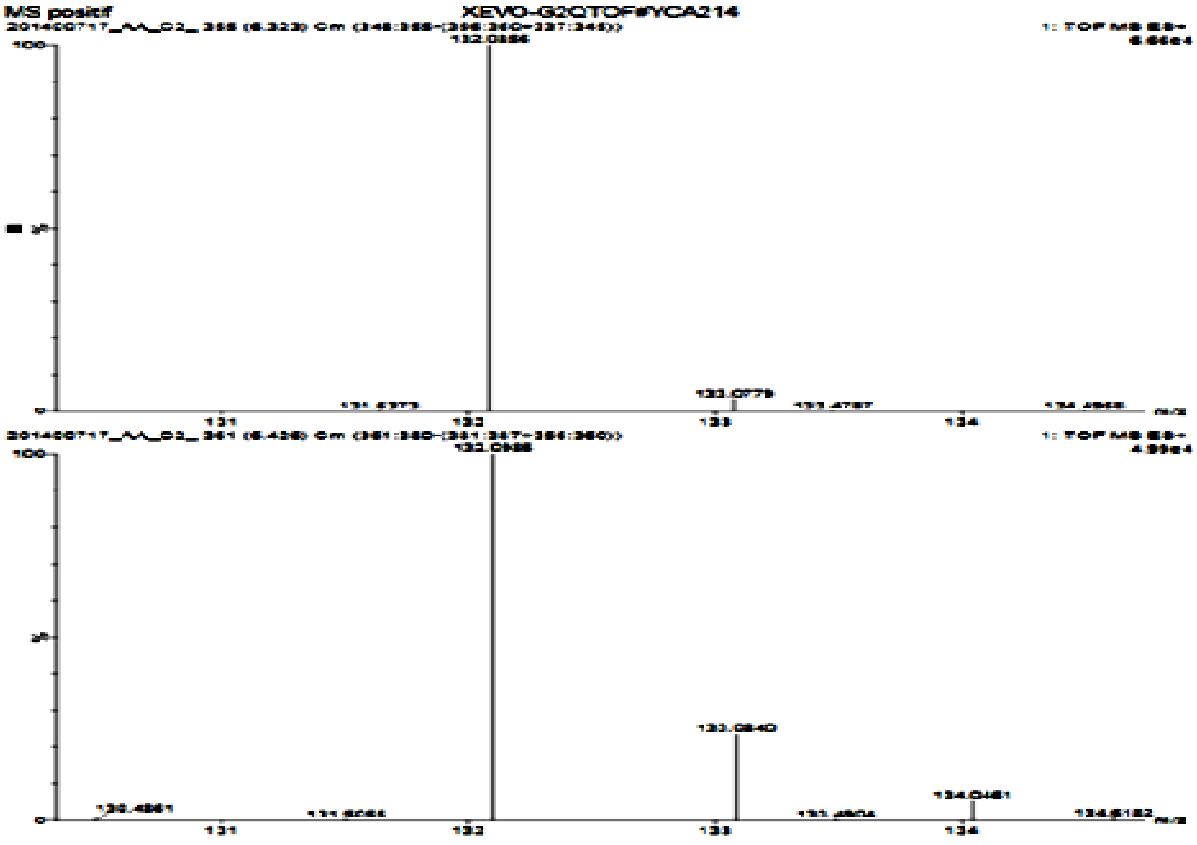

Figure 7(i). Mass Spectra of Amino Acid- Leucine, Isoleucine

Conclussions

The growth of microalgae *C. pyrenoidosa* in MBM was found to be influenced by Mg^{2+} and Fe^{2+} ions. The group with 1 g/L of Mg^{2+} and 0.00035 g/L of Fe^{2+} showed the highest cell count ($96{,}39\times10^6$ cell/mL) and the group with 1.0 g/L of Mg^{2+} and 0.0005 g/L of Fe^{2+} showed the highest biomass (15.27 ± 3.516 g). Variations in the concentrations of Mg^{2+} and of Fe^{2+} were found to influence the formation of CGF. The highest total content of protein and glucose in CGF (6.35 ± 0.108 mg/mL and 2.21 ± 0.156 mg/mL, respectively) were obtained for the group with 1.5 g/L of Mg^{2+} and 0.00035 g/L of Fe^{2+}. The levels of protein and glucose showed an increase of 110.96% and 137.63%, respectively, as compared to the standard MBM. Amino acid analysis via LC-MS showed EPS components as a source of some important amino acids.

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