Egyptian J. of Phycol. Vol. **9**, 2008

SEASONAL PRODUCTIVITY AND CHEMICAL COMPOSITION OF MASS CULTURED *ANABAENA WISCONSINENSE* AND *SPIRULINA PLATENSIS* UNDER EGYPTIAN CONDITIONS

Nabil Fahmy Abd El Hakim[1] and Tartiel El-Sayed Mohamed Badawy[2]

1- Faculty of Agriculture, Al Azhar University.
2- *Central Laboratory for Aquaculture Research, Abbassa, Ecology and Biology Department, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.*

Abstract

The growth of two blue-green algae species *Anabaena wisconsinense* and *Spirulina platensis* under outdoor conditions (Egyptian conditions) was studied; variation in chemical composition of algal cells due to season of cultivation was also investigated. Average cell mass productivity reached the maximum in summer followed by autumn and spring in both *Anabaena* and *Spirulina* The decline in crude protein with ageing of culture was accompanied by an increase in fat and total carbohydrates contents and by the decrease of nucleic acid and mineral contents. Amino acid analysis showed that both species were deficient in methionine. Methods of drying significantly affected amino acid composition. Growth and chemical composition of *Spirulina* cells are maximum crude protein content was around 63% for all seasons. This was attained at the age of 12 days for spring and summer and at 18 days in autumn seasons, the calculated average and maximum productivity of algal cells and crude protein showed the highest value in summer culture followed by autumn and spring seasons. Summer temperature naturally ranged higher than the other two seasons and consequently, resulted in higher growth rate and protein productivity. However, analysis of variance comparing algal growth rate and yields of spring, summer and autumn cultures were not significant.

Key words: *Anabaena wisconsinense*, Blue-green algae, Chemical composition, Mass culture, Seasonal productivity, *Spirulina platensis*.

Introduction

A few years ago, microalgae have been increasingly produced for commercial purposes which include human and animal consumption, bioactive compounds for medicine, fuel production, biofertilizers, and as live feeds for the cultivation of filter feeding organisms. Currently, microalgal biomass production is economically feasible only when product values are relatively high, such as special chemicals and pigments, or when the microalgae play a critical role in aquaculture production (Spectorova *et al*., 1997).

(ISSN: 1110-8649)

Anabaena and *Spirulina* are of freshwater blue-green filamentous algae, and releasing increasing bioactive components such as vitamins, minerals, polyunsaturated fatty acids, carotenes and other pigments that have antioxidants activity (Cohen and Vonshak, 1991; Lin *et al*., 2007; Wang *et al.*, 2007). The previous authors suggested that blue-green algae *Anabaena wisconsinense* and *Spirulina platensis* could be used to produce a natural dietary antioxidant supplement or added to healthy food products, to prevent some chronic diseases. Moreover, *Spirulina* is a rich source of protein (60-70%), vitamins, essential amino acids, minerals and essential fatty acids such as palmitic acid, linolenic acid,and linoleic acid. Therefore, it has been used as a nutrient for fish larvae (Lu *et al*., 2002) and as an ingredient in fish diet for juveniles and adults common carp (Nandeesha *et al*., 1998).

Microalgae have been investigated as a human and animal food for over 40 years, the use of microalgae in aquaculture has several potential advantages over the production of microalgae for human foods or terrestrial animal feeds such as high conversion efficiencies and no need for harvesting, drying and storage, as the animals or food chains could use the algae as produced. However, the production of microalgae for aquaculture feeds has been relatively neglected, mainly, because the aquaculture system themselves were generally poorly developed (Benemann, 1992). Different techniques of clean algae production were developed, growing blue-green algal usually occur in abundance during the warm months of the year (Fogg *et al*., 1984). The growth of two blue-green algae species *Anabaena wisconsinense* and *Spirulina platensis* under outdoor conditions was studied. Variation in chemical composition of algal cells due to season of cultivation was also investigated.

The present study was carried out in the Central Laboratory for Aquaculture Research (CLAR) and International Central Laboratory for Aquaculture Research Management. The World Fish Center, Regional Center for Africa and West Asia, Abbassa, Abu-Hammad, Sharkia, Egypt.

Materials and Methods

Algae culture:

A pure culture of *Anabaena wisconsinese* and *Spirulina platensis* were obtained from the Department of Botany, Faculty of Science, Cairo University, Cairo, Egypt. The basal medium was adopted by Zarrouk (1966). Both examined algae were cultured throughout three seasons; spring, summer and autumn. The algae were cultivated in 50 L photo bioreactors using semi-continuous culture (Morist *et al.*, 2001). Representative samples were taken (five liters of culture) every 3 days for growth measurements and chemical analysis. To determine algal biomass, a 100 ml aliquot of the algae suspension were used Culture pH was adjusted around an optimum value of growth (7.0 for *Anabaena* and 8.3 for *Spirulina*) three times daily (at hours 9 a.m., 12 and 3 p.m.) by controlling CO_2

flow rate in the culture. Atmospheric temperature was recorded daily by thermometer at three times (at hours 9 a.m., 12 and 3 p.m.). Tap water was used to prepare the medium for all of the large scale culture experiments. Also, the large scale cultures were disinfected according to Hemerick (1973) by the addition of the commercial bleach, Clorox ® at the rate of 5.25% solution as sodium hydrochlorite (NaOCL) and kept for one day under continuous aeration, presented in Table (1).

Table (1): Amount of Clorox and sodium thiosulfate used to disinfect water.

Chemicals	Type		
	Carboy 20 L	Glass aquaria 100 L	Tank 1000 L
Clorox (ml)	5	25	100
Sodium thiosulphate (g)	0.2	1.0	5

Sodium thiosulfate is not added until 4 hours after the addition of clorox.®

Glass aquaria culture stages (outdoor):

When the carboy cultures reached the harvestable density (log phase), they were used to inoculate 100-liter glass aquaria (75 x 40 x 60 cm), which were prepared in the same way as the carboys. Carboys and mass culture aquaria using air compressor of a 5-hp air blower. This aeration keeps algal cells in suspension, supplies suitable oxygen density and carbon dioxide concentration needed for algae propagation, and helps to stabilize pH. The glass aquaria are cleaned and filled with tap water. Sodium hypochlorite is added to disinfect the system; after 24 hours the residual hypochlorite is neutralized with sodium thiosulfate.

Nutrients for outdoor algae cultures:

According to Allen and Nelson (1974) a complete trace metal mixture is also added composition of Zarrouk (1966), algae culture medium presented in Table (2). Compressed air is provided to carboys and mass culture tanks from air compressor of a 5-hp air blower. Aeration keeps algal cells in suspension, supplies carbon needed for plant growth, and helps to stabilize pH. Amount of the following salts per 1000 liters tap water, each chemical dissolved separately in 100 ml warm water before added to water tank. The Proximate chemical analysis was determined according to the methods described by Association of Official Analytical Chemists, A.O.A.C. (1995). The chemical composition of this medium is as follows in Table (2):

Table (2): Chemical composition of culture medium for outdoor cultivation.

Chemicals	Final Conc. (g/1000L)
Sodium nitrate	127
2- Ammonium sulfate	100
or substituted with potassium nitrate	187
3- Monopotassium phosphate	30
4- Fe-EDTA (Ethylene diamine tetraacetic acid disodium salt)	15
5-Minerals mixed (mg\ 1000 L) zinc sulfate Cobalt chloride	2.2

Manganese Chloride	18.0
Sodium mohybdate	0.36
Cupric sulfate	9.8

Algal biomass harvesting and Drying:

The qualities of the algal biomass dried by the two methods were compared by determining their moisture and crude protein contents. Since cells of microalgae at the stationary phase of growth tended to settle to the bottom of the cultivation tank, primary separation of the algal cells from the liquid phase was achieved by gravity separation. After the algal culture reached maximum growth, the circulation provided by the pumping system was stopped and the algal cells settled and formed thick sediment at the bottom of the cultivation tank within 24h. (Allen and Nelson, 1974).

Concentrated algal suspension:

The concentrated algal suspension was centrifuged with automatic super speed centrifuge at 5000 r.p.m for 10 min. In order to find a suitable drying method, the algal slurry obtained by centrifugation was dried either:

(1) In an Oven at 110 °C for 15–20 minutes , or oven-dried at 85° C for 4 h

(2) In air at room temperature (25 °C ±2) for 72-96 hours.

Measurement of growth parameters:

Crude protein

This method is used for algae by using microkjeldahl methods on the dried algal biomass at 110 ºC for 15 minutes according to APHA (1985).

$$\% \text{ Nitrogen} = \frac{(S - B) \times 14.007 \times N}{\text{grams of sample} \times 1000} \times 100$$

S = ml HCl to titrate sample.

B = ml HCl to titrate blank.

N = Normality of HCl

% crude protein = % nitrogen x 6.25

Total carbohydrate:

One ml of concentrated sulfuric acid was added to known weights from the oven dry algal tissue in an ice-bath and left for 20 hour and completed to 7 ml concentrated sulfuric acid .Total carbohydrate content was measured as glucose according to Dubois *et al*. (1956).

Nucleic acid contents:

The method applied for total RNA and DNA determinations in dried algal biomass is that of with some little modifications as described by APHA (1985).

Quantitative estimation of RNA:

The RNA contents were measured colorimetrically by the orcinol procedure described by Morse and Carter (1969) as follows: One ml of the RNA fraction was added to 0.2 ml of the orcinol reagent; 3 ml of ferric chloride solution were added. The mixture was heated for 30 minutes in a boiling water bath. The tubes were then cooled and its optical density was measured at 660 nm. A similarly treated blank (without the sample) was used for the zero setting.

Quantitative estimation of DNA:

The DNA contents were measured by the diphenylamine procedure described by Morse and Carter (1969) as follow: One ml of the DNA supernatant was added to 2.5 ml of the reagent and the mixture was heated for 5 minutes in a boiling water bath. The sample was cooled and the color was measured at 540 nm. Blank, laying the sample was used for the zero setting. From the standard curves of RNA and DNA, the concentrations of both were calculated.

Statistical analysis

Correlation coefficients, regression and Least Significant Difference (LSD) were carried out using the Analysis of variance (ANOVA) and Duncan's multiple Range Test (1996) to determine differences between treatments means at significance rate of $P < 0.01$. The standard errors of treatment means were also estimated. All statistics were carried out using Statistical Analysis system (SAS, 2000).

Results and discussion

The growth of algae is essentially dependent on five factors namely, nutrient composition of the medium, H-ion concentration of the medium, temperature, light and adequate agitation of the culture (Zaret *et al.*, 1981; Zaret, 1998; Edmondson, 2006; Payer *et al*., 2001). Growth curves showed a lag phase from zero to three days for spring and autumn seasons. Logarithmic growth phase continued until 15 days for spring, 30 days for summer 18 days for autumn seasons. Decline of growth rate started after 24 and 21 days for summer and autumn seasons, respectively (Fig. 1). Such differences in growth curves could be due to variation in climatic conditions (Table 3). Becker (1994) suggested that, the nutritional status as well as surrounded environmental growth conditions limited both growth and cellular component of green algae. In this respect, the environmental factors may be both physical, such as temperature and light, and chemical, which provide all raw materials used for the structural and protoplasmic synthesis of the algal cells. Richmond and vonshak (1978) stated that when irradiation is very high. *Spirulina* filaments in the upper layer suffer from over-exposure to irradiation. Macronutrients a large number of mineral elements of plant growth, six major essential required in large quantities (N, P , K, Mg , Ca, S) .Nitrogen is the major component of proteins and amino acids, and is, after C, H and O, the most abundant element in living cells (Knud-Hanssen *et al.*, 1998).

The different forms of N can be separated into organic and inorganic, as well as particulate and dissolved components. Particulate organic N is found in living biomass and detritus while soluble organic nitrogenous materials are released into the water from excretions, secretions and decomposition processes. Nitrogen and phosphorus combination have been implicated to be important in regulating the productivity of algae (Taha and Allam, 1959). The optimal temperature range for growth of *H. pluvialis* was found between 25 and 28°C. High temperature severely impaired cell division and some metabolic processes such as chlorophyll synthesis and protein content increased (Fan *et al*., 1994).

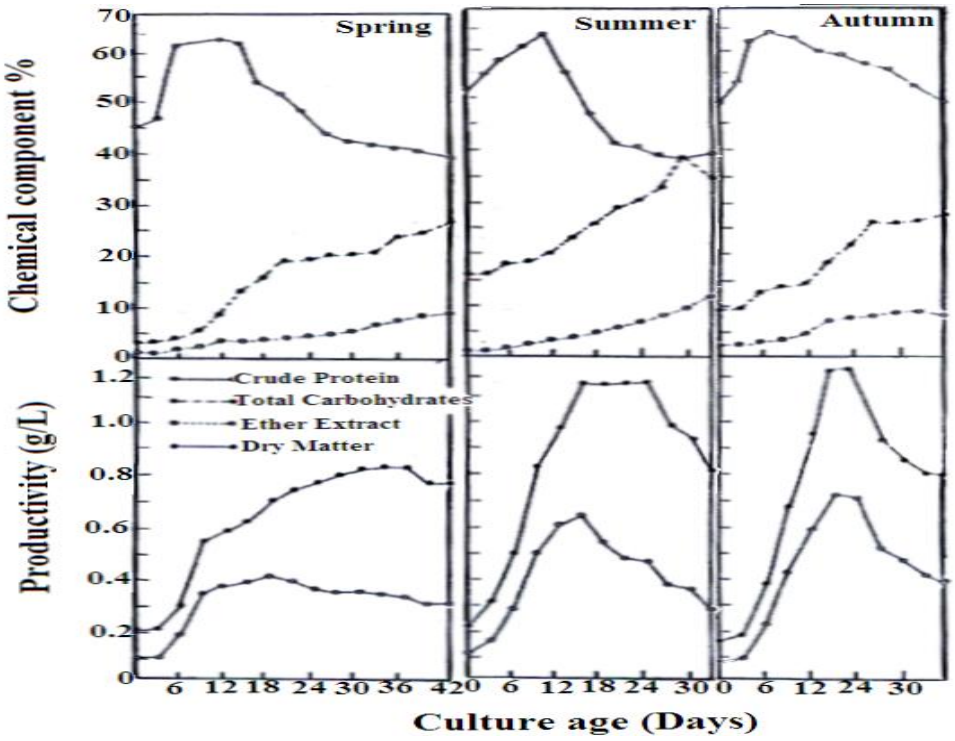

Figure (1): Seasonal variations of dry matter and chemical constituents of outdoor mass culture of *Anabaena wisconsinense* (Dry weight basis).

Borowitzka *et al*. (1991) reviewed that; commercially the large-scale production of *H. pluvialis* must also carefully controlled. Where, growth rate was reduced at higher temperature and the best growth was obtained between 15 and 25°C. However, at 28°C growth was inhibited and at 35°C the culture died. Also, relatively high culture temperature may lead to enhanced carotenoids formation and or higher reactivates of active oxygen in the algal cell (Tjahjono *et al*., 1994). In natural habitat, the aplanospore formation in *H. lacustris* in small ponds

Table (3): Seasonal changes as monthly means of some physico-chemical properties of water samples for use in outdoor mass culture. (Data are represented as mean of three samples replicates ± standard deviation).

Season	Spring			Summer			Autumn		
Months / Parameters	March	April	May	June	July	August	September	October	November
Temperature ^{O}C	18.3 ± 1.1	22.36 ± 2.0	25.8 ± 3.2	27.2 ± 4.3	28.2 ± 0.9	29.46 ± 3	25.4 ± 12	24.26 ± 11	22.7 ± 3.6
Dissolved Oxygen (mg/L)	6.1 ± 0.3	7.13 ± 0.45	5.3 ± 0.9	4.73 ± 0.6	3.76 ± 0.2	3.2 ± 0.47	6.3 ± 1.7	5.16 ± 2.3	6.73 ± 1.8
PH	8.03 ± 2.4	8.13 ± 1.01	8.60 ± 0.7	8.56 ± 0.9	8.7 ± 1.32	8.7 ± 1.2	8.67 ± 0.9	8.36 ± 3.8	7.93 ± 2.9
Salinity ppm	0.21 ± 0.03	0.2 ± 0.00	0.21 ± 0.0	0.22 ± 0.01	0.23 ± 0.02	0.23 ± 0.0	0.2 ± 0.01	0.22 ± 0.0	0.19 ± 0.02
Total Solids (mg/L)	264.6 ± 24	267.3 ± 12	271 ± 18.5	275.6 ± 28	274.6 ± 33	271 ± 26.6	272.3 ± 39	266 ± 23.1	265.3 ± 27
Total suspense (mg/L)	133 ± 0.9	125 ± 3.25	134.6 ± 6.3	137.3 ± 8.6	144.6 ± 13	132 ± 12	145 ± 25.7	138 ± 14.5	151 ± 37.4
NH_4 (mg/L)	0.496 ± 0.1	0.68 ± 0.04	0.45 ± 0.02	0.54 ± 0.03	0.59 ± 0.02	0.68 ± 0.07	0.58 ± 0.14	0.47 ± 0.07	0.54 ± 0.05
NH_3 (mg/L)	0.033 ± 0.0	0.04 ± 0.01	0.036 ± 0.0	0.05 ± 0.00	0.066 ± 0.0	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.00
NO_3 (mg/L)	0.18 ± 0.01	0.193 ± 0.1	0.12 ± 0.0	0.09 ± 0.01	0.09 ± 0.01	0.07 ± 0.03	0.1 ± 0.00	0.11 ± 0.01	0.13 ± 0.04
Alkalinity (mg/L)	319 ± 18.6	332 ± 11.7	346 ± 31.2	357 ± 22.5	368.3 ± 39	387.3 ± 28	340 ± 24.5	348.6 ± 16	358 ± 34.5
Hardness (mg/L)	237 ± 27.4	231 ± 9.20	251.3 ± 26	243.6 ± 17	268 ± 23.5	272.3 ± 18	229.3 ± 26	241.3 ± 18	249.6 ± 31
Total phosphorus (mg/L)	0.32 ± 0.01	0.22 ± 0.04	0.286 ± 0.1	0.26 ± 0.01	0.36 ± 0.10	0.35 ± 0.02	0.28 ± 0.05	0.22 ± 0.03	0.29 ± 0.07

Proximate chemical analysis was determined according to the methods described by Associated of Official Analytical Chemists, A.O.A.C. (1995).

is directed more or less at with standing during out, high irradiation and light (Wong and Chan, 1990).

The growth of algae can be limited by insufficient illumination, low temperature, and low concentration of a biologically important element on the medium or its low rate of diffusion from the medium into the cells. It is suggested that the variation in temperature and light intensities resulted in lower productivity in spring season (Tables 4 and 5). Saleh *et al*. (1979) reported that green algae cultured in summer have been characterized by higher growth rate, followed by autumn and winter. Data of growth and chemical composition of *Spirulina* cells are given in (Fig. 2).

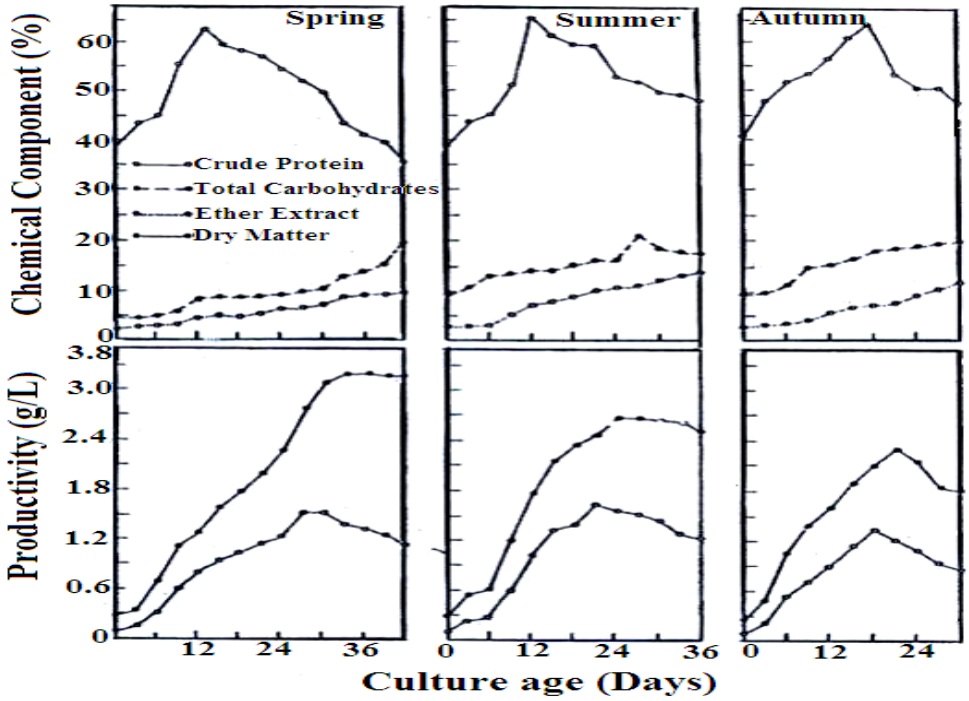

Figure (2): Seasonal variations of dry matter and chemical constituents of outdoor mass culture of *Spirulina platensis* (Dry weight basis).

This phenomenon has been attributed to the decrease of temperature in these seasons. *Anabaena* cultured in the spring season continued growing until 42 days while the summer and autumn cultures bleached and consequently died after 33 and 30 days respectively (Behrenfeld and Flakowski, 1997; Boyd, 1973; Nalewjako *et al.*, 1976; Munoz *et al.*, 2004). Maximum crude protein content as around 63% for all seasons. This was attained at the age of 12 days for spring and summer and at 18 days in autumn seasons. Tables (4 and 5) indicates that the

Table (4): Average productivity* of algal cells and crude protein of outdoor *Anabaena wisconsinense* and *Spirulina platensis* cultures (dry weight basis).

Season / Algae	Average Productivity								
	Spring			Summer			Autumn		
	Day	D. M. mg/L	C. P. mg/L	Day	D. M. mg/L	C. P. mg/L	Day	D. M. mg/L	C. P. mg/L
Anabaena wisconsinense	34	19.5	7.8	16	65	35.5	23	59	34.7
Spirulina platensis	33	87.4	36.5	25	112.7	64.0	21	92.7	51

D. M. = Dry matter mg/L C. P. = Crude protein mg/L

*Average productivity = the change in the dry weight of algal cells (mg/L) from initial culture of the end of the log phase divided.

Table (5): Maximum productivity* of algal cells and crude protein of outdoor *Anabaena wisconsinense* and *Spirulina platensis* cultures (dry weight basis).

Season / Algae	Average Productivity								
	Spring			Summer			Autumn		
	Day	D. M. mg/L	C. P. mg/L	Day	D. M. mg/L	C. P. mg/L	Day	D. M. mg/L	C. P. mg/L
Anabaena wisconsinense	7-9	88.0	52	8 and 9	107	74	9	93	61
Spirulina platensis	25-27	154	98	8-10	204	113	5-7	185	104

*Maximum productivity = the maximum yield (mg/l/day) obtained during the log phase.

calculated average and maximum productivity of algal cells and crude protein showed the highest value in summer culture followed by autumn and spring seasons (Felts and Heath., 1984; Dam *et al*., 2002; Yang and Huang, 2007).

Summer temperature naturally ranged higher that other two seasons and consequently, resulted in higher growth rate and protein productivity. However, analysis of variance comparing algal growth rate and yields of spring summer and autumn cultures were not significant. Maximum protein productivity value of *Anabaena* culture was obtained in summer season followed by autumn and spring respectively, (Tables 4 and 5). Such data agree with higher growth rate in summer of green algae, investigated by Shaheen *et al.* (1984). Results of Figure (2) illustrate that *Anabaena* and *Spirulina* cultures exhibited a decline in crude protein content continued until the end of the cultures. The start of the protein and the extent to which it declined varied with the season of cultivation. Lipids extracted by ether and total carbohydrates in the cells showed a spontaneous increase during the same period.

This metabolic shift from protein synthesis to fat and carbohydrate synthesis has been attributed to deficiency in nitrogen content of the medium due to culture ageing. This phenomenon has been reported by El-Fouly *et al.* (1979) and El-Bastawy *et al.* (1996). Tables (6 and 7), Protein chemical score of samples taken from culture of spring and summer seasons was nearly the same and was relatively higher in the autumn season.

The time required for air-drying the algae was dependent on some environmental factors such as relative humidity, temperature, evaporation rate and light intensity. Qualities of the algal biomass dried by the two methods were compared by determining the moisture and crude protein contents of the dried algal biomass. Oven drying at 110ºC was for drying the harvested algal cells because a much shorter drying time was required. El-Fouly *et al*. (1979) reported that total ash of *Chlorella* decreases proportionally with the decrease in crude protein. However, we might explain these results on the basis that *Anabaena* culture was uncovered. Consequently contamination with atmospheric dust was possible.

Table (8) showed that the analysis of variance of these data indicates no significant difference between the three amino acid patterns. This finding evidenced the stability of amino acid composition of *Anabaena and Spirulina* with change of culturing conditions. Two samples of spring season culture containing maximum and minimum crude protein content (63.6 and 39.2% in *Anabaena*, 63.1 and 35.5% in *Spirulina*), respectively, were analyzed for their amino acids content. Persoone *et al.* (1988) stated that the ash content of *Spirulina* in outdoor culture in was 5%. Venkataraman *et al.* (1980) reported that the total ash of *Spirulina* algae in outdoor culture ranged between 10-12%.

Table (6): Effect of seasonal variation on the combined amino acid content along with their percentage of participation in total amino acid content of *Anabaena wisconsinense* grown at outdoor conditions. (Data expressed as g/100g protein).

Amino acid	Season						FAO pattern **
	Spring	* S.%	Summer	S.%	Autumn	S. %	
Threonine	6.4	7.4	6.2	6.6	6.6	7.4	2.8
Isolecucine	4.8	5.5	5.8	6.1	4.6	5.1	4.2
Leucine	9.7	11.2	8.2	8.7	7.8	8.6	4.8
Lysine	4.2	4.9	5.4	5.7	4.0	4.5	4.2
Methionine* **	0.1	0.12	0.1	0.1	1.0	0.01	2.2
Phenylalanine***	2.8	3.2	4.2	4.4	3.5	3.9	2.8
Valine	4.9	5.7	6.7	7.0	6.2	6.9	4.2
Tryptophane	1.4	1.6	1.5	1.0	1.5	1.7	1.2
Histidine	1.2	1.4	1.3	1.4	1.4	1.6	
Arginine	6.4	7.4	6.5	6.9	6.3	7.0	
Aspartic acid	8.5	9.8	10.0	10.5	10.0	11.2	
Serine	3.2	3.7	5.0	5.3	4.1	4.6	
Glutamic acid	12.0	13.9	12.4	13.1	10.4	11.6	
Proline	4.0	4.6	4.2	4.5	4.4	4.9	
Glycine	5.1	5.9	4.8	5.0	5.1	5.7	
Alanine	8.2	9.5	8.1	8.6	8.3	9.3	
Tyrosine	3.5	4.0	3.8	4.02	4.1	4.6	
Ornithine	0.1	0.1	0.2	0.2	0.2	0.22	
Total amino acids	86.5		94.4		89.5		

***S. % Score% of total amino acids * *Recommended essential amino-acid content for an ideal protein for animal consumption (WHO/FAO) (Bhumiratana, 1976). * **Essential amino acids in *Anabaena* g/100g protein. Algal samples containing crude protein level (dry weight basis).**

The amino acid composition of the 3 species compared well with the FAO amino acid pattern except for methionine and isoleucine, in the study carried by Saleh *et al*. (1979). They reported that bioassay evaluation of the algal proteins gave the following values: protein efficiency ratio 1.9 to 2.1, net protein ratio 2.4 to 2.8, biological value 75 to 78, digestibility coefficient 88 to 89 and calculated net protein utilization 67 to 69.

Total nucleic acid content was about 4%. Uric acid concentration in plasma of rats fed on the algae was significantly higher than in plasma of rats fed on a casein diet. Tartiel (2005) found that algal growth at final density and biomass yield of examined algae grown in outdoor culture, comparison of moisture and crude protein contents of the algal biomass dried by Oven were 5.8 % of *Chlorella and* 4.9 % of *Scenedesmus*, while crude protein content were 46.7 % and 52.3 % respectively, and air drying at about 25ºC temperature room were found to be 9.8 % and 8.7 %, while crude protein content were 48.2 % and 53.1 % respectively.

Table (7): Effect of seasonal variation on the combined amino acid content along with their percentage of participation in total amino acid content of *Spirulina platensis* grown at outdoor conditions. (Data expressed as g/100g protein).

Amino acid	Season						FAO Pattern
	Spring	* S. %	Summer	*S. %	Autumn	*S. %	
Threonine	6.1	6.9	6.8	6.9	5.5	6.06	2.8
Isolecucine	6.2	7.02	6.0	6.08	6.3	6.9	4.2
Leucine	9.2	10.4	9.7	9.8	10.1	11.1	4.8
Lysine	5.3	6.0	4.8	4.9	4.3	4.7	4.2
Methionine	1.0	1.4	1.2	1.2	1.2	1.3	2.2
Phenylalanine	4.0	5.7	5.4	5.5	5.8	6.4	2.8
Valine	6.2	7.02	7.0	7.09	6.3	6.9	4.2
Tryptophane	1.4	1.6	1.5	1.5	1.4	1.5	1.2
Histidine	1.3	1.5	1.2	1.2	1.4	1.5	
Arginine	6.3	7.1	6.8	6.9	5.9	6.5	
Aspartic acid	7.4	8.3	9.2	9.3	8.0	8.8	
Serine	4.1	4.6	4.3	4.4	3.3	3.6	
Glutamic acid	12.4	14.0	13.1	13.3	11.2	12.3	
Proline	4.8	5.4	4.7	4.8	3.7	4.07	
Glycine	5.9	7.8	5.8	5.9	5.3	5.8	
Alanine	4.2	4.8	7.2	7.3	7.5	8.3	
Tyrosine	2.2	2.5	3.5	3.5	3.2	3.5	
Ornithine	0.3	0.33	0.4	0.40	0.3	0.33	
Total amino acids	88.3		98.6		90.7		

Table (8): Effect of drying methods on moisture and protein for examined algae grown in outdoor cultures.

Drying methods (Dry matter)	Oven dry at 110 ºC for 15-20 minutes		Air at room temperature for 72 – 96 hours	
	Moisture %	Protein%	Moisture %	Protein %
A. wisconsinense	63.6±0.5	5.8±1.3	63.8±0.75	9.8±2.7
S. platensis	63.1±1.6	4.9±0.05	63.5±0.94	8.7±0.33

@ Data are represented as mean of three samples replicates ± standard deviation

Methionine was the limiting amino acid in both samples (Table 9) showed no significant difference between the two amino acid patterns. These results are in agreement with those obtained by Woynaravich (1968); Vollenweider (1969) and Fngerhut (1985). Results obtained from studies on the green algae by El-Fouly *et al.* (1998) are in agreement with the reported findings on the amino acids composition as affected by seasonal variation or protein level.

Samples of *Anabaena* and *Spirulina* were dried by oven drying method at 100°C overnight or by drum drying at 110° C and analyzed for amino acid composition (Table 10). Oven drying led to decreases in the contents of some amino acids, in particularly methionine, phenylalanine, aspartic acid, serine and glycine in *Anabaena* and methionine, serine, glyeine and alanine in *Spirulina*.

Table (9): Variation in the amino acids composition with level of crude protein in *Anabaena wisconsinense* and *Spirulina platensis* grown at outdoor conditions. (Data expressed as g/100g protein).

Amino acid (a. a.)	FAO pattern	Algae							
		Anabaena wisconsinense				*Spirulina platensis*			
		Maximum protein		Minimum protein		Maximum protein		Minimum protein	
		a. a.	s.%	a. a.	s.%	a. a.	s.%	a. a.	s.%
Threonine	**2.8**	**6.4**	**228**	**5.1**	**182**	**6.1**	**217**	**5.5**	**196**
Isolecucine	**4.2**	**4.8**	**30.7**	**3.7**	**88**	**6.2**	**147**	**6.1**	**145**
Leucine	**4.8**	**9.7**	**202**	**7.5**	**156**	**9.2**	**191**	**9.0**	**187**
Lysine	**4.2**	**4.2**	**100**	**3.0**	**71**	**5.3**	**126**	**4.3**	**102**
Methionine	**2.2**	**0.1**	**4.5**	**0.7**	**31**	**1.0**	**54**	**1.4**	**63**
Phenylalanine	**2.8**	**2.8**	**100**	**2.9**	**103**	**4.0**	**178**	**4.8**	**171**
Valine	**4.2**	**4.9**	**116**	**4.6**	**109**	**6.2**	**147**	**6.0**	**142**
Tryptophane	**1.2**	**1.4**	**117**	**1.2**	**100**	**1.4**	**117**	**1.3**	**108**
Histidine		**1.2**				**1.3**			
Arginine		**6.4**				**6.3**			
Aspartic acid		**8.5**				**7.4**			
Serine		**3.2**				**4.1**			
Glutamic acid		**12.0**				**12.4**			
Proline		**4.0**				**4.8**			
Glycine		**5.1**				**5.9**			
Alanine		**8.2**				**4.2**			
Tyrosine		**3.5**				**2.2**			
Ornithine		**0.1**				**0.3**			

***Recommended essential amino-acid content for an ideal protein for animal consumption (WHO/FAO) (Bhumiratana, 1976). * S. % Score% of FAO pattern.**Essential amino acids in *Anabaena wisconsinense* g/100g protein.**

Fee (1970); Allen and Garret (1977); De Pauw *et al*. (1984) stated that drum dried green algae gave a significant higher nutritive value and essential amino acids pattern as compared with other processing methods. They also stated that better performance of drum dried algae might be attributed to the cell wall drying, which results in better digestibility and utilization of protein. Becker (1987) mentioned that algae contained significantly less nucleic acids (about 4% of the dry weight) than the yeast (11%) and bacteria (up to 18 %). These values of

nucleic acids content in blue-green algae *Anabaena and Spirulina* are acceptable from nutritional point of view. FAO/WHO Committee (1983) considered 5% nucleic acids (dry wt. basis) in food products a safe level. Total ash in algal cells unexpectedly increased with the decrease in crude protein content.

Table (10): Effect of drying methods on the amino acid composition of *Anabaena wisconsinense* and *Spirulina platensis* grown at outdoor conditions. (Data expressed as g/100g protein).

Amino acid	*Anabaena wisconsinense*		*Spirulina platensis*	
	Drum dried	Oven dried	Drum dried	Oven dried
Threonine	**7.2±0.60**	**6.2±0.32**	**7.3±0.17**	**6.8±0.16**
Isolecucine	**6.0±0.13**	**5.8±1.52**	**6.4±2.56**	**6.0±0.14**
Leucine	**9.3±0.10**	**8.2±0.24**	**11.1±0.55**	**9.7±0.66**
Lysine	**4.9±1.06**	**5.4±0.29**	**4.6±0.84**	**4.8±0.70**
Methionine	**1.6±0.49**	**0.1±0.11**	**1.8±0.61**	**1.2±0.16**
Phenylalanine	**6.0±0.36**	**4.2±0.19**	**5.6±0.52**	**5.4±0.75**
Valine	**7.2±0.54**	**6.7±0.59**	**7.4±0.42**	**7.0±1.28**
Tryptophane	**1.5±0.66**	**1.5±1.00**	**1.6±0.33**	**1.5±1.80**
Histidine	**1.4±0.38**	**1.3±0.32**	**1.6±0.79**	**1.2±0.23**
Arginine	**7.7±0.43**	**6.5±0.69**	**8.2±0.19**	**6.8±0.44**
Aspartic acid	**8.4±1.33**	**10.0±0.65**	**10.3±0.76**	**9.2±0.68**
Serine	**12.8±0.37**	**5.0±1.22**	**7.8±0.92**	**4.3±0.88**
Glutamic acid	**15.1±0.70**	**12.4±0.20**	**14.5±0.96**	**13.1±1.45**
Proline	**5.7±0.13**	**4.2±0.69**	**4.8±1.22**	**4.7±0.37**
Glycine	**9.3±0.28**	**4.8±0.73**	**9.8±0.59**	**5.8±0.87**
Alanine	**9.3±0.55**	**8.1±0.70**	**9.5±0.33**	**7.2±0.36**
Tyrosine	**4.2±1.44**	**3.8±0.35**	**4.5±0.47**	**3.5± 1.60**
Ornithine	**0.2±0.84**	**0.2±0.39**	**0.3±0.51**	**0.4±0.21**

***Recommended essential amino-acid content for an ideal protein for animal consumption (WHO/FAO) (Bhumiratana, 1976). **Essential amino acids in *Anabaena* g/100g protein. @ Data are represented as mean of three samples replicates ± standard deviation**.

On the other side, the obtained correlation coefficient ($P< 0.01$) evidences the negative correlation between cellular protein content from one side and media nitrogen during logarithmic growth phase and cellular fat and total carbohydrates during nitrogen deficiency period. All values were significant for spring, summer and autumn cultures except the value of *Spirulina* obtained in autumn season. It is considered that insignificance of correlation coefficient value in the autumn season is related to the low number of samples used ($n = 5$), y = regression line, r = correlation coefficient and p = significant relationship $p< 0.01$.

Wang *et al.* (2005) stated that *Spirulina* grown in synthetic media contained about 60% protein, and it decreased to only 28% for stationary phase culture. Figures (3 and 4) show the linear regression line equation and the correlation coefficient of available inorganic nitrogen content in media and algal

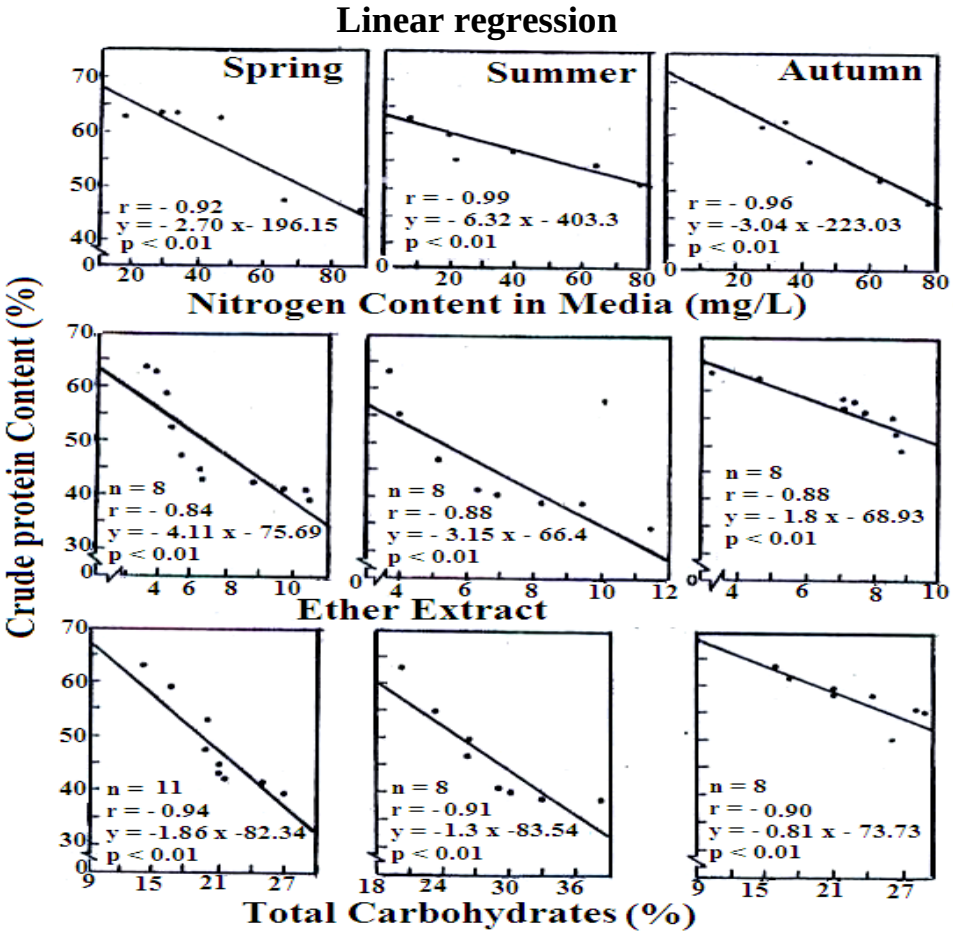

Figure (3): Correlation between crude protein content and nitrogen content, ether extract and total carbohydrates in outdoor mass culture of *Anabaena wisconsinense* (Dry weight basis).

crude protein during the logarithmic phase. Also correlation between cellular crude protein content of *Anabaena and Spirulina* and the other chemical components (total carbohydrate and ether extract) during nitrogen deficient period were calculated. Methionine was the sole limiting amino acid as compared to the FAO provisional amino acid pattern (FAO 1983; Wong and Chan 1990; Vymazal 1995; Zaghloul 1997). This reveals the fact of the dependence of chemical availability of amino acids on method of drying. Statistical analysis, evidences this finding and indicates significant difference between the two patterns ($P<0.01$).

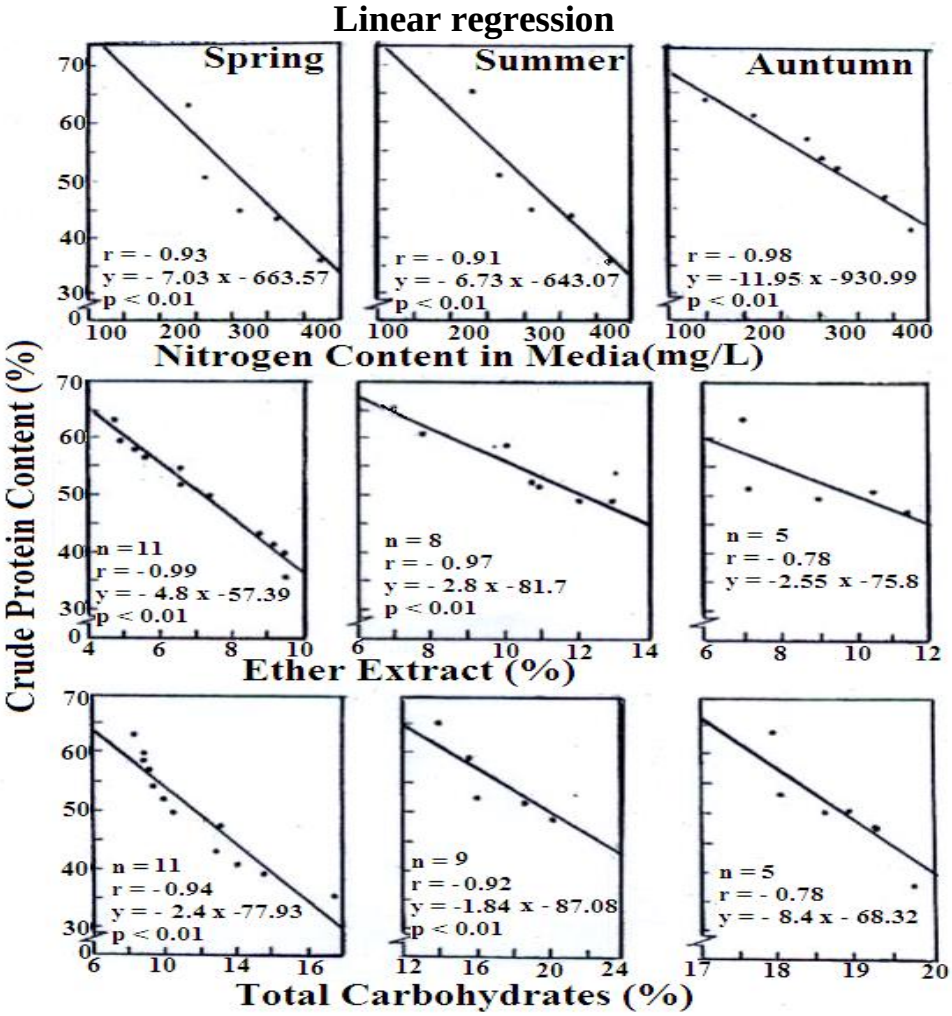

Figure (4): Correlation between crude protein content and nitrogen content, ether extract and total carbohydrates in outdoor mass culture of *Spirulina platensis* (Dry weight basis).

Variations in some chemical components accompanying maximum and minimum crude protein content are shown in Tables (11 and 12). Decrease in cellular crude protein was accompanied with 15 and 20% decrease in nucleic acids content in both strains respectively. Indigestible polysaccharides Tables (11 and 12) were higher in samples containing minimum protein compared with samples containing maximum.

These varying increases are proportional with decreases of crude protein content of algal cells cultivated in different seasons. Meanwhile, the increase of indigestible polysaccharides content is related to the increase of carbohydrates in the same samples. The high percentage of silica content (about 50% of total ash as shown in Table (11) might stress this probability. Wu and Chung (1964) and Forstner and Witman (1983) stated that the surface of ponds containing algae tend to absorb air born particles such as dust and pollen, which increase the apparent ash content of algae.

In this respect De Pauw *et al.* (1998) mentioned that site selection is one of the most important factors which can increase net productivity of algae, added that micro algae plant should be located at site where climatic conditions provide optimal growth conditions for the longest possible period. The yield from 3 large-scale cultures of *Scenedesmus acutus, Chlorella vulgaris and Coelastrum proboscideum* was drum dried. Algae are autotrophic organisms, which have potential as food and feed for man and animals. They are rich in protein (50-60%), lipids (2-22%), vitamins and minerals.

With a mixed algal culture (*Chlorella* and *Scenedesmus)* grown in shallow polytene basins, daily yield of algal suspension was 95 tons or 247 kg dry substances per hectare. Also, the same authers added that the estimated cost is about $1.25/ton of algal suspension at 10% of their live weight. In comparison to oilcake (0.5 kg/day), algal suspension supplemented to a basal straw diet increased fiber digestibility 81%, growth rate 458 g/day and feed conversion efficiency. Results were also in accordance with those obtained by Wang *et al.* (2007), they reported that the context of threats to fragile environments, there is a need in animal production to identify alternative feed resources, which are environmentally friendly, but at the same time utilize natural resources efficiently.

When a culture grows under optimum conditions its growth curve follows a sigmoid pattern in which at least five phase in the life of the culture may be recognized. They are namely: log phase, logarithmic phase, linear phase, stationary phase and death phase (Venkataraman *et al*., 1980). This phenomenon could be attributed to high temperature and high sunlight intensity during these seasons, which led to photo-inhibition. Other studies on growth of green and blue-green algae under outdoor cultivation conditions gave similar results as reported by El-Fouly *et al.* (1998). The green algae require, for optimum growth, temperature in the culture liquid between 26°C and 32°C, the irradiation should exceed 600 Cal/m^2 per day. Conditions like these exist in Egypt all the round, which allow high and stable yield with optimum efficiency of equipment utilization. On the other hand, it was observed that the amount of protein was within the same range for all cultures (1.3 – 1.6 g/L) in spite of the differences in algal yields. The latter values were 3.18, and 1.8 g/L. for spring and autumn

Table (11): Variation of chemical components with level of protein in *Anabaena wisconsinense* (Dry weight basis).

Chemical comp. (Dry wt.)	Cultures					
	Spring		Summer		Autumn	
	Mx.	Mn.	Mx.	Mn.	Mx.	Mn.
Crude protein (%)	62.9±3.6	39.2±0.08	63.6±0.98	34.3±1.33	62.7±0.77	48.2±1.6
Nucleic acids (%)	4.8±0.36	4.1±0.12	4.7±0.22	4.0±0.22	4.7±0.23	3.9±0.04
Carbohydrate (%)	8.2±0.11	5.7±0.54	8.0±0.37	7.8±0.61	7.0±0.41	3.4±0.01
Crude fat (%)	10.0±0.28	9.4±0.36	11.0±0.04	9.6±0.05	10.3±0.68	9.8±0.08
Total ash (%)	9.1±0.10	8.3±0.05	9.8±0.74	8.1±0.43	9.2±0.84	7.7±0.02
Crude fiber (%)	5.2±0.01	5.0±0.0	5.2±0.32	4.9±0.07	5.3±0.44	4.1±0.07
Phosphorus (%)	1.1±0.09	0.8±0.00	1.2±0.04	0.9±0.01	1.3±0.02	1.0±0.06
Calcium (%)	1.4±0.05	0.9±0.04	1.4±0.06	1.3±0.05	1.6±0.07	1.5±0.00
Iron (%)	0.5±0.01	0.5±0.01	0.6±0.01	0.5±0.0	0.6±0.06	0.5±0.01
Copper (ppm)	1.2±0.03	1.2±0.06	1.0±0.02	1.0±0.01	1.2±0.04	1.2±0.01
Zinc (ppm)	3.0±0.41	2.5±0.03	3.0±0.00	2.6±0.03	2.9±0.41	1.8±0.02
Manganese (ppm)	1.4±0.07	1.4±0.07	1.6±0.3	1.2±0.08	1.5±0.02	1.5±0.05
Magnesium (ppm)	15.0±0.92	14.0±0.88	15.0±0.90	13.0±0.56	12.0±0.45	11.0±1.3

*** Mx. And Mn. Are samples containing maximum and minimum crude protein, respectively. * Mean value ± standard deviation**

Table (12): Variation of chemical components with level of protein in *Spirulina platensis* (Dry weight basis).

Chemical comp. (dry wt.)	Cultures					
	Spring		Summer		Autumn	
	Mx.	Mn.	Mx.	Mn.	Mx.	Mn.
Crude protein (%)	60.1±1.6	41.5±0.98	63.1±1.14	47.5±0.43	60.7±1.54	47.2±1.22
Nucleic acids (%)	4.2±0.08	4.0±0.07	4.6±0.52	3.9±0.04	4.0±0.06	2.9±0.32
Carbohydrate (%)	8.2±0.01	6.0±0.02	8.0±0.23	7.6±0.07	8.0±0.03	6.8±0.04
Crude fat (%)	12.0±1.4	10.7±0.33	12.0±0.87	11.3±.02	12.0±0.08	11.0±0.43
Total ash (%)	9.4±0.43	8.5±0.21	9.7±0.22	8.0±0.31	8.4±0.11	8.4±0.22
Crude fiber (%)	5.4±0.06	4.9±0.28	5.5±0.07	4.2±0.02	5.0±0.21	3.9±0.08
Phosphorus (%)	1.2±0.04	0.8±0.01	1.3±0.02	0.8±0.01	1.3±0.07	1.0±0.09
Calcium (%)	1.4±0.01	0.9±0.04	1.4±0.00	1.2±0.01	1.6±0.04	1.5±0.01
Iron (%)	0.6±0.01	0.5±0.07	0.6±0.05	0.5±0.03	0.6±0.00	0.5±0.00
Copper (ppm)	1.3±0.02	1.0±0.00	1.2±0.07	1.0±0.01	1.0±0.01	0.9±0.02
Zinc (ppm)	3.0±0.00	2.4±0.04	3.4±0.09	3.0±0.07	3.0±0.02	2.8±0.01
Manganese (ppm)	1.6±0.08	1.5±0.01	1.5±0.07	1.4±0.03	1.3±0.00	1.1±0.07
Magnesium (ppm)	15±0.55	14.0±0.78	15.0±1.33	13.0±0.92	12.0±0.95	9.0±1.58

*** Mx. And Mn. Are samples containing maximum and minimum crude protein, respectively.**
*** Mean value ± standard deviation**

seasons, respectively. Lower dry matter yield in summer and autumn seasons resulted probably form the death of algal cells at the age of 36 and 30 days respectively. Death of cells could be attributed to photosynthesis inhibition which has been reported in other studies. (Fan *et al*., 1994; Yan *et al*., 2000; Delorenzo *et al.*, 2004), reported that production of *Spirulina* in Southern France was 12

g/m^2/day, and its protein content was 64 – 70 % of the dried matter and variation in the raw protein content might be observed, especially when nitrogen was lacking in the medium.

In conclusion, Egypt has an arid climate with maximum irradiation, offering the best conditions for photosynthetic productivity. Variation of day-length is not considered to be of importance with respect to algal yields. The fluctuations of temperature during the seasons might be compensated by choice of appropriate algal species.

Acknowledgments

The candidate wishes to express her deep gratitude and my deep thanks to Prof. Dr. Eglal Mohamed Zaki Harb, Department of Botany, Cairo University, special thanks and deepest gratitude to Prof. Dr. Nabil Fahmy Abdel Hakim, Al-Azhar University for his great help and would like to thank Prof. Dr. Gamal El-Naggar, for his great help the whole work with continuous support, World Fish Center, Regional Center for Africa and West Asia, Abbassa, Abu- Hammad, Sharkia, Egypt.

References

Allen, E. J. and Nelson, E.W. (1974). On the artificial culture of marine plankton organisms. *J. Mar. Biol. Assoc*., **8:421-474**.

Allen, M. D. B. and Garret, M. K. (1977). Bacterial changes occurring during the culture of algae in the liquid phase of animal slurry. *J. Appl. Bacteriol*., **42:27-43**.

American Public Health Association (APHA) (1985). Standard methods for the examination of water and wastewater, edition American Public Health Association, Washington, D. C., USA.

Association of Official Analytical Chemists (A.O.A.C.) (1995). Official Methods of Analysis Association of Official Analytical Chemists. Edit., KHL Rich. Arlington Vargenia, USA.

Becker, E. W. (1987). Biotechnology and exploitation of the green alga *Scenedesmus obliquus* in India. *Biomass*, **4 (1):1-19**.

Becker, E.W. (1994). Microalgae, Biotechnology and Microbiology. *Cambridge Univ. Press*., **pp. 9-39**.

Behrenfeld, M. J. and Flakowski, P.G. (1997). Photosynthetic rates derived from satellite-based on chlorophyll concentration. *Limnol. Oceanogr*., **42:1-20**.

Benemann, J. R. (1992). Microalgae aquaculture feeds and environmental. *J. Appl. Phycol*., **4: 233-245**.

Bhumiratana, A. (1976). The production and utilization of Algae as a Protein source in Thailand. *Kasetsart University, Bangkok*, **90 pp**.

Boyd, C. E. (l973). Summer algal communities and primary productivity in fish ponds. *Hydrobiol*., **41: 357-390**.

Cohen, Z. and A. Vonshak. (1991). Fatty acid composition of *Spirulina* sp like cyanobacteria in relation their chemotaxonomy. *Phytochemistry*, **30:205-206**.

Dam, R.; Lee, S. K.; Fry, P.C. and Fox, H. M. (2002). Utilization of algae as a protein source for humans. *J. Nutr*., **86:376-382**.

De Pauw, N.; Verboven, J. and Claus, C. (1984). Large-scale microalgae production for nursery rearing of marine bivalves. *Aqua Cultural Engineering*, **116/ 117:121-134**.

DeLorenzo, M. E.; Leatherbury, M.; Weiner, J. A. and Fulton, M. H. (2004). Physiological factors contributing to the species-specific sensitivity of four estuarine microalgal Exposed to the Herbicide Atrszine. *Aquat. Ecosyst. Health Manage*, **7:137-146**.

Dubois, M.; Gilles, K. A.; Hamilton, J. K. and Smith, F. (1956). Colorimeteric methods for determination of sugars and related substances. *Analyt. Chem*., **18:350-356**.

Duncan, D. B. (1996). Multiple range and multiple F test. *Biometrics*, **11:1-42**.

Edmondson, W. T. (2006). Eutrophication in North America, eutrophication causes consequences. Washington, D.C., USA., **124-149**.

El-Bastawy, E.; Belinger, E. G. and Sigee, D. C. (1996). Elemental composition of phytoplankton in a subtropical lake, *Spirulina platensis* (Cyanophyta) *Eur. J. Phycol*., **31:157-166**.

El-Fouly, M. M.; Abdalla, F. E. and Saleh, A. M. (1979). Studies on out-door mass culture of green alga *Chlorella vulgaris*. Effect of nitrogen efficiency in growth medium. *Al-Azhar Agric. Bull*., **22:l-14**.

El-Fouly, M. M.; Soeder, C. J.; Mohn, F. H. and Greoneweg, J. (1998). Open door mass production, chemical composition and biological evaluation of different algal species. *Bull. Egypt*, **2: 149-165**.

Fan, L.; Vonshak, A. and Boussiba, S. (1994). Effect of temperature and irradiance on growth of *Haematococcus pluvialis*. *J. Phycol*., **30:829-833**.

FAO (1983). Compilation of legal limits for hazardous substances in fish and fishery products. *FAO Fishery Circular No. 464.*

Fee, E.J. (1970). A relation between lake morphometry and primary productivity and its use in interbreeding whole. Lake eutrophication experiments. *Limnol. Oceanger*., **24: 401-416**.

Felts, P.A. and Heath, A.G. (1984). Interactions of temperature and sublethal environmental copper exposure on the energy metabolism. *J. Fish Biol*., **25: 445-453**.

Fingerhut, U. (1985). Wachstum und Zusammenwirken von Scenedesmus falctus und Pseudomonas aeroginosa bei chemostatischer Kultur in acetathaltigem Modellabwasser., Phytochemistry.,205-209.

Fogg, G.E. (1984). Algal cultures and phytoplankton ecology. *University of Wisconsim Press*, **pp. 37-51**.

Forstner, U. and Witman, G.T. (1983). A histological examination and analysis for polychlorinated hydrocarbons in shore atlantic cod (*Gadus morhua*). *Arch. Environ. Contam. Toxicol*., **12:627-632**.

Lin, W.; Pan, B.; Xu, J. and Q. Hu. (2007). Antioxidant activity of *Spirulina platensis* extracts by supercritical carbon dioxide extraction. *Food chem*., **105:36-41**.

Lu, J., Yoshizaki, M. Sakai, K. and Takeuchi, T. (2002). Acceptability of raw *Spirulina platensis* by larval tilapia . *Fish. Sci*., **68:51-58**.

Morse, M. L. and Carter, C. F. (1969). The synthesis of nucleic acid in cultures of *Escherchia coli* Strains B and B\r. *J. Bacteriol*., **58:317-326**.

Munoz, R.; Kollner, C.; Guieysse, B. and Mattiasson, B. (2004). Photosynthetically oxygenated salicylate biodegradation in a continuous stirred tank photobioreactor. *Center for Chemistry and Chemical Engineering, Lund University*, **6:797-803**.

Nalewjako, C; Dunstall, T. G. and Shear, H. (1976). Kinetics of extra-cellular release in axenic algae and in mixed algal-bacterial cultures: Significant in the estimation of total phytoplankton excretion rates. *Bull. Egypt*, **4:134-139**.

Nandeesha, M. C.; Gangadhara, B.; Varghese, T. J. and Keshavanath, P. (1998). Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp. *Aquaculture Res*., **29:305-312**.

Payer, H. D.; Chiemvichak, Y.; Hosakul, K. and Kongpanich, J. (2001). Temperature as an important climatic factor during mass production of microalgae.Algae Biomass. *Elsevier/North-Holland Biomedical Press, New York.,USA*., **pp. 389-399**.

Persoone, G.; Morales, J. and Claus, C. (1988). Mass culture of algae bottleneck in the nursery culturing of molluscs. *Algae biomass. Elsevier/North-Holland Biomedical Press, New York., USA*.

Saleh, A. M.; Abdalla, F.E. and El- Fouly, M. M. (1979). Studies on outdoor mass-culture of green alga . Seasonal variation of growth and crude Protein content. *Al-Azhar Univ. Press. Cairo*.

Spectorova, A.; **Montesinos, J. L.; Cusido J. A. and Godia, F.** (1997). Recovery and treatment of *Spirulina platensis* cells cultured in a continuous photobioreactor to be used as food. *Process Biochem*., **37: 535-547**.

Taha, E. M. and Allam, A. M. (1959). Physiological and biochemical studies on Egyptian fresh water algae. V. Growth and cell protein influenced by culture conditions. *Arch. Microbiol*., **34:393-400**.

Tartiel, M. Badawy (2005). Physiological studies on some green algae. Ph.D. Thesis, Faculty of Agriculture, Cairo University. Egypt.

Venkataraman, L.V.; Nigam, B.P. and Ramanathan, P. K. (1980). Rural oriented fresh water cultivation and production of algae m India. In: G. Algae Biomass. Elsevier/North- Holland Biomedical Press, New York, USA., **pp.81-95**.

Vollenweider, S. K. (1969). Method for determination of Chlorophyll in microplants and phosphoproteins in algae. *J. Bid, Chem*., **161:81**.

Vymazal, J. (1995). Algae and element cycling in wet lands .Duke Univ. North Calorina .*CRC press*.

Wang, Q.L.; Liu, Y.D.; Shen, Y.W. ; Jin, C.Y. and Zhu, J.M. (2005). Studies on mixed mass cultivation of *Anabaena* spp. (nitrogen-fixing blue-green algae) on a large scale. *Hydrobiol. Acad. Sinica, China*. **3:221-228**.

Wang, S.M.; Wang, Q.L.; Li, S.H. and Zhang, J. R. (2007). A study of treatment of spring wheat with growth promoting substances from nitrogen-fixing blue-green algae. *Academia Sinica, Wuhan, Hubei, China*,**1:45-52**.

Wong, P.K. and Chan, K.Y. (1990). Growth and value of *Chlorella salina* grown on highly saline sewage effluent. *Department of Biology, Chinese University of Hong Kong, Environment*, **4:234-250**.

World Health Organization (WHO) (1984). Guide lines for drinking water quality. Geneva.

Woynarovich, E. (1968). New systems and new fishes for culture in Europe. *FAO Fish. Rep*., **44 (5): 162-181**.

Wu, H.W. and Chung, L. (1964). Progress and achievements in the artificial propagation of four farm fishes in China. *Gen*., **160: 203-218**.

Yan, G.; Yu-Jing, Y.; Wang, X. and Wang, Y.X. (2000). The effects of pH and temperature on orthophosphate removal by immobilized *Chlorella vulgaris. Biotechnology Letters*, **18(8):893-896**.

Yang, J. X. and Huang, X.F. (2007). Effects of environmental factors on the hatching rate of eggs (*Rotatoria: Monogononta*). *Acta Hydrobiologica Sinica*, **4: 331-339**.

Zaghloul, K. H. (1997). Studies on the effect of water pollution along different sites of the River Nile on the survival and production of some freshwater fishes. Ph. D. Thesis. Zoology, Cairo Univ. Egypt.

Zaret H. D. (1998). The effect of phosphorus and nitrogen on phytoplankton dominace in fish ponds. *Aquaculture Research*, **28:591- 597**.

Zaret T. M.; Devol, A. H. and Dos Santos, A. (1981). Nutrient addition experiment in Lago Jacaretinga, Central Amazon Basin, Brazil. *Verh. Internat. Verein. Limnol.*, **21:721-724**.

Zarrouk, C. (1966). Contribution a` l'étude d`une cyanophycee. Influence de divers facteurs physiques photosynthe *Spirulina* sp. Ph D Thesis, Université de Paris, France.

الا نتـــاجيـــــة الموسـميـــة والتركـيب الكيمــاوى لطحلبى الا نا بينـــا والا سبيرولينـــا تحـــت الظـــروف البيـــئيـــة المصـــر يـــة

نبيل فهمى عبد الحكيم[1] و ترتيل السيد محمد بدوى[2]

1- كلية الزراعة- جامعة الأزهر.

2- المعمل المركزى لبحوث الثروة السمكية بالعباسة – قسم بحوث البيئة والبيولوجى – مركز البحوث الزراعية - وزارة الزراعة – الدقى – مصر.

يمثل النتروجين المثبت من الجو اكثر من 90% من المحتوى النتروجينى على سطح الارض ، وتعتبر الطحالب الخضراء المزرقة اكثرالاحياء الدقيقة نشاطا لتثبيت النتروجين، ويتناول هذا ا لبحث د راسة نمو جنسين من الطحالب الخضراء المزرقة تحت ظروف الجو الخارجى (الظروف البيئية المصرية)، وتحت تقدير الاختلافات فى التركيب الكيماوى للطحالب النامية فى المواسم المختلفة 0وقد أظهرت النتائج ارتفاع متوسط الانتاجية فى شهور الصيف يليها الخريف ثم الربيع فى كلا الطحلبين . كذ لك أوضحت النتائج انه بزيادة عمر المزرعة يقل محتوى الخلايا الطحلبية من البروتين وكان هذا مصحوبا بزيادة فى محتوى الدهن والكربوهيدرات الكلية ونقص فى الرماد والاحماض النووية، وكذلك قد اظهرت طرق التجفيف المختلفة تأثيرات على محتوى الاحماض الامينية ، وكان لطحلب الانابينا قد رة عالية فى تبيت النتروجين ولذ لك ينصح بأستخدامه فى التسميد الحيوى biofertelization ، اما طحلب الاسبيرولينا فكان لديه القد رة على افراز أحماض امينية فى البيئة يعتبر البعض منها كمواد مضادة لنمو بعض انواع البكتريا، وأظهرت طرق التجفيف المختلفة تأثيرا واضحا على محتوى الخلايا من البروتين وكذلك نسبة الاحماض الامينية المختلفة.