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

NUTRITIONAL VALUE AND FATTY ACID COMPOSITIONS OF *CHLORELLA VULGARIS* GROWN UNDER AUTOTROPHIC AND HETEROTROPHIC CONDITIONS

Mostafa El-Sheekh[1] and Alaa A. Fathy[2*]

[1]Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.
[2] (corresponding author) Botany Department, Faculty of Science, Alexandria University, Alexandria, Egypt.*

Abstract

Chlorophyll -a content of autotrophic cells of *Chlorella vulgaris* was double that estimated in heterotrophic cells, while chlorophyll -b content of autotrophic cells was nearly half the value recorded for heterotrophic cells. Carotenoids content of heterotrophic cultures decreased by 30.82% compared to that value of autotrophic cells. There was a slight decrease in the protein content of *C. vulgaris* under heterotrophic conditions. When the composition of total free amino acids and proline of *C. vulgaris* grown under autotrophic conditions is compared to that grown heterotrophically, it was observed that a significant increase in total free amino acids and proline in heterotrophic cultures. The percentage of most fatty acids of heterotrophic cells was relatively higher than autotrophic ones. There was no qualitative difference between autotrophic and heterotrophic cultures, except for the fatty acid 16:02 which was absent under autotrophic conditions.

Keywords: Fatty acids, *Chlorella vulgaris*, autotrophic**,** heterotrophic.

Introduction

Natural products play an important role in the drug production (Cragg *et al.*, 1997). Thus, the investigation of new algal chemical compounds, as natural source of natural products, has proved to be a promising area of pharmaceutical study (Blunt *et al*., 2005; Singh *et al*., 2005; Cardozo *et al.*, 2007).

Carotenoids are natural pigments exercising important biological functions in algae, plants and animals (Polivka and Sundström, 2004; Cardozo *et al*., 2007). For human nutritional purposes, some carotenoids offer provitamin A activity (Mayne, 1996). They are directly providing photoprotection against UV light photooxidation in the skin beside their key role factor in reducing the incidence of many diseases (Cantrell *et al.*, 2003; Astley *et al.*, 2004; Sies and Stahl, 2004; Aust *et al.*, 2005).

(ISSN: 1110-8649)

Polyunsaturated fatty acids (PUFAs), especially the essential omega-6 and omega-3 fatty acids, play key roles in nearly all cellular metabolic process in human body because there is no synthetic mechanism for their production inside the body (Funk, 2001; Sayanova and Napier, 2004). Thus an external source is needed to provide the human body with its need of such essential fatty acids. Most of fatty acids production processes investigated to date have been based on photoautotrophic growth (Sánchez *et al.*, 2002; Molina *et al.*, 2003).

It is well known that algae are at the bottom of the aquatic food chain. The primary determinant in establishing the food quality transferred through successive levels of the food web appears to be the biochemical composition of the algae (fatty acids, amino acids, protein and other pharmaceutical products) (Droop, 1974; Brown and Miller, 1992; Morimoto *et. al.*, 1995; Shibata *et. al.*, 2003; Cardozo *et al*., 2007).

Algae are considered as the main natural source for both carotenoids and essential fatty acids beside other valuable nutritional value. The green alga *Chlorella* has attracted considerable interest for commercial production of functional food such as polyunsaturated fatty acids by *C. sorokiniana* (Chen and John, 1991) and leutin by *C. protothecoides* (Shi *et. al*., 2002). *Chlorella zofingiensis* has been proposed as a promising producer of the high-value carotenoids (Orosa *et. al.,* 2000; Ip and Chen, 2005). The correlations between cultivation conditions and percentage of carotenoids, protein and fatty acids contents were subjects of many studies (Piorreck *et. al.,* 1984; Vladimirova *et. al.*, 2000; Petkov and Garcia, 2007).

Fatty acids yield and productivity in photoautotrophic systems are low, because the insufficiency of light caused by mutual shading of cells (Barclay *et al*., 1994; Chen *et al.*, 1996). For enhancement of fatty acid production by microalgae, the development of a heterotrophic growth is desirable (Wen, 2001). The concept that autotrophic microorganisms can utilize only inorganic carbon dioxide as a carbon source has been modified over years (Shugarman and Appleman, 1966; Bennett and Hobbie, 1972; Cho *et al.*, 1981; Feng *et al.*, 2005).

In this study an attempt was made to evaluate the nutritional value of *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions concerning their content of carotenoids, protein, proline, total free amino acids and fatty acids. It was an attempt too, to answer the question: Does heterotrophy enhance fatty acids production? Also, validity of fatty acids composition as stable taxonomic feature for the genus *Chlorella* was briefly discussed as aside issue.

Materials and Methods

Culture conditions:

The modified basal medium (Chen *et al.,* 1996) (described in Wu and Shi, 2007) containing (mg L^{-1}) KH_2PO_4 1250, $MgSO_4$ 1000, EDTA 500, H_3BO_3 114.2, $CaCl_2$ 111, $FeSO_4$ 49.8, $ZnSO_4$ 88.2, $MnCl_2$ 14.2, MoO_3 7.1, $CuSO_4$ 15.7 and $Co(NO_3)_2$ 4.9 and supplemented with 10 g L^{-1}glucose (as the carbon source) was used for heterotrophic cultivations of *Chlorella vulgaris*. Cultivation of axenic *C. vulgaris* was carried out in 250-ml flasks (each containing 100 ml of medium). All media in the flasks were sterilized in autoclave at 121°C for 20 min. The cultures were incubated at 30°C with orbital shaking at 130 rpm under darkness. Autotrophic cultures were continuously illuminated with 40 W fluorescent lamps (3500 lux). Experiment for each trial was carried out in triplicates. Each culture was tested occasionally for contamination by bacteria or fungi by incubating an aliquot with peptonic agar at 30 ºC for at least 2 days in the dark. Cultures used for chemical analysis were harvested after nine days during the exponential phase.

Estimation of pigments:

Algal cells were homogenized at 1000 rpm for one min, using 100% acetone (50 ml for each g sample). The homogenate was filtered through two layer cheese cloths, and was centrifuged at 2500 rpm for ten min. The supernatant was separated and the absorbance was read at 662, 645 and 470 nm for chl-a, chl-b and carotenoids, according to the method described by Dere *et al.,* (1998). The amount of these pigments was calculated according to the formulas of Lichtenthaler and Wellburn (1985):

Chl-a = 11.75 A662 – 2.350 A645

Chl-b = 18.61 A645 – 3. 960 A662

Carot. = 1000 A470 – 2.270 Chl-a – 81.4 Chl-b / 227

Estimation of total free amino acids:

Total free amino acids were estimated according to the method of Yem and Cocking (1955). One ml aliquot of the acidified extract was mixed with 2 ml sodium acetate buffer (pH 6.5) and 1 ml freshly prepared ninhydrin reagent. The resulting colour was then read at 570 nm with Perkin Elmer spectrophotometer. Leucine was used as a standared.

Estimation of free proline:

Free proline estimation was carried out using the acid ninhydrin method of Bates *et al.,* (1973). The absorbance was read at 520 nm using Perkin Elmer spectrophotometer, and the proline concentration was calculated from a proline standard curve.

Estimation of protein:

The protein was estimated according to Folin-Ciocalteu method described by Hartree (1972), and the resulting color was measured at 650 nm using Perkin Elmer spectrophotometer, related to a standard curve of bovine serum albumin.

Determinations of lipids and fatty acid composition:

Fatty acids were analyzed by Gas Liquied Chromatography (GLC) (Schimadzu GC4-CM). The relative peak areas on the chromatogram were estimated after tracing them on sectional paper and thus the content (%) of different fatty acids was estimated. The conditions for GLC analysis were: Column size:3 mm x 3 mm id; carrier gas: nitrogen; fuel: hydrogen-air mixture; hydrogen flow rate: 1 ml min^{-1}; air flow rate: 0.5 ml min^{-1}; detector: FID; detector temperature: 270°C; column temperature: 180°C. Extraction and methanolysis were performed according to the method described by Chalvardjian (1964) and Moneam and Ghoneim (1986).

Statistical analysis:

Data were analyzed by means of correlation coefficients, using COSTAT 2.0 statistical analysis software.

Results and Discussion

The pigment contents of *Chlorella vulgaris* cells grown under autrotrophic and heterotrophic conditions were shown in Table 1. Chlorophyll "a" content of autotrophic cells was double that estimated in heterotrophic cells (4.481 and 2.672 mg g^{-1}dw, respectively). On contrary, chlorophyll "b" content of autotrophic grown cells was nearly half the value recorded for heterotrophic cells (0.563 and 1.071 mg g^{-1}dw, respectively). The heterotrophic ratio of chl-a to chl-b lost about 68.65% of its value in autotrophic grown cells. Carotenoids content of heterotrophic cultures decreased by 30.82% compared to its value for autotrophic cultures. The results in Table 1(A &B) showed that the significantly enhancement effect of light on chlorophyll "a" and carotenoids synthesis seemed remarkably under photoautotrophic conditions. However, under heterotrophic conditions, chl-b content was significantly increased on the expense of the production of other pigments. These results suggest that when there is an organic carbon source like glucose in the culture medium and in absence of light, *C. vulgaris* favor the formation of chl-b. This agree with the work of Misonou and Pachlavuni (1986), who suggested that under heterotrophic conditions the synthesis pathways of chl. "a" and carotenoids are blocked or at least slow down, while the synthesis pathway of chl. "b" was greatly promoted. Another possible explanation was driven by Vladimirova (1976), who found ultra structural alterations in the photosynthetic apparatus of *Chlorella* sp. grown heterotrophically. It is possible that our result of the variation in pigment content under different trophic conditions, in this study, is related to such suggestion.

Table 1. Contents (mg g^{-1}dw) (A) and Correlation coefficients (B) of chlorophyll – a, chlorophyll -b, and carotenoids of *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions for 9 days .

(A)

Pigment	Autotrophic	Heterotrophic
Chl-a	4.481 ± 0.7	2.672 ± 0.3
Chl-b	0.563 ± 0.2	1.071 ± 0.2
Chl-a / Chl-b ratio	7.959 ± 0.7	2.495 ± 0.5
Carotenoids	6.064 ± 0.8	4.195 ± 0.4

(B)

Variables		Degree of significance
Chl-a	Chl-b	**
	Chl-a / chl-b	***
	Carto.	***
Chl-b	Chl-a / chl-b	**
	Carto.	*
Chl-a / chl-b	Carto.	***

0.05<* means significant at p
0.01<** means significant at p
0.01<*** means highly significant at p

On comparing protein content of *C. vulgaris* cells grown under autotrophic and heterotrophic conditions, there was a slight decrease (6.05%) in the protein content (Table 2 A & B). When the composition of total free amino acids and proline of *Chlorella* grown autotrophically is compared to that grown heterotrophically, it was found that a significant increase in total free amino acids and proline (65.86 and 42.86 %, respectively) (Table 2 A & B). It seemed that heterotrophy negatively affect the protein synthesis, while positively affect and promoted synthesis of total free amino acids and proline. That is may be due to the heterotrophic cells did not need to use the available amino acids to build up protein molecules or due to degradation of protein molecules. At the same time, the increase of amino acids and proline was expected, due its known accumulation behavior in plants subjected to different environmental stresses as light availability (Hashimoto *et. al.*, 1982; Borowitzka, 1988; Sansawa and Endo, 2004).

Table 2. Contents (A) and Correlation coefficients (B) of protein, proline, and total free amino acids (TFAA) of *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions for 9 days.

(A)

Growth condition	Protein (mg g^{-1}dw)	Proline (mg g^{-1}dw)	Total free amino acids (mg g^{-1}dw)
Autotrophic	388.95 ± 21.3	301.97 ± 16.1	6.438 ± 0.9
Heterotrophic	365.4 ± 11.5	704.59 ± 23.4	9.776 ± 2.2

(B)

Variables		Degree of significance
Protein	Proline	***
	TFFA	***
Proline	TFAA	***

*** means highly significant at $p < 0.01$

The composition of fatty acids of *C. vulgaris* is presented in Figures 1& 2. The percentage of most fatty acids of heterotrophy cells was relatively higher than autotrophic cells (Fig. 2). Only, three fatty acids (12:0, 14:0, and 20:0) showed different trend, where they increased under autotrophic conditions rather than heterotrophic conditions. However, except for the fatty acid 16:02 which was absent under autotrophic conditions, there was no qualitative difference between autotrophic and heterotrophic cultures, (Figures 1 & 2). Such quantitative differences could be accepted as temporary physiological response to variation in trophic status (Shinichi *et. al.*, 1983; Zhukova and Aizdaicher, 1995; Rosa *et. al.*, 2005). The biological importance of the utilization mechanism of glucose by *Chlorella* is not completely understood, but whatever, the natural circumstances which make autotrophy grown *Chlorella* switch to heterotrophy, a supply of exogenous carbon source might be provided (White, 1974 Cho *et al.*, 1981; Hashimoto *et. al.*, 1982; IP and Chen, 2005). In the presence of exogenous carbon

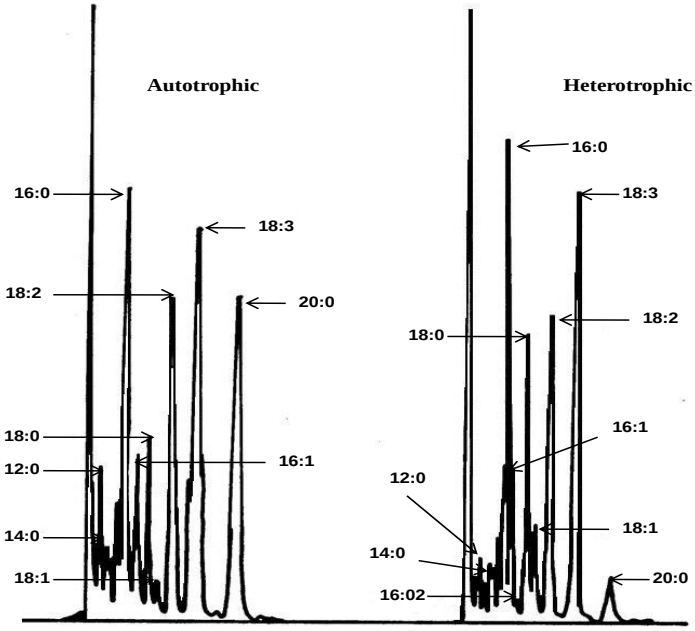

Figure 1. GLC chromatogram of fatty acids extracted from *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions for 9 days.

like glucose, the growth and fatty acids production were enhanced (Cho *et. al*., 1981; Feng *et. al*., 2005).

However, the most important significant question is how one explains the qualitative variations in fatty acids of different species/phenotypes of the genus *Chlorella*? Our results showed that *Chlorella vulgaris* has quite a simple qualitative fatty acids composition compared to other chlorophycean species. The qualitative composition of fatty acids varied not only at species level, but also varied in different phenotypes of the same species of *Chlorella* (Nichols, 1965; De Mort *et. al.*, 1972; Antonyan *et. al.*, 1986; Vladimirova et al., 2000; Petkov and Garcia, 2007). Petkov and Garcia (2007) stated that there is no difference in

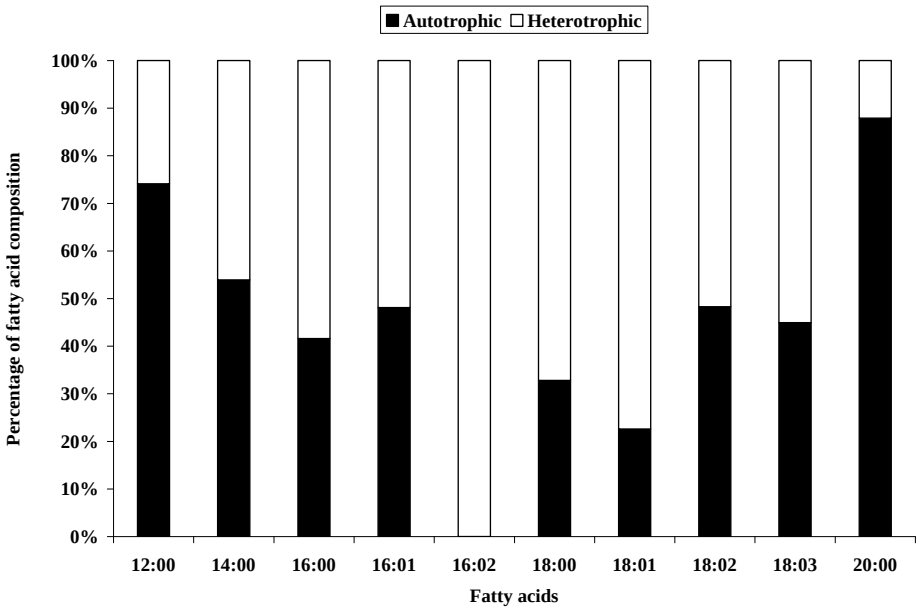

Figure 2. Difference in percentage composition of individual fatty acids extracted from *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions for 9 days.

Table 3. Correlations coefficients for fatty acids extracted from *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions for 9 days.

Fatty acids	Variables								
	20:00	18:03	18:02	18:01	18:00	16:02	16:01	16:00	14:00
12:00	***	***	**NG**	***	***	**	*	***	*
14:00	**	**	**NG**	**	**	**	*	**	
16:00	***	***	**NG**	***	***	***	*		
16:01	*	*	**NG**	*	*	*			
16:02	***	***	**NG**	***	***				
18:00	***	***	**NG**	***					
18:01	***	***	**NG**						
18:02	**NG**	**NG**							
18:03	***								

NG means not significant
0.05<* means significant at p
0.01<** means significant at p
0.01<*** means highly significant at p

qualitative composition of fatty acids of the genus *Chlorella*. Although they believed in the stability of qualitative fatty acids composition as a characteristic feature of the genus *Chlorella*, they drove a lot of evidences that showed a great variability in qualitative fatty acid composition in different species of *Chlorella*. Meanwhile, they accept the idea of using qualitative fatty acids composition as a taxonomic feature of particular species of *Chlorella*, they presented many explanations of the variability in qualitative fatty acids composition. For example, they revealed the presence of the fatty acids 15:0, 17:0 and 17:1 as results of culture contamination with bacteria, in agreement with others (De Mort *et al*., 1972; Wacker *et al.,* 2002). Also, they explained the presence of the fatty acids 20:0, 20:1 and 20:2 on the basis of the presence of impurities with similar retention time in GLC which agreed with the results obtained by Antonyan *et al.,* (1986) and Homova *et al.,* (1986).

Due to the obvious variability in both qualitative and quantitative fatty acid compositions of *Chlorella* in this study compared to other recent reports, one should pay a lot of attention to come into acceptable conclusion for such issue. Thus, it is better to perform much detailed investigations on qualitative fatty acids composition for different *Chlorella* species using more sophisticated and precise techniques to come into acceptable conclusion.

Finally, considering production of natural food supplements or / and natural pharmaceutical products, it's strongly recommended using autotrophic cells of *Chlorella* rather than using those of heterotrophic cells for such purpose. This is because the richness in carotenoids, protein and fatty acids contents of autotrophic *Chlorella*.

Acknowledgement: The authors are much grateful to Dr. Mohamed Shiboob (Faculty of Agriculture, University of Alexandria) for his help in essential oil analysis.

References

Antonyan A. A.; Meleshko G. I.; Pepelyaev Y. V.; Naidina V. P. and Sukhova N. I. (1986). Comparative characterization of fatty acid of lipids from various algae. Prikl. Biokim. Mikrobiol. **22:570-576**.

Astley S. B.; Hughes D. A.; Wright A. J. A.; Elliott R. M. and Southon S. (2004). DNA damage and susceptibility to oxidative damage in lymphocytes: effect of carotenoids *in vitro* and *in vivo*.Br. J. Nutr. **91:53-61**.

Aust O.; Stahl W.; Sies H.;Tronnier H. and Heinrich U. (2005). Supplementation with tomato-based products increases lycopene, phytofkuene, and phytoene levels in human serum and protects against UV-light-induced erythema. *Int. J. Vitam. Nutr. Res*. **75:54-60**.

Barclay W. R.; Meager K. M. and Abril J. R. (1994). Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. *J. Appl. Phycol*. **6:123-129**.

Bates L. S.; Waldes R. P. and Teare I. D. (1973). Rapid determination of free proline for water stress studies. Plant Soil **39:205-207**.

Bennett M. **E. and Hobbie J. E.** (1972). The uptake of glucose by *Chlamydomonas* sp. *J. Phycol*. **8: 389-392**.

Blunt J. W.; Copp B. R.; Munro M. H. G.; Northcote P. T. and Prinsep M. R. (2005). Marine natural products. Nat. Prod. Rep. **22:15-61**.

Borowitzka M. **A.** (1988). Vitamins and fine chemicals from microalgae, p. 153-196. In Borowitzka M. A. and Borowitzka L. J. (ed.), Micro-algal biotechnology, vol. 7. Cambridge Univ. Press, Cambridge.

Brown M. R. and Miller K. A. (1992).The ascorbic acid content of eleven species of microalgae used in marine culture. *J. Appl. Phycol*.**4:205-215**.

Cantrell A.; McGarvey D. J.; Truscott T. G.; Rancan F. and Böhm F. (2003). Singlet oxygen quenching by dietary carotenoids in a model membrane environment. Arch. Biochem. Biophys.**412:47-54**.

Cardozo K. H. M.; Guaratini T.; Barros M. P.; Falcão V. R.; Tonon A. P.; Lopes N. P.; Campos S.; Torres M. A.; Souza A. O.; Colepicolo P. and Pinto E. (2007). Metabolites from algae with economical impact. Comp. Biochem. Physiol. **146:60-78**.

Chalvardjian A. M. (1964). Fatty acids of brown and yellow fat in rats. Biochem. J. **90:518-521**.

Chen F. and John M. R. (1991)**.** Effect of C/N ratio and aeration on the fatty acid composition of heterotrophic *Chlorella sorokiniana*. *J. Appl. Phycol*. **3:203-209**.

Chen F.; Zhang Y. and Guo S. Y. (1996). Growth and phycocyanin formation of *Spirulina plantensis* in photoheterotrophic cultures. Biotechnol. Lett. **18:603-608**.

Cho B.; Sauer N.; Komor E. and Tanner W. (1981). Glucose induces two amino acid transport systems in *Chlorella*. Proc. Natl. Acad. Sci. **78(6):3591-3594**.

Cragg G. M.; Newman D. J. and Snader K. M. (1997). Natural products in drug discovery and development. *J. Nat. Prod*. **60:52-60**.

De Mort C. L.; Lowry R.; Tinsley I. and Phinney H. K. (1972). The biochemical analysis of some estuarine phytoplankton species. I. Fatty acid composition. *J. Phycol*. **8:211-216**.

Dere A.; Güneş T. and Sivaci R. (1998). Spectrophotometric determination of Chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Tr. J. Bot*. **22:13-17**.

Droop M. R. (1974). Heterotrophy of carbon, p. 530-559. In Stewart, W. D. P. (ed.), Algal physiology and biochemistry. Blackwell Scientific Press, Oxford.

Feng F.; Yang W.; Jiang G.; Xu T. and Kuang T. (2005). Enhancement of fatty acid production of *Chlorella* sp. (Chlorophyceae) by addition of glucose and sodium thiosulphate to culture medium. **40:1315-1318**.

Funk C. D. (2001). Prostaglandins and leukotrienes: advances in eicosanoids biology.Sci. **294:1871-1875**.

Hartree E. F. (1972). A modification of the lowery method that gives a linear photometric response. Analy. Biochem. **48:422-426**.

Hashimoto S.; Setoyama Y.; Yokokura T. and Mutai M. (1982). Effects of *Chlorella* phospholipid on the aortic collagen and elastin metabolism and the serum lipid content in rats with experimental atheriosclerosis. Exp. Mol. Pathol. **37:150-155**.

Homova T.; Gussakova S.; Glushenkova A. and Travkina I. (1986). Lipidis of *Chlorella vulgaris* extracts. Khim. Prir. Soedin. **3:284-288**.

Ip P. F. and Chen F. (2005). Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. Process Biochem. **40:733-738**.

Lichtenthaler H. K. and Wellburn A. R. (1985). Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biol. Soc. Trans. **11:591-592**.

Mayne S. T. (1996). β-Carotenoid, carotenoids and disease prevention in humans. FASEB J. **10:690-701**.

Misonou T. and Pachlavuni I. K. (1986). Photosynthetic pigments of *Chlorella* sp. K cultured under photoauto-, mixo- and chemohetero-trophic growth conditions. Jap. *J. Phycol*. **34:163-170**.

Molina G. E.; Belarbi E. H.; Acién F. F.G.; Robles M. A. and Chisti Y. (2003). Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. **20:491-515**.

Moneam N. M. A. and Ghoneim T. (1986). Gas chromatographic analysis of total fatty acids extracted from *Schinus terebenthifolius* berries. J. Chromatograpgy **361:391-395**.

Morimoto T.; Nagatsu A.; Murakami N.; Sakaibara J.; Tokuda H.; Nishino H. and Iwashima A. (1995). Anti-tumor promoting glyceroglycolipids from green alga, *Chlorella vulgaris*. Phytochem. **40:1433-1437**.

Nichols B. (1965). Light-induced changes in the lipids of *Chlorella vulgaris*. Biochem. Biophys. Acta. **106:274-279**.

Orosa M.; Torres E.; Fidalgo P. and Abalde J. (2000). Production and analysis of secondary carotenoids in green algae. *J. Appl. Phycol*. **12:553-556.**

Petkov G. and Garcia G. (2007). Which are fatty acids of the green alga *Chlorella*? Biochem. Syst. Ecol. **35:281-285**.

Piorreck M.; Baasch K. H. and Pohl P. (1984). Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochem. **23:207-216**.

Polivka T. and Sundström V. (2004). Ultrafast dynamics of carotenoid excited states-from solution to natural and artificial systems. Chem. Rev. **104:2021-2071.**

Rosa A.; Deidda D.; Serra A.; Dessi M. A. and Pompei R. (2005). Omega-3 fatty acid composition and biological activity of three microalgae species. *J. Food Agric. Environ*. **3:381-389**.

Sánchez M. A.; Cerón G. M.C.; García C. F.; Molina G. E. and Chisti Y. (2002). Growth and biochemical characterization of micoalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture. Enzy. Microb Technol.**31:1015-1023**.

Sansawa H. and Endo H. (2004). Production of intercellular phytochemicals in *Chlorella* under heterotrophic conditions. *J. Biosci. Bioeng*. **98(6):437-444**.

Sayanova O. V. and Napier J. A. (2004). Eicosapentaenoic acid: biosynthesis routs and the potential for synthesis in transgenic plants. *Phytochem*. **65:147-158**.

Shi X. M.; Jiang Y. and Chen F. (2002). High-yield production of leutin by the green microalga *Chlorella protothecoides* in heterotrophic fed-batch culture, Biochem. Prog. **18:723-727**.

Shibata S.; Oda K.; Onodera-Masuoka N.; Matsubara S.; Kikuchi-Hayakawa H.; Ishikawa F.; Iwabuchi A. and Sansawa H. (2003). Hypocholestrolemic effect of indigestible fraction of *Chlorella vulgaris* in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol*. **47:373-377**.

Shinichi T.; Shigehisa Y.; Kanazawa A. and Hachiro H. (1983). Effects of water temperature and salinity on eicosapentaenoic acid level of marine *Chlorella*. Bull. Jap. Soc. Sci. Fish. **49:805-809**.

Shugarman P. M. and Appleman D. (1966). Chlorophyll synthesis in *Chlorella*. II. Effect of glucose and light intensity on the lag phase. *Plant Physiol*. **41:1701-1708**.

Sies H. and Stahl W. (2004). Nutritional protection against skin damage from sunlight. Annu. Rev. Nutr. **24:173-200**.

Singh S.; Kate B. N. and Banerjee U. C. (2005). Bioactive compounds from cyanobacteria and microalgae: an overview. Crit. Rev. *Biotechnol*. **25:73-95.**

Vladimirova M. G. (1976). Changes in ultrastructure of the cell of *Chlorella* sp. K. during its functional reorientations. Fiziologia Rastenii **23:1180-1187**.

Vladimirova M. G.; Klyachko-Gurvich G. L.; Maslova I. P.; Zholdakov I. A and Bartsevich E. D. (2000). A comprehensive study of *Chlorella* sp. IP-PAS C-48 and revision of its taxonomic position. Russ. *J. Plant Physiol.* **47:644-654**.

Wacker A.; Becker P. and Elert E. V. (2002). Food quality effects of unsaturated fatty acids on larvae of the Zebra mussel *Dreissena polymorpha*. *Limnol. Oceanogr*. **47:1242-1248**.

Wen Z. Y. (2001). A high yield and productivity strategy for eicosapentaenoic acid production by the diatom *Nitzschia laevis* in heterotrophic culture. Ph. D. Dissertation. Hong Kong: The University of Hong Kong. In: Wen Z. Y.; Chen F. 2003. Heterotrophic production of eicosapentaenoic acid by microalgae. Biotechnol. Adv. **21: 273-294**.

White A. W. (1974). Growth of two facultative heterotrophic marine centric diatoms. *J. Phycol*. **10:292-300**.

Wu Z. and Shi X. (2007). Optimization for high-density cultivation of heterotrophic *Chlorella* based on a hybrid neural network model. Lett. Appl. Microbiol. **44:13-18**.

Yem E. W. and Cocking E. C. (1955). The determination of amino-acids with ninhydrin. Analy. **80:209-213**.

Zhukova N. and Aizdaicher N. (1995). Fatty acid composition of 15 species of marine microalgae. *Phytochem*. **39:351-356**.

القيمة الغذائية و تركيب الأحماض الدهنية لطحلب كلوريللا فولجاريس النامي في ظروف ذاتية و غير ذاتية التغذية

مصطفى الشيخ[1] و علاء فتحي[2]

قسم النبات – كلية العلوم – [1] جامعة طنطا و [2]جامعة الإسكندرية

كان محتوى الكلوروفيل "أ" في الخلايا ذاتية التغذية ضعف ما هو موجود في الخلايا غير ذاتية التغذية، في حين كانت قيمة الكلوروفيل "ب" في الخلايا ذاتية التغذية تقرب من نصف القيمة المسجلة للخلايا غير ذاتية التغذية. وقد نقص محتوى الكاروتينات في المزارع غير ذاتية التغذية بنسبة 30،82 ٪ بالمقارنة مع قيمتها في المزارع ذاتية التغذية. وكان هناك انخفاض طفيف في محتوى البروتين في طحلب **الكلوريللا** غير ذاتي التغذية. كما كانت هنالك زيادة كبيرة في محتويات إجمالي الأحماض الأمينية الحرة و البرولين في طحلب **الكلوريللا** الذاتي التغذية، عندما قورنت مع مثيلاتها في الطحلب الذي نمى بطريقة غير ذاتية التغذية. كما كانت نسبة معظم الأحماض الدهنية في الخلايا غير ذاتية التغذية اعلي نسبيا من مثيلاتها في الخلايا ذاتية التغذية. و لم يكن هناك فارق نوعي بين المزارع الذاتية و الغير ذاتية التغذية، فيما عدا الحمض الدهني 16:02 و الذي كان غائبا في ظل ظروف التغذية الذاتية.