Research Article

Potential Production of Omega Fatty Acids from Microalgae

Sayeda, M. Abdo[1], Gamila H. Ali[1], Farouk K. El-Baz[2]*
[1]Water pollution Research Department, National Research Centre, 33 El Bohouth st. (former El Tahrirst.)-Dokki, Giza, Egypt.
[2]Plant Biochemistry Department, National Research Centre, 33 El Bohouth st. (former El Tahrirst.)-Dokki, Giza, Egypt.
***Corresponding author's E-mail:** fa_elbaz@hotmail.com

Accepted on: 31-08-2015; **Finalized on:** 30-09-2015.

ABSTRACT

The aim of the current study is to investigate the production of essential omega fatty acids 3, 6 and 9 from four species of microalgae which were *Chlamydomonas variabills*, *Chlorella vulgaris*, *Haematococcus pluvialis* and *Spirulina platensis*. The results showed that *Chlamydomonas variabills* has the highest lipid content (21%) with high concentration of omega 6 (29.24%). *Chlorella vulgaris* showed 12% total lipid with high concentration of omega3 (21.17%). *Spirulina platensis* is a highly important cyanobacteria species, it contains 15.8% lipid with 4.9% omega 3 fatty acid. In addition, omega 9 was detected only in *Spirulina platensis* with percentage 3.22%. Although, *Haematococcus pluvialis* has the lowest total lipid percentage (10%), it revealed a detectable amount of omega 6 fatty acids (14.83%). The ratio of omega 3 to omega 6 depends on the algal species, in *Chlorella vulgaris* and *Chlamydomonas variabills*, it is in the recommended healthy range. The most common fatty acids methyl ester of the algal species were also identified.

Keywords: Omega fatty acids, polyunsaturated fatty acids, *Chlamydomonas variabills, Chlorella vulgaris*, *Spirulina platensis*, *Haematococcus pluvialis*.

INTRODUCTION

Fatty acids are organic compounds formed by long chain hydrocarbon and carboxylic group.[1] Based on their nature hydrocarbon chain, fatty acids can be classified into saturated and unsaturated. Omega-3 fatty acids (PUFA n-3) are unsaturated fatty acids and contained in food as α-linolenic acid (ALA, C18:3, n-3).[2,3] ALA is the shortest chain of n-3 and mainly found in vegetables oil and nuts.[2,3] Eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) are derivatives product from n-3 found in fish and many other microorganisms, such as microalgae and bacteria.[1,3,4] Omega-6 fatty acid (PUFA n-6) are polyunsaturated fatty acid contained in food as Linoleic Acid (LA, C18:2, n-6).[3,5] Linoleic acid (LA) is the shortest chain from n-6 and arachidonic acid (AA, C20:4, n-6) is one of derivatives product from n-6.[6]

Furthermore, polyunsaturated fatty acids (PUFA) are considered as essential fatty acids and it is important to human health and must be consumed regularly, since they cannot be synthesized by humans.[7-10] Since PUFA (EPA and DHA)are considered essential for early infant development and important for the prevention of chronic disease and can markedly reduce the risk of heart disease.[11,12]

Many national and international health organizations recommend daily intake for PUFA in parallel with daily intakes for essential nutrients. The World Health Organization (WHO) recommends the intake of 2 portions of oily fish per week in order to obtain sufficient dietary EPA and DHA (200-500 mg).[13] PUFAs can be obtained from fish or extracted from fish oil and other marine organisms such as algae. Although, there is a possibility of accumulated toxin in the PUFAs derived from fish sources, unpleasant smell, taste and poor oxidative stability has limits the application of fish oil as food additive.[7,14]

Thus there is a need for alternative sources of long chain PUFA in order to meet commercial demands and health-related requirements and hence prevent the onset of chronic disease. Macroalgae, microalgae and microbes present a spacious and relatively unexploited resource of fatty acids, thus providing an alternative source to declining fish stocks and a solution to increasing PUFA demand.[15] It is believed that the PUFA yields from microorganism are in the order microalgae > fungi > bacteria.[16] The average composition of proteins, fats, and carbohydrates in microalgae are 12-35%; 7,2-23%; 4,6-23% (%dry weight) respectively.[17,18]

Different nutrition, environment, and growth phases can influence the nutritional composition in microalgae, including fatty acid composition.[17,19]

Microalgae are widely used to meet food needs and public's health. Nutritional supplement products from microalgae can be found in form of powders, tablets, capsules, and concentrates. Algal PUFA production is more economical than biofuel production and hence a number of large-scale producers are now focusing on nutritional PUFA production as opposed to biofuels.[20,21]

The overall aim of this point of research was to select and compare algal species containing significant quantities of PUFAs that might be useful for further commercial exploitation.

MATERIALS AND METHODS

The organisms and culture medium

The algae species (isolated from the Egyptian ecosystem) are *Chlamydomonas variabills*, *Chlorella vulgaris* and *Heamtococcus pluvialis* which are unicellular green micro algae belonging to Division Chlorophyta. In addition, *Spirulina platensis* a blue green alga belonging to Cyanophyta (Figure 1) was also used. It was selected due to its high growth potentially. The algae were grown on BG11 media in a small open pond with capacity of 25L (Figure 2). It consists of a mixing chamber 50cm length and 15cm depth coupled with a sedimentation part 15cm length and 20cm depth. The system is equipped with diffusion aeration system which provides the necessary oxygen and mixing required. The BG11 media content are**:** 1.5g/L $NaNO_3$, 40mg/L K_2HPO_4, 75mg/L $MgSO_4.7H_2O$, 36mg/L $CaCl_2.2H_2O$, 6mg/L Citric acid, 20mg/L Na_2CO_3, 1mg/L Na_2EDTA, 6mg/L Ferric ammonium citrate, 2.86mg/L H_3BO_3, 1.81mg/L $MnCl_2.4H_2O$, 0.222mg/L $ZnSO_4.7H_2O$, 0.39mg/L $Na_2MoO_4.2H_2O$, 0.079mg/L $CuSO_4.5H_2O$ and 0.0494mg/L $Co(NO_3)_2.6H_2O$.[22] Algae were harvested at stationary phase, dried at 40 C^o and subjected to extraction.

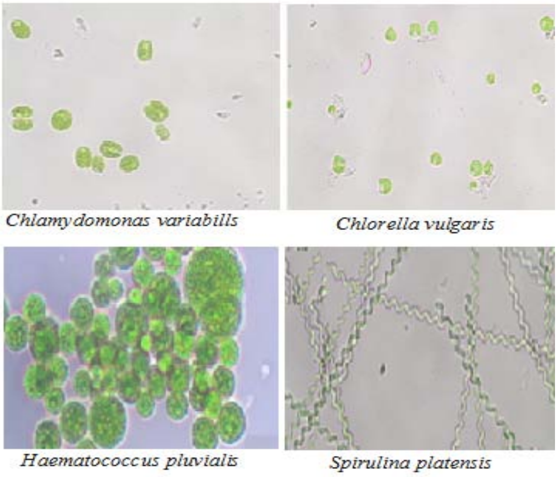

Figure 1: Candidate algal species

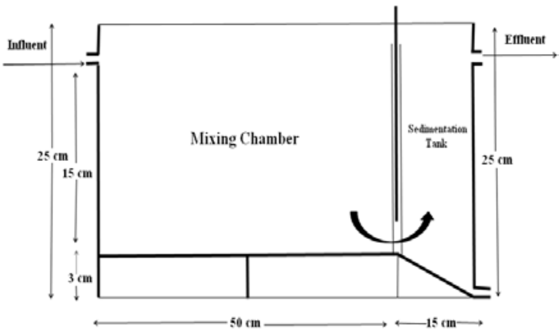

Figure 2: Schematic diagram of the continuous flow tank (side view)

Algal Biomass Harvesting

A suitable harvesting method was applied according to the algal species. Filter press operating under pressure was used with *Chlamydomonas variabills* and *Chlorella vulgaris* through membrane filter 0.8µm. *Haematococcus pluvialis* was harvested through settling then centrifugation. *Spirulina platensis* was harvested using simple method using a handmade thieve consists of a phytoplankton net with a pore size of 30µm mesh size.

Lipid Extraction

Hexane-Isopropanol Extraction Method

The method of Bligh and dyer[23] was used. The dried biomass of microalgae was ground into homogenous fine powder. The dry cells were mixed with hexane-isopropanol (3:2, v/v) as a co-solvent using homogenizer for minutes at 800 rpm in a proportion of 1 gm in 75 ml of solvent mixture. The homogenate mixture was subjected to a magnetic stirrer at 30°C for 2hr. Cell residue was removed by filtering. The filtrate was transferred into a separating funnel and sufficient water was added to induce biphasic layering. After settling the solvent mixture was partitioned into two distinct phases, top dark green hexane layer containing most of the extracted lipids and bottom light green layer containing most of the co-extracted non-lipids. The hexane layer was collected in a pre-weighted flask and evaporated using a rotary evaporator.

Total lipid content (%) = oil weight/dry cell weightx100

Preparation of fatty acids

The lipid samples were methylated by heating under reflux using methanolic HCL (5%) at 60 °C. The fatty acids were extracted with petroleum ether 40-60 °C. The ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered off.

Gas chromatography/mass spectrometry (GC/MS)

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 150°C (hold 4 min) to 280°C as a final temperature at an increasing rate of 5°C/min (hold 4min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

RESULTS AND DISCUSSION

Four microalgae species were investigated to study their lipids and essential omega fatty acids (3, 6 and 9) production capacity. Figure (1) showed the morphological characteristics of the selected algal species. Three of them are belonging to green microalgae (*Chlamydomonas variabills, Chlorella vulgaris* and *Haematococcus pluvialis*) and one belongs to blue-green microalga (*Spirulina platensis*).

Total lipid content of isolated species

The results in table (1) showed that the total lipid content of the isolated microalgae species varied from 10-21 %. *Chlamydomonas variabills* showed the highest oil content (21%). These results are similar to those recorded by Feinberg,[24] who found that *Chlamydomonas* species demonstrated 23% total lipid content. Blue-green algae *Spirulina platensis* contained 15.8 % lipid. These results were confirmed by Mata[19], as they stated that *Spirulina platensis* lipid content varied from 4-16.6%. In this concern Damiani[25] recorded that the total lipid of *Haematococcus pluvialis* grown under nitrogen starvation conditions was 34.85%, as compared to 15.61%, in the same strain grown under normal conditions. They also declared that, the fatty acids profile was similar under all conditions and plamitic, stearic, oleic and linoleic acids were the main components. Moreover, *Chlorella vulgaris* exhibited12% total lipid content. Gouveia and Oliveira[26] reported that *Chlorella vulgaris* has total lipid content varied from 14% to 56% depending on nutritional and growth conditions. Table (1) also showed that the lowest lipid content in the algal species under investigation was detected in *Haematococcus pluvialis* (10%).

Table 1: Total Lipid Percentage of Microalgae Isolates

Algal Species	Total lipids (%)
Chlamydomonas variabills	21± 0.1
Chlorella vulgaris	12±0.2
Haematococcus pluvialis	10±0.3
Spirulina platensis	15.8±0.3

Polyunsatyrated omega fatty acids composition

Polyunsaturated Fatty Acids (PUFAs) include docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA), γ-linolenic acid (GLA), alpha linolenic acid (ALA) and have been widely recognized as beneficial towards human health.[27] The results presented in Table (2) showed that *Chlamydomonas variabills* has a considerable concentration of omega 6-GLA (29.24%) and omega 3 - EPA (5.84%). In addition, *Chlorella vulgaris* demonstrated a low and moderate concentrations of omega 6 (1.2 and 6.37%, respectively), as well as a high concentration of omega 3 (21.17%), while omega 9, was not detected. As for *Haematococcus pluvialis* it contains a high concentration of omega 6(14.83%) and a detectable concentration of (8,11-Eicosadienoic acid) omega 6 (1.54%). On the other hand in *Spirulina platensis,* omega 3 and omega 9 were detected (4.9% and 3.22%, respectively), while omega 6 was not existed. Tsurkan[28] found that sum of total PUFAs in green microalgae varied from 55.9 to 64.5%.

Hence, our results confirmed the production of omega fatty acids especially omega 3 and 6 from microalgae. Taken together, microalgae are considered also the principal organisms in that they produce a distinct range of chemical and biological compounds, principally vitamins, pigments, proteins, minerals, lipids and polysaccharides. In comparison to other living sources, algae are very rich in some kinds of fatty acids such as polyunsaturated fatty acids (PUFA), δ-linoleic acid (GLA).[29,30]

From the present results it could be concluded that, the highest percentage of omega 3 was detected in *Chlorella vulgaris* (21.17). *Chlorella* has a fast growth rate and high PUFA content[31] and could be another important source of a PUFA-rich nutraceuticals supplements especially arachidonic (omega 6) and eicosapentaenoic acids EPA (omega 3). Omega-3 long chain fatty acids are of significant commercial interest due its role in preventing arteriosclerosis and coronary heart disease, beside alleviating inflammatory conditions such as arthritis.[12] Several countries, including the Scandinavian countries and Canada, have now established daily recommended dietary intakes values for omega-3 fatty acids.[32] The British Nutrition Foundation Nicol,[33] has also published recommendations for dietary intake of these unsaturated fatty acids. In addition, DHA Docosahexaen acid 22:6 (n-3) has been recognized recently as an important component of human breast milk that contributes to normal development of infant's brains and nervous systems.[34]

In the present study the highest percentage of omega 6 (29.24%) was found in *Chlamydomonas variabills* followed by *Haematococcus pluvialis* (14.83%). γ-Linolenic acid (GLA), (omega 6) has attracted attention worldwide because of its medicinal value with regard to cardio-vascular diseases, hyper-cholesterolaemia, Horrobin menstrual disorders, skin diseases (atopic eczema) and other disorders.[35-38]

The balance of omega fatty acids is important to consider. The so-called omega-3:omega-6 ratio has become a model for gauging the proper balance of these fats in oils and the diet.[39] Diets with greater than a 1:10 ratio of omega-3 to omega-6 are not recommended, whereas a 1:1 ratio is considered perfect. Very unhealthy ratios of 1:25 and 1:50 are common, especially with regular consumption of 'fast-food', high amounts of fried food, and low intake of fresh whole foods. Thus, 'Eating to live and not living to eat' becomes an important consideration with increases in modern, convenient, non-functional food choices. In the present study the ratio of omega-3 to omega-6 depends on the algal strains. In *Chlamydomonas variabills* it showed 1:5, however in *Chlorella vulgaris* this ratio is within the recommended range (1: 0.05).

As indicated in Table (2) omega 9 was only detected in *Spirulina platensis* with moderate percentage (3.22%). *Spirulina platensis* is a highly important cyanobacteria species in that it contains high levels of gamma-linoleic acid (omega 6).[40] Therefore, it is widely cultivated to be used commonly as a human, and in cosmetics, pharmaceuticals, and other various industrial fields.[41-43]

Microalgae have multiple potential to produce high-valued products and there is an urgent need of awareness makes these biomolecules popular in the world to meet the increasing demands with respect to population. Most of these biomolecules are not produced in the animal/human body but termed as essential; therefore, it is highly recommended to make these biomolecules available for food and feed purposes.[14] Table (3) showed the most common fatty acids methyl ester identified and detected in the isolated species of algae (*Chlamydomonas variabills, Chlorella vulgaris, Haematococcus pluvialis* and *Spirulina platensis*).

Table 2: GC/MS chemical profile of alge species omega fatty acids methyl ester

Isolates	RT[a]	% area[b]	Compound name	Molecular formula	M.W
Chlamydomonas variabills	14.99	5.84	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) omega 3	$C_{21}H_{32}O_2$	316
	19.91	29.24	6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6	$C_{19}H_{32}O_2$	292
Chlorella vulgaris	19.58	1.2	6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6	$C_{19}H_{32}O_2$	292
	19.76	6.37	9.12-Octadecadienoic acid methyl ester, omega 6	$C_{19}H_{32}O_2$	292
	19.91	21.17	6,9,12,15-Docosatetraenoic acid omega 3	$C_{23}H_{38}O_2$	346
Haematococcus pluvialis	13.83	1.54	8,11-Eicosadienoic acid, methyl ester, omega 6	$C_{21}H_{38}O_2$	322
	19.93	14.83	6,9,12-Octadecatrienoic acid, methyl ester, omega 6	$C_{19}H_{32}O_2$	292
Spirulina platensis	14.5	4.9	9,12,15-Octadecatrienoic acid (ALA) omega 3	$C_{28}H_{40}O_4$	440
	15.46	3.22	9-Octadecenoic acid - Oleic acid omega 9	$C_{34}H_{64}O_2$	504

a: Retention time (as minutes); b: The percentage composition was computed from the GC/MS peak area.

Table 3: The most common fatty acids methyl ester and derivatives extracted from the isolated alge species

RT[a]	% area[b]	Compound name	Molecular formula	MW
Chlamydomonas variabills				
13.59	0.15	n-Butylricinoleate	$C_{22}H_{42}O_3$	88
15.88	24.84	Pentadecanoic acid - 13 methyl - methyl ester (CAS)	$C_{17}H_{34}O_2$	270
21.65	2.07	Hexadecanoicacid,2(octadecyloxy) ethylester- palmitic acid derivative	$C_{36}H_{72}O_3$	552
28.08	1.12	Octadecane, 3ethyl5(2ethylbutyl)	$C_{26}H_{54}$	366
Chlorella vulgaris				
15.87	7.85	Pentadecanoic acid - 14 methyl - methyl ester (CAS)	$C_{17}H_{34}O_2$	270
20.47	1.95	Octadecanoic acid methyl ester (CAS) - stearic acid Octadecanoic derivative	$C_{19}H_{38}O_2$	298
Haematococcus pluvialis				
9.45	1.48	Benzenedodecanoic acid, 3methoxy 2 (methoxy carbonyl), methyl ester (CAS)	$C_{22}H_{34}O_5$	378
15.90	14.25	Pentadecanoic acid, 14methyl, methyl ester (CAS)	$C_{17}H_{34}O_2$	270
16.46	4.27	Octadecanoic acid, 2hydroxyethylester (CAS)	$C_{20}H_{40}O_3$	328
16.99	3.9	Hexadecanoic acid, 2 (octadecyloxy) ethyl ester (CAS) - palmitic acid derivative	$C_{36}H_{72}O_3$	552
18.48	7.77	9Hexadecenoic acid, 9octadecenyl ester, (Z,Z)(CAS) - palmitoleic acid derivative	$C_{34}H_{64}O_2$	504
19.13	12.84	2H-Pyran, tetrahydro2 (12pentadecynyloxy)	$C_{20}H_{36}O_2$	308
19.79	4.4	9,12Octadecadienoic acid (Z,Z), 2,3 dihydroxypropylester – linoleic derivative	$C_{21}H_{38}O_4$	354
19.93	14.83	6,9,12Octadecatrienoic acid, methyl ester (CAS) linolenic acid (GLA) derivative	$C_{19}H_{32}O_2$	292
20.49	3.89	Octadecanoic acid, methyl ester (CAS)- stearic acid derivative	$C_{19}H_{38}O_2$	298
35.38	0.69	5,6,8,13Tetrahydro2,3,9,10tetramethoxy8oxo13aHdibenzo[a,g]quinolizine13acarboxylic acid	$C_{22}H_{23}N_7$	413
Spirulina platensis				
14.86	5.43	9 hexadeceneic acid, 9 octadecenyl ester, palmitoleic acid derivative	$C_{34}H_{64}O_2$	504
15.93	4.45	Octadecanoic acid, 3 hydroxy, 2 tetradecyl methyl ester, stearic acid derivative	$C_{33}H_{66}O_3$	510
16.47	7.43	Hexadecanoic- palmitic acid derivative	$C_{36}H_{56}O_6$	584

a: Retention time (as minutes); b: The percentage composition was computed from the GC/MS peak area

As shown in table (3) the most common fatty acids and their derivatives detected in *Haematococcus pluvialls* were linolenic acid (14.83) followed by pentadecanoic acid (14.25%), plamitoleic (7.77%), linoleic acid (4.4%), stearic acid (3.89%) and plamitic acid (3.90%). These results are agreed with those obtained by Gacheva.[44]

However, in *Chlamydomonas variabills*, pentadecanoic acid and plamitic acid (hexadecanoic acid) showed the highest content (24.84 and 2.07%, respectively), In *Chlorella vulgaris* two common fatty acids were detected, the pentadecanoic acid - 14 methyl ester (7.85%) and octadecanoic acid methyl ester (1.95%).

Three common fatty acids derivatives were identified in *Spirulina platensis* as indicated in Table (3), palmitoleic (5.43%), stearic (4.45%) and plamitic acid (7.43%).

In this concern, Petkov and Garcia[40] confirmed the composition of 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3 for *Chlorella vulgaris*, the fatty acid with 20 carbon atoms and four or five double bonds are considered not originating from *Chlorella* and this confirm and agree with the results of the present study in which two acids with C_{17} and C_{19} were detected.

CONCLUSION

The use of microalgae for large scale production of omega-3, 6 and 9 fatty acids and biomolecules has recently attracted a lot of interest. There is confidence among companies producing microalgae that the production of a high value product, such as omega-3 from microalgae, will further assist in the establishment of the microalgae industry.

Acknowledgement: This work was supported and funded by the project entitled "Biodiesel production from algae as a renewable energy source". Funding organization: Research Development and Innovation (RDI), Funding Program: EU-Egypt Innovation Fund, 2014-2016.

REFERENCES

1. Ward OP, and Singh A, Omega-3/6 fatty acids: Alternative source of production. Process Biochemistry, 40, 2005, 3627-3652.
2. Chew YL, Lim YY, Omar M, Khoo KS, Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT, 41, 2008, 1067-1072.
3. Kalogeropoulos N, Chiou A, Gavala E, Christea M, Andrilkopoulos NK, Nutritional evaluation and bioactive micro constituents (carotenoids, tocopherols, sterols and squalene) of raw and roasted chicken fed on DHA-rich microalgae. Food Research International, 43, 2010, 2006-2013.
4. Guedes AC, Amaro HM, Barbosa CR, Pereira RD, Malcata FX, Fatty acid composition of several wild microalgae and cyanobacteria, with a focus on eicosapentaenoic, docosahexaenoic and linolenic acids for eventual dietary uses. Food Research International, 44, 2011, 2721-2729.
5. McCusker MM, Healing fats of the skin: the structural and immunologic roles of the ω-6 and ω-3 fatty acids. Clinics in Dermatology, 28, 2010, 440-451.
6. Handayania NA, Ariyantib D, Hadiyanto, Potential production of polyunsaturated fatty acids from microalgae. Open Access Scientific Reports, 1(2), 2012.
7. Spolaore P, Joannis-Cassan C, Duran E, Isambert A, Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 101, 2006, 87-96.
8. Ruiz-Rodriguez A, Reglero G, Ibanez E, Recent trends in the advanced analysis of bioactive fatty acids. Journal of Pharmaceutical and Biomedical Analysis, 51, 2010, 305-326.
9. Liou YA, King DJ, Zibrik D, Innis SM, Decreasing linoleic acid with constant α-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. Journal of Nutrition, 137, 2007, 945-952.
10. Parsaeimehr A, Sun Z, Dou X, Chen Yi-F, Simultaneous improvement in production of microalgal biodiesel and high-value alpha-linolenic acid by a single regulator acetylcholine Biotechnology for Biofuels, 8, 2015, 1-10.
11. Fleith M, Clandinin MT, Dietary PUFA for preterm and term infants: review of clinical studies. Critical Reviews in Food Science and Nutrition, 45(3), 2005, 205-29.
12. Barclay WR, Meager KM, Abril JR, Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. Journal of Applied Phycology, 6, 1994, 123-129.
13. FAO/WHO. Diet, Nutrition and the prevention of chronic diseases. Geneva, Switzerland, 2003.
14. Yaakob Z, Ali E, Zainal A, Mohamad M, Takriff MS, 1 An overview: biomolecules from microalgae for animal feed and aquaculture. Journal of Biological Research, 21, 6, 2014, 1-10.
15. Ratledge C, Fatty acid biosynthesis in microorganisms being used for Single Cell Oil production. Biochimie, 86(11), 2004, 807-815.
16. Dominguez LA, Polyunsaturated fatty acids in bacteria, algae and fungi-a review. Environmental Engineering and Management Journal, 3, 2012, 160.
17. Anonymous, Nutritional value of micro-algae. www.fao.org, Akses: 24 Juni 2011, Pukul 10.00 WIB, 2011.
18. Farina G, Cattaneo D, Lecchi C, Invernizzi G, Savoini G, Agazzi A, A Review on the role of EPA and DHA through goat nutrition to human health: Could they be effective both to animals and humans? Journal of Dairy, Veterinary & Animal Research, 2(2), 2015.
19. Mata TM, Martins AA, Caetano NS, Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews, 14, 2010, 217-232.
20. Ratledge C, Cohen Z, Microbial and algal oils: do they have a future for biodiesel or as commodity oils? Lipid Technology, 20, 2008, 155-160.
21. Robertson R, Guihéneuf F, Schmid M, Stengel DB, Fitzgerald G, Ross P, Stanton C, Algae-derived polyunsaturated fatty acids: implications for human health.

Publisher: Nova Sciences Publishers, Inc., Hauppauge, NY 11788 USA., Editors: Angel Catalá, 2013, 1-54.

22. Stanier RY, Kunisawa MM, Cohen-Bazir G, Purification and properties of unicellular blue-green algae (order Chlorococcales). Bacteriology Reviews, 35, 1971, 171-201.

23. Bligh EG, Dyer WJA, Rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 1959, 911-917.

24. Feinberg DA, Fuel options from microalgae with representative chemical compositions. National Technical Information Service, SERI, 1984, 1-23.

25. Damiani MC, Popovich CA, Constenta D, Leonardi, Lipid analysis in *Hematococcus pluvialis* to assess its potential use as a biodiesel feedstock, Bioresource Technology, 101, 2010, 3801-3807.

26. Gouveia L, Oliveira AC, Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology and Biotechnology, 36, 2009, 269-274.

27. Givens DI, Gibbs RA, Very long chain n-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal-derived foods to increase intake. Nutrition Bulletin, 31, 2006, 104-110.

28. Tsurkan Y, Karpenyuk T, Guschina I, Orazova S, Goncharova A, Beisembaeva R, Identification of newly-isolated microorganisms containing valuable polyunsaturated fatty acids. Journal of Biotech Research, 6, 2015, 14-20.

29. Borowizka MA, Algal biotechnology products and processes: matching science and economics. Journal of Applied Phycology, 4(3), 1992, 267-279.

30. Cohen Z, The Chemicals of *Spirulina*, In: Vonshak, A (Ed.) *Spirulina platensis (Arthrospira)*: Physiology, Cell-biology and Biotechnology, Taylor & Francis Ltd. 1997, 175-204.

31. Akira S, Wang HL, Hesseltine CW, Culture conditions affect eicosapentaenoic acid content of Chlorella minutissima. Journal of the American Oil Chemists Society, 61(5), 1984, 892-894.

32. Health & Welfare Canada, Nutrition Recommendations. Ottawa, Canada, 1991, 40-45.

33. Nicol WM, Nutritional and physiological significance of unsaturated fatty acids. Food Safety Notebook, 3(6), 1992, 57-58.

34. Nettleton JA, Are n-3 fatty acids essential nutrients for fetal and infant development? Journal of the American Dietetic Association, 93, 1993, 58-64.

35. Horrobin DF, Huang YS, The role of linoleic acid and its metabolites in the lowering of plasma cholesterol and the prevention of cardiovascular disease. International Journal of Cardiology, 17(3), 1987, 241-55.

36. Puolakka J, Makarainen L, Viinikka L, Ylikorkala O, Biochemical and clinical effects of treating the premenstrual syndrome with prostaglandin synthesis precursors. Journal of Reproductive Medicine, 30, 1985, 149-153.

37. Schalin-Karrila M, Mattila L, Jansen CT, Uotila P, Evening primrose oil in the treatment of atopic eczema: effect on clinical status, plasmaphospholipid fatty acids and circulating blood prostaglandins. British Journal of Dermatology, 1987, 117, 11-19.

38. Carter JP, Gamma-linolenic acid as a nutrient. Food Technology, 42, 1988, 72-82.

39. Harris WS, The omega-6/omega3 ratio and endovascular disease risk: uses and abuses. Current Atherosclerosis Reports, 8(6), 2006, 453-459.

40. Petkov G, Garcia G, Which are fatty acids of the green alga Chlorella?. Biochemical systematic and ecology, 35, 2007, 281-285.

41. Cirik S, Zengin Bir Bitkisel Gıda Spirulina, Tübitak Bilim ve Teknik dergisi, (Nisan): 1989, 19-20.

42. Chen F, Zhang Y, Guo S, Growth and phycocyanin formation of Spirulina platensis in photoheterotrophic culture, Biotechnology Letters, 18(5), 1996, 603-608.

43. Glazer AN, Phycobiliproteins. In: Cohen Z (ed) Chemicals from Microalgae, Taylor and Francis Ltd, UK, 1999, 262-280.

44. Gacheva G, Dimitrova P, Pilarski P, New strain Hematococcus Cf. pulvialis rhozin-12 growth, biochemical, characteristics and future perspectives. Genetic and Plant Physiology, 5(1), 2015, 29-38.

Source of Support: Nil, **Conflict of Interest:** None.