BIOGLOBIA Vol. 7 (2): 15 –23, 2020 **ISSN: 2349-5626**

Estimation of Solid Protein and PUFA Contents of *Scenedesmus abundans* and *Chlorella pyrenoidosa* for Nutritional Benefits.

ONWURAH CHRISTIAN OBIEKWE

Department of Food Technology Federal Polytechnic, Oko Anambra State, Nigeria.
Author's e-mail: christianobiekwe2007@yahoo.com
Received: 10.06.2020 **Revised: 26.06.2020** **Accepted: 12.07.2020**

ABSTRACT

The two Chlorophyta *Scenedesmus abundans* and *Chlorella pyrenoidosa* were collected from National Collection of Industrial Microorganism (NCIM), Pune, India. They were cultured in Fog's media and experimental condition was maintained at temperature of 28^0C with 12:12 hrs. photo and dark periods until stationary growth phase was reached. The cultures were harvested by centrifugation and the supernatant was discarded. The pellets were dried overnight in air oven at temperature of 40^0C. The dried powder was stored at airtight plastic container for subsequent nutritional analysis, for solid protein content and for PUFA contents. The result of this study showed that *S. abundans* had protein contents of 51.02 ± 0.15% in comparison with *C. pyrenoidosa* whose protein content was of 53.05 ± 0.2%. The result of fatty acid composition showed that *S. abundans* had PUFA content of 7.19mg/g^{-1}, GLA content of 21.30 mg/g^{-1}, omega-3 content of 4.80mg/g^{-1}, while omega -6 content of 3.60mg/g^{-1} in comparison with *C. pyrenoidosa* whose PUFA content was of 5.65mg/g^{-1}, GLA content of 18.35 mg/g^{-1}, omega-3 content of 2.90mg/g^{-1} and omega-6 content of 5.10mg/g^{-1}. Thus the result showed both had great potential for nutritional benefits.

Keywords: *Scenedesmus abundans, Chlorella pyrenoidosa, Nutritional benefits, PUFA, Omega-3, Omega-6.*

INTRODUCTION

Malnutrition continues to be a major public health problem throughout the developing world, particularly in Southern Asia and sub- Sahara Africa. (Schofield and Ashworth, 1996; WHO, 2002, Brabin and Coulter 2003; WHO, 2004; FAO, 2004). Diets in population are frequently in deficiency of macronutrients (like protein, carbohydrates and fat) leading to protein-energy malnutrition (PEM), micronutrients (like electrolytes minerals and vitamins) leading to specific micronutrients deficiencies or both (Pinstrup-Andersen *et al*., 1993; Levin *et al.*, 1993; Brabin and Coulter, 2003 and Millward and Jackson, 2004). In the developing countries, countless population are suffering from malnutrition, not due to lack of food, but due to inadequate, nutritionally void, toxic modern junks food with various nutritional nutrients deficiencies resulting in various health problems. (Singh *et al*., 2005). Lack of protein and fatty Acid (PUFA) is a major malnutrition deficiency. Lack of protein leads to edema and kwashiorkor (Brabin and Coulter, 2003). Fatty acid deficiency will result to metabolic disturbances and because of lack of substrate (*e.g.* tyrosine) and coenzymes (Lerner, 1971), will lead to synthesis of pigments in the hair and skin (*e.g.* hair colour may change and skin become hyper pigmented). Nowadays, food habits in society are characterized by a high consumption of fast food which contain low portion of PUFAs but that can be obtained from varieties of fishes. However, its availability may dependent on season and that may hamper the continuity of food. Therefore, it is necessary for encouraging the discovery of alternative sources of PUFA which is microalgae, (Rubio-Rodriguez *et al*., 2010; Kamei *et al*., 2002 and Kalogeropoulos, 2010).

During the recent year, concern grew about the "Food gap" between the industrialized and less industrialized parts of the world, especially as there was rapid and continuing population growth. As a result of this concern, alternative and unconventional sources of foods were sought (Nduka Okafor, 2005). Algae cultivation for feeding of world growing population becomes very eminent due to climatic changes. Land that would have been used for proper agricultural practices were destroyed due to effect of climatic changes. Algae are an important source of vitamins, proteins, polyunsaturated fatty acids (PUFA) and antioxidants (Pulz and Gross, 2004; Svircew, 2005; Blazencic, 2007 and Gouveia *et al*., 2008b).

They can be cultivated in areas unsuitable for plants with less or no seasonality required and in comparison with plants, some species have showed several fold higher production (Pulz and Gross, 2004) Algae will be a very efficient source of these elements that causes malnutrition in developing countries (Pulz and Gross, 2004).

So we in this study, have endeavored to find an alternative source of protein and PUFA in algae class and trying to compare two species of Chlorophyta- *Chlorella pyrenoidosa* and *Scenedesmus abundans* to ascertain the better species that will be used to combat malnutrition in developing countries of the World.

MATERIALS AND METHODS

The green Algae, *Chlorella pyrenoidosa* (NCIM# 2738) and *Scenedesmus abundans* (NCIM#2897) were obtained from National Collection of Industrial Microorganism (NCIM), Resource Centre Pune, India. They were cultured with Fog's medium. All media were prepared fresh from respective dry chemical. About 5ml of the each starter cultures were inoculated into 50ml medium containing in 100ml conical flasks and kept on a glass shelves illuminated from beneath with 70-80$\mu EM^{-2}S^{-1}$ white fluorescent light on a 12:12 hour light: dark cycle. Temperature of species were maintained at 28^0C. The cultures were not aerated, but were gently swirled on an orbital Shaker (Volkman *et al*., 1989 & 1993; Brown, 1991; Brown and Jeffrey, 1992a and Dunstan *et al*., 1992). The cultures were harvested towards the end of log phase (usually 4-6 hours into the light cycle) and in some later studies (Brown and Miller, 1992 and Brown and Farmer, 1994) stationary phase cultures also were examined. Cells were counted (six replicate counts per species) with Neubauer haemocytometer. Algae density assessed by spectrophotometer for optical density (O.D.) at 660 nm for growth rate (Lucia *et al*., 2011). The remainder of the culture was harvested by centrifugation (5000 revolution for 10 min). The supernatant was discarded. The Cell pellet was freeze-dried, and then stored at -20^0C for the samples of algae to be analyzed for protein and fatty Acids (PUFAs).

Protein Analysis: The protein content of freeze-dried algae was determined by Pierce Test kit using spectrophotometer based on the procedure of Lowry et al., 1951 and Kreeger *et al*., 1997. The assay performed in micro plates using Lowry's Methods purchased in a kit (catalogue No.-690-A: Sigma Chemical St. Louis Mo). A standard Curve was prepared as follows. Bovine serum albumin (BSA) powder was dissolved in distilled water and diluted to a concentration of 1μg/μL. A series of dilutions (0, 1, 2.5, 5, 10 and 20 μg/well) were made; samples were diluted such that they would fall within the BSA standard range (0-25 μg/100μL). After the dilution of standards and samples they were transferred to the micro plate, 200μL of biuret reagent was added to each well and mixed thoroughly with repeated

pipetting. Biuret reagent was prepared by mixing 0.5ml of 1% cupric sulfate with 0.5ml of 2% sodium potassium tartrate, followed by the addition of 50ml of 2% sodium carbonate in 0.1N NaOH. The mixture was then allowed to incubate at room temperature for 10-15 minutes prior to the addition of 20µL per well of 1.0N Folin Ciocalten's reagent. Samples were mixed immediately with repeated pipetting with each addition. Colour was allowed to develop for 30 minutes at room temperature and the absorbance measured at 650nm and blanked on the water only as control. All absorbance determination were made using an ELX808 Micro plate Reader (Bio Tek Instruments Winooski, VT) and 4mg freeze dried algae sample and the protein content interpolated from the constructed graph.

Fatty Acid Analysis: Fatty acid methyl esters (FAMEs) were prepared by dissolving the dried extracts or algae in 100µL chloroform and 300µl methanol containing 1.25MHC1 and then incubated at 50^0C for 24 hours. Samples were then dried under a stream of nitrogen for 2 hrs. Dried transmethylated samples were re-suspended in 70µL pyridine and derivatized in 30µL N-methyl-N-trifluoroacetamide (MSTFA) with 1% Trichloromethylsilane (TMCS). It would be incubated at 50^0C for 2 hrs. FAME later would be analyzed by using an Agilent 5973 series high temperature gas chromatography-mass spectrometer (GC-MS), fitted with an auto injector. GC-MS analysis capillary column (DB-5MS, 30mX0.25mmX0.25µm film thickness) (J and W scientific) was used. The injector and detector temperature were set at 280^0C while the initial column temperature was set at 80^0C. A 1µL sample volume was injected into the column and ran using a 100:1 split ratio. After 3 min the oven temperature was raised to 315^0C at a ramp rate of 5^0C min^{-1} and finally maintained at this temperature for 12 mins. The helium carried gas was programmed to maintain a constant flow rate of 2ml min^{-1} and the mass spectra were acquired and processed using both Agilent Chemstation (Agilent, USA) and AMDIS 32 software. (Chin Ming *et al*., 2012)

RESULTS AND DISCUSSION

Protein Content: The result of protein content using Lowry method using Bovine serum

Table 1

Lowry method of Protein Determination using (BSA) Protein Standard.

S. No.	Vol. of BSA (Ml)	Vol. of Distilled Water	Conc. Of BSA (mg/mL)	Vol of reagent D	Incubation of room temp for 10 min	Vol of reagent FCR	Incubation at dark for 30 min	O.D at 660 nm
1	B	1	0	4 mL	10'	.4 mL	30'	0
2	0.1	0.9	100 µg		incubation		dark	0.26
3	0.2	0.8	200 µg				Incubation	0.506
4	0.3	0.7	300 µg					0.696
5	0.4	0.6	400 µg					0.985
6	0.5	0.5	500 µg					1.126
7	0.6	0.4	600 µg					1.13
8	0.7	0.3	700 µg					1.284
9	0.8	0.2	800 µg					1.392
10	0.9	0.1	900 µg					1.44
11	1	0	1 mg=1000 µg					1.552
S	0.4	0.6						0.215
S_2	0.4	0.6						0.207

Albumin (BSA) standard Protein and samples *C. pyrenoidosa* (S_1) and *S. abundans* (S_2) is tabulated on Table 1.

From the result of Table 1, a graph of O.D against concentration was plotted in Fig 1 using BSA Standard Protein.

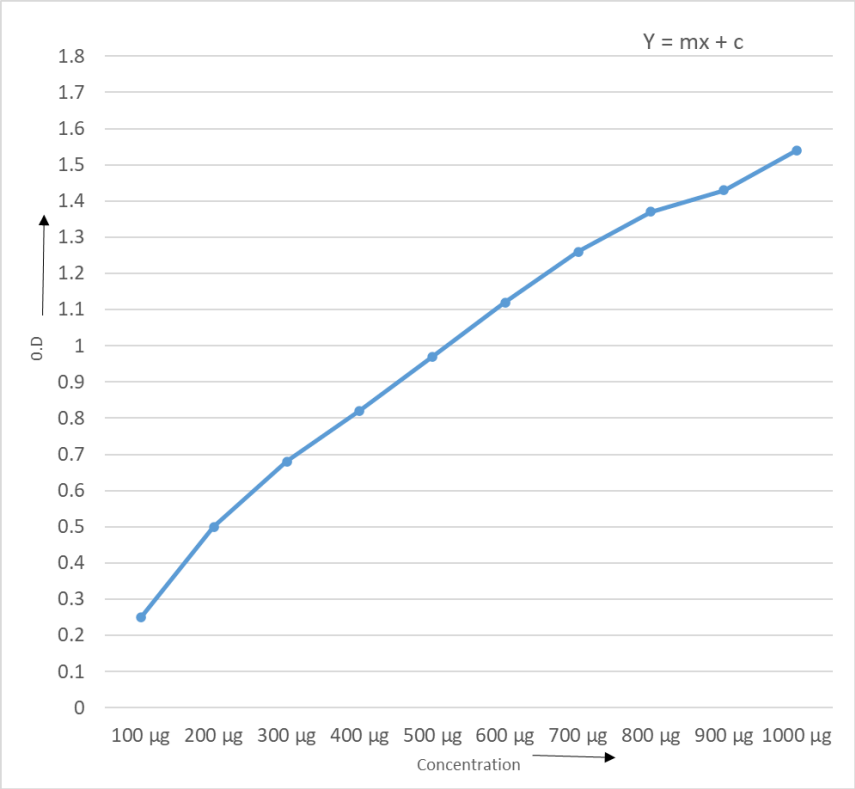

Fig 1. Graph of O.D against concentration using BSA Standard Protein (Protein Standard Curve).

From the graph the Protein content of the two samples is calculated using the formula

Y= mx + *C.*

where,

Y = Absorbance

M =Slope of the Line

X = Concentration when X = 0.

x = Y/M

The slope here is 0.001.

To calculate Protein for *C. pyrenoidosa* (sample S_1)

= 0.215/0.001

$= \left(\frac{215}{20}\right) X \left(\frac{5}{1}\right)$

= 53.75%

To calculate Protein content for *S. abundans*

= 0.207/0.001

$= \left(\frac{207}{20}\right) X \left(\frac{5}{1}\right)$

= 51.75%

The results were done in triplicate and the mean was represented on Table 2. The result showed that *C. pyrenoidosa* had total protein content of 53.05 ± 0.12% while *S.*

abundans had protein content of 51.05 ± 0.15% (Table 2). From published literature it was found that *S. platensis* had protein content of 60.32 ± 0.15% (Bensehaila *et al*., 2015; Tri Winarni *et al.*, 2014 and Holman and Malau-Aduli, 2013).

Table 2
Protein Content of Microalgae

S. No	Samples	Protein %
1	*Chlorella pyrenoidosa*	53.05±0.12
2	*Scenedesmus abundans*	51.02±0.15

The protein content of the two microalgae is illustrated on Fig 2.

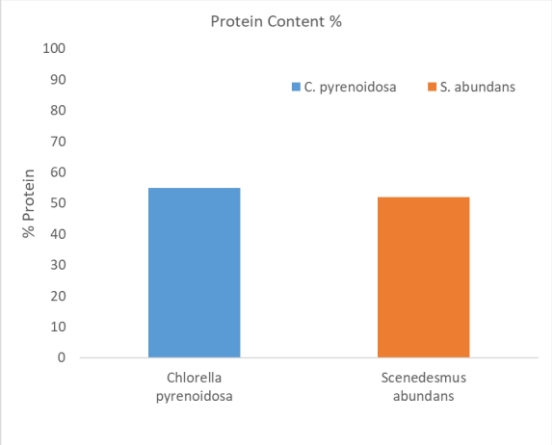

Fig 2. Protein Content of two Microalgae

PUFA (Polyunsaturated Fatty Acid) of Algae oil

The result of Fatty acid composition is showed at Fig. 3a for *C. pyrenoidosa* Chromatogram for fatty acid and Fig. 3b for Chromatogram for fatty acid for *S. abundans.*

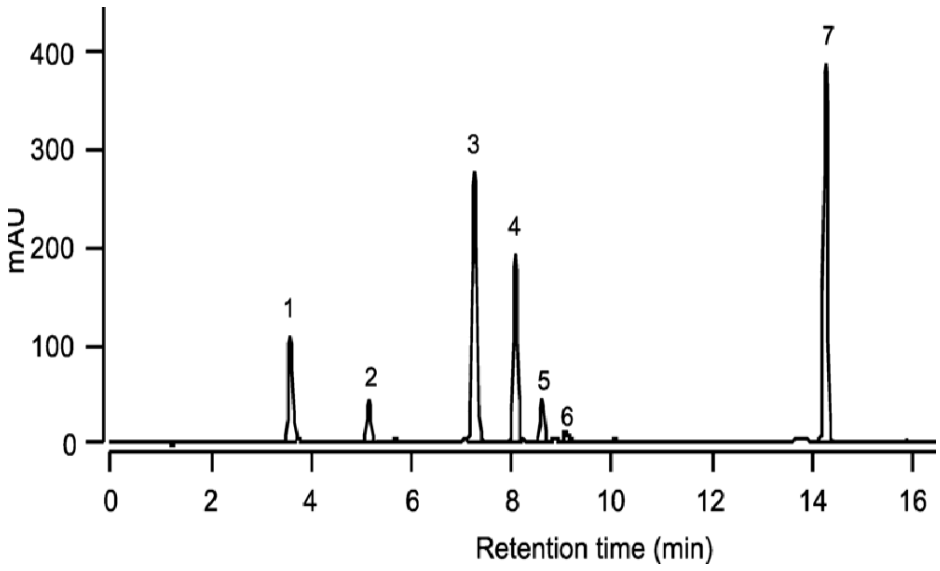

Fig. 3a. Chromatogram for fatty acid composition of *C. pyrenoidosa*

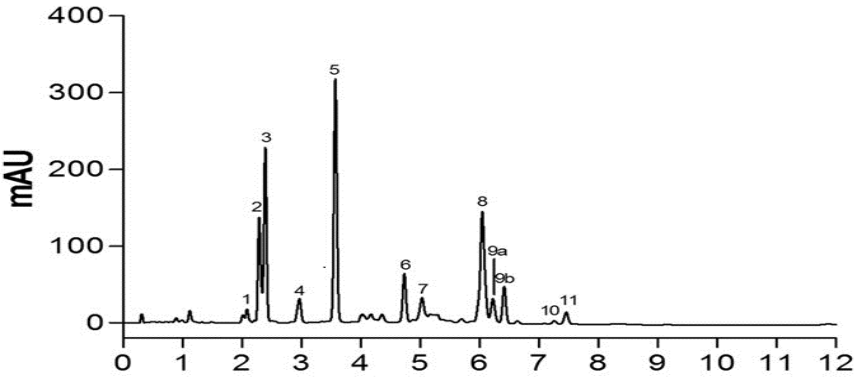

Fig. 3b. Chromatogram for fatty acid composition of *S. abundans.*

The result of fatty acid composition showed that *S. abundans* had PUFA content of 7.19 mg/g^{-1}, GLA content of 21.30 mg/g^{-1}, omega-3 content of 4.80 mg/g^{-1}, Omega-6 content of 3.60mg/g^{-1} in comparison to *C. pyrenoidosa* with PUFA content of 5.65 mg/g-1, GLA content of 18.35 mg/g^{-1}, Omega-3 content of 2.90 mg/g^{-1}, Omega-6 content of 5.10 mg/g^{-1} in Table 3. *S. abundans* had total fatty acid content of 36.89 mg/g^{-1}, in comparison to C. pyrenoidossith total fatty acid content of 32.0 mg/g^{-1}. From published literature it was found that *S. platensis* had PUFA content of 5.089 mg/g^{-1}, GLA content of 0.986 mg/g^{-1}, Omega-3 content of 2.761 mg/g^{-1}, Omega-6 content of 5.523 mg/g^{-1} (Aly *et al*., 2011; Holman and Malau-Aduli, 2013 and Kent *et al*., 2015).

Table 3
Fatty Acid Composition mg/g^{-1}

S. No.	Parameters	Scenedesmus abundans	Chlorella Pyrenoidosa
1	PUFA	7.19	5.65
2	GLA	21.3	18.35
3	Omega-3	4.8	2.90
4	Omega-6	3.6	5.10
5	Total FA	36.89	32.00

The result of total fatty acid composition of the two microalgae is illustrated in Fig. 4.

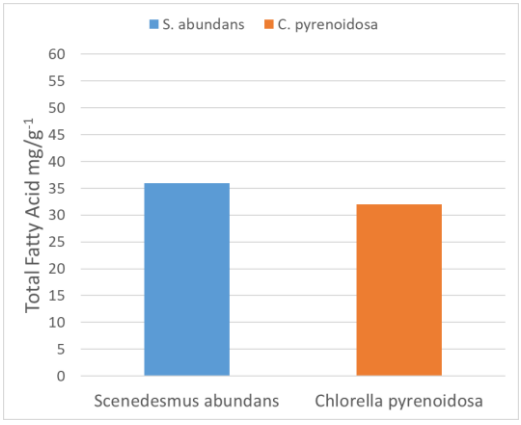

Fig. 4. Total Fatty Acid Composition of Microalgae

Proteins form the major structural components of all the cells of the body. They are the building blocks of all unit of life. They perform important notes as enzymes, membrane carriers, and hormones. Protein requirement for a healthy adult individual is 0.8g/kg of the body mass (Bilsborough and Mann, 2006). The result of protein estimation indicates that *S. abundans* had protein content of ~51.02% (% of the dry weight) of its mass which is at per with commercial products like *C. pyrenoidosa* and *Spirulina* spp. and can very well meet the daily requirement of an individual if taken as supplement (Kent *et al*., 2015 and Benesahaila *et al*., 2015. Holman and Malau-Aduli, 2013). The values are much higher than as recommended by the Ontario Ministry of Agriculture and Food for animal feeds (20% of dry weight) (Wright and lackey, 2008) but no established numerical baseline is available to define human protein supplement.

Fat is a major source of fuel energy for the body and acts in the absorptions of fat-soluble vitamins and other food components, such as carotenoids (NRC, 1989). Essential FA's are essential for maintaining human health. The result of this study shows that *S. abundans* contains all the essential FA's especially Ω3, Ω6 and PUFA's in the optimum quantity (Table 3). The desirable Ω6/Ω3 ratio is 1:1 (Simopoulos, 2008). In case of *S. abundans* we do have a similar ratio which makes it a very suitable material for human consumption and health benefits. The PUFA and GLA quantity was also comparable to the quantities available in *C. pyrenoidosa* and *Spirulina* spp. (Aly *et al*., 2011; Holman and Malau-Aduli, 2013 and Kent *et al*., 2015).

CONCLUSION

Nutritional analysis of both the algae *S. abundans* sand *C. pyrenoidosa* have proved the suitability of them to be used as food supplement with the following characteristics:-

- Rich source of good PUFA with relatively high level of alpha linolenic acid (ω-3) which is regarded beneficial to health.
- Good source of protein edible nontoxic suitable for human consumption.

The results of the present study show that *S. abundans* and *C. pyrenoidosa* reveal biochemical profile that indicates that both contains good quantity of high-quality protein, fatty acids (PUFA) which were at par or better than other algae species used as food supplement. Therefore, it points towards the potential for both for human use to solve malnutrition problem in developed and developing world if used as food supplement.

REFERENCE

1. Aly, M. S., Gad, E. I., Sayed, M and Kamel, P. 2011. Production and application of *Spirulina platensis* rich in fatty acids and vitamins. *J. American Sci*, 7(2):36-45 http://www.americanscience.org
2. Bensehaila, S., Doumandji, A., Boutekrabt, L., Manafikahi, H., Peluso, I., Bensehaila, K., Kouache, A. and Bensehaila, A. 2015. The nutritional quality of *Spirulina platensis* of transerasset Algeria. *Afri. J. Biotech*, 14(19):1649-1654.
3. Bilsborough, S. X. and Mann, N. 2006. A review of issues of dietary protein intake in humans. *Int. J. Sports Nutrition Exercise Metabolism*, 1 b (2):129-152.
4. Brabin, B. J. and Coulter, J. S. B. 2003.Nutrition-associated disease. In: Cook, G. C., Zumla, Al, editors Manson's tropical diseases. London: Saunders, *p*. 561-580.

5. Brown, M. R., Jeffrey, S. W., Volkman, J. K. and Dunstan, G. A. 1997. Nutritional properties of microalgal for mariculture. *Elsevier Aquaculture*, 151:315-331.
6. Brown, M. R and Miller, K. A. 1992. The ascorbic acid content of eleven species of microalgae used in mariculture. *J. Appl. Phycol*, 4:205-215.
7. Brown, M. R, and Farmer, C. A. 1994. Riboflavin content of six species of microalgae used in mariculture *J. Appi. Phycol*, 6:61-65.
8. Brown, M. R. and Jeffrey, S. W. 1992 a. Biochemical composition of Microalgal from the classes of chlorophyceal and prasinophyceae .1, Amino acids, sugars and pigments. *J. Exp. Mar. Biol. E. Col*, 161:91-113.
9. Chin Ming, L. R., Nurliyana, A., Bazli Syah, M., Noor Azizah, H., Li Sim and Hivzum, M. V. 2012. Identification and Biochemical composition of a green microalgae. *Asian J. Biotec*, 4:38-45.
10. Dunstan, G. A., Volkman, J. K., Jeffrey, S. W., and Barretf, S. M. 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae 2. Lipid classes and fatty acid. *J. Exp. Mar. Biol. Ecol*, 161:115-134.
11. Food and Agriculture Organization of the United Nations. 2004. Undernourishment around the world. In: The state of food insecurity in the world 2004. Rome: The organization.
12. Gouveia. L., Batista, A. P., Sousa, I., Raymund, A. and Bandarra, N. M.2008a. Microalgal in Novel food products in K. Papadopoulos food chemistry research developments. *Nova Science Publishers*, *p.*75-112.
13. Holman, B. W. B. and Malau-Aduli, A. E. O. 2013. *Spirulina* as a livestock supplement and animal feed. J. Animal Physiol. Animal Nutri, 97:615-623 Doi 10.1111 [1439-396.2012. 0. 1328X].
14. Kalogeropoulos, N., Chiou, A., Gavala, E., Christea, M. and Andrikopoulos, N. K. 2010. Nutritional Evaluation and bioactive micro-constituents (carotenoids tocopherol sterols and squalene) of raw and roasted chicken fed on DHA rich microalgae. *Food Res. Inter*, 43:2006-2013.
15. Kamei, M., Ki, M., Kawagoshi, M. and Kawai, N. 2002. Nutritional Evaluation of Japanese Take-out Lunches compared with western style fast foods supplied in Japan. *J. Food Comp. Analysis*, 15:35-45.
16. Kent, M., Welladsen, H. M., Mangolt, A. and Li, Y. 2015. Nutritional evaluation of Australian. Microalgae as potential Auman Health supplements. *Plos One* 10(2): EO 118985. Doi; 10:1371. *J. Pone*, 011985.
17. Kreegev, D. A., Goulden, C. E., Kilham, S. S., Lynn, S. A., Datta, S. and Interlandi, S. J. 1997. Seasonal Changes in the Biochemistry of Lake Sexton. *Freshwat. Biot*, 38:539-554.
18. Lerner, A.B.1971. On the etiology of Vitiligo and gray hair. *Am. J. Med*, 51:14 1-7 [Pub med].
19. Levin, H. M., Pollitt, E., Galloway, R. and McGuire, J.1993. Micronutrient deficiency disorders. In: Jamison, D. T., Mosley, W. H., Measham, A. R. and Bobadilla, J. Disease control priorities in developing countries 2Id ed. oxford (UK): Oxford University Press,*p.* 421-451.
20. Lowry, O. H., Rogebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein Measurement with the Folin Phenol reagent. *J. Biol. Chem*, 193:265-275.
21. Lucia Helena Ribeiro Teresa Raya- Rodriguez and Nelson Ferreira Fontoura. 2011. Algal density assessed by spectrophotometry; A calibration curve for the unicellular algal pseudo kirchneviclia subcapifata. *J. Environ. Chem. Ecotoxicol*, 3(8):225- 228.
22. Miliward, D. J. and Jackson, A. A.2004. Protein/energy ratios of current diets in developed and developing countries compared with a safe protein/energy ratio: implications for recommended protein and amino acid intakes. *Public Health Nutr*,7: 387-405.[Pub Med].
23. Nduka Okafor. 2005. Single cell protein in modern industrial Microbiology and Biotechnology Enfield Plymouth USA,*p.*293.
24. Pinstrup-Andersen, P., Burger, S., Habicht, J. P. and Peterson, K. 1993.Protein- energy malnutrition. In: Jamison, D. T., Mosley, W.H., Measham, A.R. and Bobadilla, J.L. Disease control priorities in developing countries 2nd ed. oxford (UK): Oxford University Press, *p.* 391-420.

25. Pulz, 0. and Gross, W. 2004. Valuable products from Biotechnology of Microalgae. *Applied Microbiol. Biotech*, 65 (6):635-648.

26. Rubio-Rondriguez, N., Beltran, S., Jaime, I., De Diegos, Sanz M. T. 2010. Production of omega-3 polyunsaturated fatty acid concentrates: A review. *Innova. Foods Sci. Emerg. Techno*, 11:1-12.

27. Schofield, C. and Ashworth, A. 1996. Why have mortality rates for severe malnutrition remained so high? *Bull. World Health Organ*, 74: 223-229 [PMC free article Pub Med]

28. Simopoulos, A. P. 2008. The importance of the Omega-6/Omega-3 fatty acid ratio is cardiovascular diseases. *Exp. Biol. Med*, 233:674-688. Doi: 10.3181/0711-MR-311PMID:18408140.

29. Singh, S., Kate, B. N. and Banerjee, A. C. 2005. Bioactive compounds from Cynobacteria and microalgae-An over view. *Critical Reviews in Biotechnology*, 25 (3) : 73-95.

30. Svircev, Z. 2005. Mikroalgalicijanoba Krerije U biotechnology Nov. Sad Prirodna-Matenaticki Fakwifet.

31. Tn Winarni, Agustini., Meiny, Suzery., Denny, Sutrisnants; Wido do Farid Marut; Fadiyants. 2015. Comparative study of Bioactive Substances Extracted from fresh and dried Spirulina *sp. Procardia Environ. Sci*,23:282-289.

32. Volkman, J. K., Jeffrey, S. W., Nichols, P. D., Rogers, G. I. and Garland, C. D. 1989. Fatty acid and Lipid composition or 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol*, 128:219-290.

33. Volkman, J. K., Brown, M. R., Dunstan, G. A. and Jeffrey, S. W. 1993. The microalgae from the class eustigmatophyeae *J. Phycol*, 29:69-78.

34. World Health Organization.2004. United Nations Children's Fund. Joint Statement on the management of acute diarrhea. Geneva: The Organization.

35. World Health Organization.2002. World health export. Geneva: The organization.

36. Wright, T. and Lackey, R. 2008. Definitions of feed manufacturing and livestock nutrition terms Ontario Ministry of Agriculture, Food and Rural Affairs, July. Factsheet order no 08-39AGDEX400/50.