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Review Article

Spirulina: A Source of Gamma-linoleic Acid and Its Applications

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Abstract

The human body needs essential nutrients in order to function, grow, and stay healthy. Our bodies cannot make these nutrients, so get them from our diet. On the other hand, some diet-related diseases can be caused by certain improper food ingredients and body inability of absorbing them. Then the idea of purifying beneficial ingredients formed. Poly-unsaturated fatty acid such as gamma-linoleic acid (GLA) is a group of essential fatty acids particularly favorable for its application in nutraceutical and pharmaceutical industries. GLA plays significant roles in improving human body functions. It has gained its importance in the last four decades for having a positive effect on the most of the chronic diseases of modern society, including cancer, diabetes, heart disease, arthritis, Alzheimer's disease, etc. Then, it has been used as a dietary supplement for the treatment of various health problems and have inflammatory component. One of the richest sources of GLA is a kind of microalgae; *Spirulina*. *Spirulina* is a blue-green alga primarily originated from two species of cyanobacteria and is believed to be the first form of plant life on the earth. This article reviews GLA applications and properties; favorable conditions for increasing its amount within *Spirulina*; and how to extract it from the algae.

Keywords: Algae, *Spirulina*, Gamma-linoleic Acid, Fatty Acid, Extract

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Introduction

Structure of γ-linolenic acid (GLA)

Among Poly-unsaturated fatty acid (PUFAs), one of the important essential fatty acids (EFA) from omega-6 series is γ-linolenic acid (GLA). The name of EFA was introduced in 1929 and refers to the fatty compounds important for functioning of the human body. GLA or "cis 6, 9, 12-octadecatrienoic acid"18:3 n-6 belongs to tri unsaturated acids (triene) which contain three double bonds. Figure 1 shows the γ-linolenic acid structure [1].

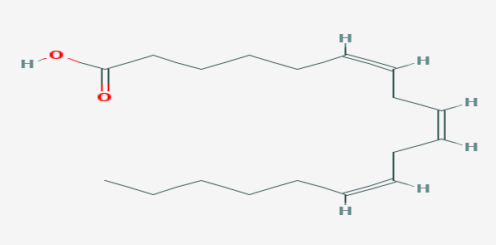

Figure1. Structure of γ-linolenic acid.

Biosynthesis and mechanisms of GLA

GLA is formed in the pathway of metabolic transformations of linoleic acid (LA , all cis 6,9-octadecadienoic acid), as a result of delta-6-desaturase enzyme [1]. This reaction is very slow and further restricted during nutritional deficiencies of vitamins and minerals (zinc, cobalt, etc.). GLA is further metabolized to Dihomo-γ-linolenic acid (DGLA 20:3 n-6) which undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce eicosanoids and prostaglandins [2]. γ-linolenic acid is the first intermediate in the bioconversion of linolenic acid to long-chain polyunsaturated fatty acid, arachidonic acid (AA, 20:4 n-6) [3]. Studies by Hassam *et al*., (1975) have shown that the desaturation steps tend to be very slow but the elongation steps are rapid. Their results also suggest that the conversion of LA to GLA by 66 desaturases is the rate-limiting step in the conversion of LA to AA *in vivo* [4].

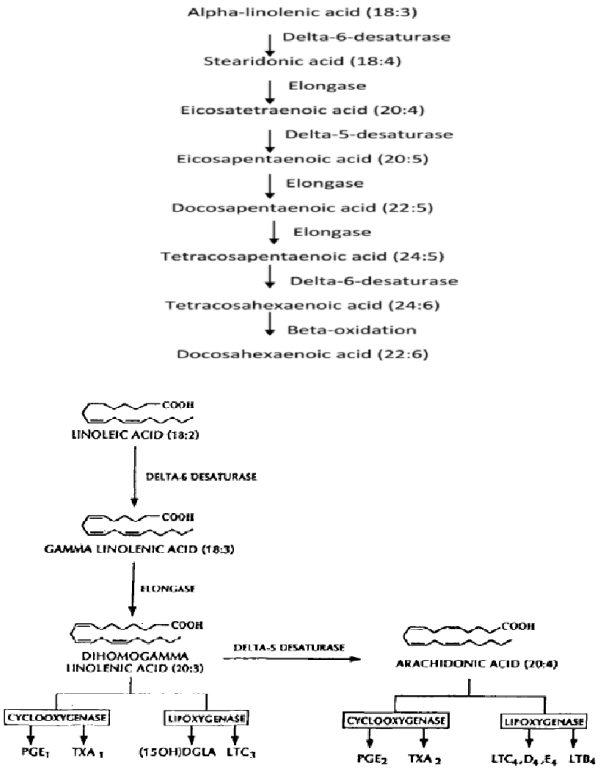

Figure 2. Biosynthesis of γ-linolenic acid [12].

The role of GLA in Medicine

γ-linoleic acid is absorbed easily by human cells and helps in energy release [5]. GLA also helps in different defense mechanisms including killing of invading microorganisms, damaged cells, wound healing, tissue repair, etc. Also, in the last five decades, inflammation has been confirmed in various chronic diseases and GLA has shown anti-inflammatory actions [2]. Because of its metabolism to dihomo-γ-linolenic acid dietary, GLA has the potential to prevent the formation and therefore the negative inflammatory effects of arachidonic acid [6]. There are promising studies which suggest that supplementation with GLA and particularly combining it with (n3) long chain-PUFAs have great potential to dampen inflammatory processes [7]. GLA improves the processes of β-oxidation of free fatty acids in fatty liver and facilitates reduction of body weight by increasing the activity of carnitine palmitoyl and intensification of peroxisomal β oxidation [8]. γ-linolenic acids are physiological components in inner and outer cell transport complexes of cell membranes or mitochondria membranes for transmission of signals in the neuronal lattice of brain in human [1]. GLA plays an important role in the treatment of wide variety of pathologies such as atherosclerosis, normalizes nerve conduction velocity, sciatic endoneurial blood flow, atopic eczema, Parkinson's disease, premenstrual syndrome, multiple sclerosis, lowers low-density lipoprotein, cardio-circulatory, diseases coronary, heart diseases, arthritis and zinc deficiency [9, 10, 11]. Treatment with 2.8 g of GLA per day (as the free fatty acid) for 6 months resulted in clinically relevant and statistically significant reduction in the signs and symptoms of disease activity in patients with rheumatoid arthritis [12, 13, 14].

Immune cells including lymphocytes, polymorphonuclear leukocytes, monocytes, splenocytes, kuppfer cells, etc, have a high content of polyunsaturated fatty acids in their membrane phospholipids. GLA is taken up by inflammatory cells and is rapidly elongated to DGLA. In some species, it can be desaturated to AA; but, in human immune cells, it is not desaturated probably because of very limited to any presence of delta-5-desaturase in immune cells. Dietary administration of GLA-rich oils has a potential in modulating immune function. Several *in vitro* and *in vivo* studies have investigated the effect of GLA on immune functions.

GLA and DGLA inhibit protein kinase C (PKC) activity in PMA-stimulated T-lymphocytes; however, only GLA inhibit basal PKC activity. In the same study, both fatty acids stimulated translocation of PKC from cytosol to membrane. GLA and DGLA inhibited early and late rise in intracellular calcium induced by anti-CD3 monoclonal antibody in T cells and also inhibited a rise in inositol-1,4,5-triphosphate (IP3) production [15].

Source of GLA

γ-linolenic acid have been reported to be produced by several families principally *Boraginaceae*, as well as *Caryophyllaceae*, *Scrophulariaceae,Cannabinaceae*, *Saxifragaceae*, *Onagraceae,Liliaceae*, *Aceraceae*, *Scrophulariaceae*, *Ranunculaceae*, *Primulaceae*, *Asteliaceae* and also Evening-primerose (*Oenotherabiennis* L.) and Black-current (*Ribesnigrum* L.) [3, 15]. The level of gamma-linolenic acid in Evening-primrose oil varies from 7 to 10% of total fatty acids, whereas in borage oil it ranges from 17 to 25%, and in biotechnology-derived safflower it is 35%, in canola oils about 36-40% and hemp seed oil ~15%. GLA is also found in some fungal sources i.e. *Mucor javanaicus* and *Mortierella isabellina*.

On the other hand, a minimal amount is produced in the body as a downstream metabolite conversion from the EFA linoleic acid. GLA is also present naturally in the form of triglycerides. Cyanobacteria are also included in microorganisms that produce GLA (*Spirulina maxima* and *Spirulina platensis*) [8, 16].

Microalgae and GLA

GLAs and their limited availability triggered the search for the potential new sources of these fatty acids [17]. The cyanobacteria (blue–green algae) are capable of accumulating 1% of GLA in the dry cell mass. Under certain environmental conditions, viz, high intensity and low temperature, the GLA to total fatty acid ratio could be enhanced up to 31% [18]. The first use of microalgae by human dates back to 2000 years ago from the Chinese who used Nostoc to survive during famine [19]. Microalgae are considered a potential source of a wide spectrum of long-chain PUFAs [20]. For instance, arachidonic acid (AA, 20:4w6) from *Porphyridium*, eicosapentaenoic acid (EPA, 20:5w3) from *Nannochloropsis*, *Phaeodactylum*, *Nitzschia*, *Isochrysis*, *Diacronema*, and docosahexaenoic acid (DHA, 22:6w3) from *Crypthecodinium*, *Schizochytrim* has been detected [21].

The production of highly purified γ-linolenic acid from a microalgal source would be promising. The advantages of these strains include their higher oil productivity, fast reproduction, and non-food source. They are economically cultivable, as they require only light energy, air, and minerals to grow. They require much less land areas compared to conventional crops and could be simultaneously used for the extraction of biotin, vitamin B-12, folic acid, other vitamin B complexes, phycobili proteins, and other proteins [22, 23]. Also, added to these advantages, the isomer of GLA (α-linolenic acid) is found to be absent in total fatty acids of the cyanobacterium. The absence of the fatty acid isomer makes purification much more simple when GLA is the fatty acid of interest [24].

Spirulina, a source of GLA

Microalgae *Spirulina* is rich in fatty polyunsaturated acids, mainly γ-linolenic acid [25] and can be considered as a source of γ-linolenic acid [26]. Semih Otle and Ruhsen Pire [11] experimented on three samples of *Spirulina* and expressed that although high proportions of GLA were found, no ALA was found in these samples. Half of the total *Spirulina* lipids are fatty acids, the rare polyunsaturated fatty acid γ-linolenic acid with putative medicinal properties represents 10–20% of the fatty acids in *S. maxima*, compared to 49% in *S. platensis* [27]. The oil of Evening-primrose is currently the major source of GLA which may be safely used for supplement diets, but it is by far the most expensive edible oil of commerce. Since it has frequently been claimed recently, *Spirulina* seems likely would turn out to be a less expensive source of GLA [28].

Spirulina microalgae

Spirulina, newly named *Arthrospira*, are filamentous cyanobacteria, a microscopic photosynthetic bacterium, that derives its name from the spiral or helical nature of its filaments [27]. It is found in warm water alkaline volcanic lakes and possesses an amazing ability to live in extremely harsh conditions [29]. Two species of *Spirulina* that are most commonly used in nutritional supplements are *Spirulina platensis* and *Spirulina maxima* [30]. *Arthrospira platensis* is found in Africa, Asia and South America, whereas *Arthrospira maxima* is confined to Central America [31]. As early as over 400 years ago, *Spirulina* was eaten as food by the Mayas, Toltec's, and Kanembu in Mexico during the Aztec civilization [32]. The first documented report on *spirulina* dates back to the 16th century, when Spanish invaders Mexico, and discovered that the Aztecs use a blue green cake known as tecuitlatl as food. *Spirulina* was discovered in the mid-1960s, when a french botanist, Jean Leonard, described a blue-green cake sold in the food market of Fort Lamy, Chad. In the local dialect,it was called as Dihe [33].

Figure 4. Microscopic view of *Spirulina*.

Medical and dietary forms of spirulina

Spirulina is a source of nutraceutical, with antioxidants, probiotics properties and biological activities such as prevention of anemia (due to high iron and vitamin contents), also it is an important source of the protein C-phycocyanin, which has antioxidant and anti-inflammatory properties [34], but, the phycocyanin isolated from *Spirulina platensis* extract is capable of binding to ferrous and ferric ions, probably causing decrease in the absorption of iron from food [35]. Recently, large studies have been devoted to therapeutic benefits of *Spirulina* on various diseased conditions including hypercholesterolemia, hyper- glycerolemia, cardiovascular diseases, inflammatory diseases, cancer, and viral infections hypolipidemic [36]. The safety of *Spirulina* consumption for human has been established through toxicological studies and sold as a health drink or pills in tablet form for more than 10 years without any undesirable effect. A hot water extract of *Spirulina* has been orally administered to patients as an anticancer and antiviral agent. The molecular mechanism and experimental immunomodulatory function of *Spirulina* have been first reported in mice, 1994 [37]. *Spirulina* can improve hemoglobin, protein, and vitamin levels in malnourished children, alleviate vitamin-A deficiency through provision of bioavailable β-carotene, and favorably effect on antioxidant capacity, immune function, and anemia status. Positive health influences of cyanobacteria, including *Spirulina*, have been attributed to its fiber components, phycocyanin, γ-linolenic acid, vitamins, phenolic compounds, and minerals [38].

Figure 5. Medical and dietary forms of *Spirulina*.

Spirulina profile

we can conclude that the advantages of *Spirulina* are multiple: its high nutritional value, the availability of its nutrients, it's simple production method due to its moderate requirements for growth, its excellent conservation after recollection, and its security in relation to consumption (no toxicities), to name a few [39]. *Spirulina platentis* is rich in proteins, carotenoids, essential fatty acids, vitamin B complex, vitamin E, and mineral such as copper, manganese, magnesium, iron, selenium and zinc [40].

Table 1. Nutritional profile of *spirulina* powder (composition by 100 g) [14, 27].

Component	Value	Level
Moisture content	6-7	gr
protein	60-70	gr
Fatty acid	4-5	gr
carbohydrate	15-18	gr
chlorophyll	1-2	gr
Mixed carotenoid	350-450	mg
Beta carotene	180-190	mg
phycocyanin	8-12	gr
GLA	1-2	gr
calcium	400-600	mg
iron	50-100	mg
potassium	200-2000	mg
magnesium	200-300	mg
zinc	1.0-2.0	mg
Vitamin A1	100-200	mg
Vitamin B1	1.5-4.0	mg
Vitamin B2	3.0-5.0	mg
Vitamin B6	0.5-0.7	mg
Vitamin B12	0.05-2.0	mg
Vitamin E	5.0-20	mg

Table 2. Fatty acid composition of 35 *Arthrospira* strains grown under standard conditions [43].

Fatty acid	Value
C16:0	42.3-47.6%
C16:1	2.4-5.4%
C18:0	0.0-2.1%
C18:1	2.9-11.8%
C18:2	13.1-31.5%
γ -C18:3	12.9-29.4%

Review and enrich gamma from Spirulina

Ciferri was the first who suggested that *Spirulina* can be used as a source of PUFAs, especially GLA. It is well known that some environmental parameters such as temperature, light intensity, nitrogen cell concentration, growth phase, light/dark cycle, and outdoor cultivation strongly influence lipid production of *Spirulina*. Light plays an important role in the cultivation of photosynthetic microorganisms. When using mixotrophics, lipid content and GLA level was higher, that is, when both light and carbon source are provided to the microorganism [41].

The production of purified GLA is costly but Olguın *et al*., in a study on the effect of low light flux and nitrogen deficiency on *Spirulina* obtained 26-31% of gamma-linolenic acid but temperature was more important parameter than sodium nitrate (greater amounts of GLA obtained at 30°C) [42]. The results of some researchers from England on the composition of the 10 *Spirulina* strains showed highest γ-linolenic acid, and linoleic acid contents were generally found in cultures grown at 20°C and 10 μmol photon m^{-2} s^{-1} [43]. Japanese researchers found out that in *Spirulina* platensis, higher γ-linolenic acid could be effectively obtained by culturing under light and then leaving in the dark for a week. Also, decreasing the culture temperature from 30°C to 20°C had no significant effect [44]. Additionally, when the cellular mechanisms for photosynthesis are active and light and carbon sources are available, nitrogen and phosphorus limitation is an efficient trigger to increase lipid content. Brahmdutt & Pabbi (India) [45] studied three *Spirulina* strains and showed that nitrogen limitation was more effective in increasing total lipid content, but phosphorus limitation had more effect on the fatty acid profile. Under phosphorus limitation reduction in the fatty acid content occurred in all three strains. Rijn & Shilo [46] studied the fatty acid content and nitrogen source in the culture medium *Spirulina*. They showed that reserve compounds are accumulated during nitrogen depletion. Under phosphorus limitation, an increase mainly in triglyceride levels occurs. Also, in KL University (India) researcher's studies on Algal cultivation in bicarbonate enriched medium under static, continuous, and periodic operation of air exhibited that periodic sparging is suitable for GLA production. They illustrated that the commercial exploitation of *Spirulina* for GLA production can be achieved by shortening the harvesting time (between day 6 and 8) at optimum aeration rates. To understand the role of dissolved oxygen on GLA content, studies on the enzyme regulatory mechanism is necessary [47]. The common methods of producing PUFA concentrates including urea adduct formation, solvent winterization (to separate TAG, diacylglycerols, fatty acids, esters and other lipids that are soluble in organic solvents), fractional distillation, high-performance liquid chromatography, and the scalability of HPLC for purifying large quantities of fatty acids are expensive and impractical [4, 48]. Other purification processes includes crystallization method at low temperature and lipase catalyzed enzymatic purification. Although, pure GLA can be achieved by above mentioned methods but those methods are expensive and very tedious. Sajilata, *et al*., [9] employed argentite silica gel column chromatography for GLA purification. Silica gel column chromatography has been shown to be suitable for the isolation of GLA methyl ester from *S. platensis*. Using argentite silica gel chromatography was obtained GLA methyl ester with over 96% purity [48]. The simplest and most efficient technique for obtaining a GLA concentrate is urea complexation. It requires no organic solvent except than ethanol [4]. Japanese researchers concluded that urea addition step is effective for obtaining highly pure GLA, when the ratio of GLA in total fatty acid is considerably high, but the ratio of oleic or linoleic acid, which may give unfavorable effect on the concentration of 7-1inolenic acid, is relatively low. Results get better by repeating the urea addition twice [43]. An increased lipid and TFA in the solvent from biomass were observed when the extraction temperature was raised. Time and the optimum number of extraction stages were also reduced when extraction was carried out at 60°C, therefore the best procedure for extracting lipid from *Spirulina*, recommended for industrial scale-up was three-stage extraction (20 min/stage) using a sample solventratio of 1:5 at 60 °C [9]. Researchers in JSS College of Pharmacy (India) investigated a new method for producing higher concentrations of γ-linolenic acid and its ester forms from *Spirulina platensis*. The fatty acid methyl esters (FAME) were prepared from the freeze dried biomass of *Spirulina platensis*. The FAME fraction was subjected to urea fractionation by extraction with n-hexane (enriched GLA-ME fraction) and was found 57.62 % w/w of GLA-ME by using HPTLC. GLA-ME was isolated by flash chromatography system from the *Spirulina platensis* and the percentage yield is found to be 71 % [49]. Another novel method for performing preparative chromatographic separation is using Cyclograph Centrifugal Chromatography System (CCCS) [50]. The methodology of Supercritical Fluid Extraction with carbon dioxide (SC-CO_2) could be considered as a promising technique in producing solvent-free extracts of *S. platensis*. This method is non-toxic, non-flammable, non-explosive, cost-efficient and readily available. In Chiayi University (Taiwan), researchers demonstrated the capability of using SC-CO_2 in supercritical flow fractionation to continuously prepare GLA from *S. platensis*. Findings from this study showed that the extraction of GLA from *S. platensis* was optimized at 60°C at a pressure of 30 MPa and a flow rate of 3 ml/min [51].

Conclusion

Based on the result obtained, γ-linolenic acid has many medical and dietary applications. One of its rich resources is *Spirulina* microalgae. Due to the simple algae

culture conditions and also because algae are not food source, it is a better candidate than plants. The results show high efficiency gamma can be achieved of *Spirulina* by optimizing the culture conditions. But yet it is needed to search for better methods of extraction and purification of fatty acid.

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References

1. Zielińska, A., Nowak, I., Fatty acids in vegetable oils and their importance in cosmetic industry. *CHEMIK nauka-technika-rynek*, 2014, Vol. 1, pp. 103-110.
2. Kapoor, R., Huang, Y.S., Gamma linolenic acid: an anti-inflammatory omega-6 fatty acid. *Curr Pharm Biotechnol*, 2006, Vol. 7, pp. 531-534.
3. Kucukboyaci, N., Dogru Koca, A., Yildirimli, Ş., Killic, E.İ., Goren, A.C., γ-linolenic acid content and fatty acid profiles of the seed oils of some *anchuusa* species. *Turk J Pharm Sci*, 2013, Vol. 10, pp. 87-94.
4. Spurvey, S.A., Production of structured lipids via enzymatic interesterification of gamma-linolenic acid (GLA) and marine oils. Memorial University of Newfoundland, 2002.
5. Karkos, P., Leong, S., Karkos, C., Sivaji, N., Assimakopoulos, D., Spirulina in clinical practice: evidence-based human applications. *Evid Based Complement Alternat Med*, 2008, Vol. 2011, pp. 27-32.
6. Kumar, P., Desai, N., Dwivedi, M., Multiple potential roles of Spirulina in human health. CRC Press A, 2007.
7. Sergeant, S., Rahbar, E., Chilton, F.H., Gamma-linolenic acid, Dihommo-gamma linolenic, Eicosanoids and Inflammatory Processes. *Eur J Pharmacol*, 2016, Vol. 785, pp. 77-86.
8. Białek, M., Rutkowska, J., The importance of γ-linolenic acid in the prevention and treatment. *Postepy higieny i medycyny doswiadczalnej (Online)*, 2014, Vol. 69, pp. 892-904.
9. Ahmed, S.U., Reddy, K.K., Swathy, S.L., Singh, S.K., Kanjilal, S., Prasad, R.B., et al., Enrichment of γ-linolenic acid in the lipid extracted from *Mucor zychae* MTCC 5420. *Food Res Int*, 2009, Vol. 42, pp. 449-453.
10. Raja, R., Hemaiswarya, S., Ganesan, V., Carvalho, I.S., Recent developments in therapeutic applications of Cyanobacteria. *Crit Rev Microbiol*, 2016, Vol. 42, pp. 394-405.
11. Ötleş, S., Pire, R., Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. *J AOAC Int*, 2001, Vol. 84, pp. 1708-1714.
12. Zurier, R.B., Rossetti, R.G., Jacobson, E.W., Demarco, D.M., Liu, N.Y., Temming, J.E., et al., Gamma-linolenic acid treatment of rheumatoid arthritis. A randomized, placebo-controlled trial. *Arthritis Rheum*, 1996, Vol. 39, pp. 1808-1817.
13. de Jesus Raposo, M.F., de Morais, R.M.S.C., de Morais, A.M.M.B., Health applications of bioactive compounds from marine microalgae. *Life Sci*, 2013, Vol. 93, pp. 479-486.
14. Mohan, A., Misra, N., Srivastav, D., Umapathy, D., Kumar, S., *Spirulina*, the nature's wonder: A review. *Lipids*, 2014, Vol. 5, pp. 7-10.
15. Kapoor, R., Nair, H., Gamma linolenic acid oils. *Bailey's Industrial Oil and Fat Products*, 2005.
16. Goffman, F.D., Galletti, S., Gamma-linolenic acid and tocopherol contents in the seed oil of 47 accessions from several *Ribes* species. *J Agric Food Chem*, 2001, Vol. 49, pp. 349-354.
17. Cohen, Z., Didi, S., Heimer, Y.M., Overproduction of γ-linolenic and eicosapentaenoic acids by algae. *Plant physiol*, 1992, Vol. 98, pp. 569-572.
18. Sharathchandra, K., Rajashekhar, M., Total lipid and fatty acid composition in some freshwater cyanobacteria. *J Algal Biomass Utln*, 2011, Vol. 2, pp. 83-97.
19. Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., Commercial applications of microalgae. *J Biosci Bioeng*, 2006, Vol. 101, pp. 87-96.
20. Grima, E.M., Pérez, J.S., Camacho, F.G., Medina, A.R., Giménez, A.G., Alonso, D.L., The production of polyunsaturated fatty acids by microalgae: from strain selection to product purification. *Proce Biochem*, 1995, Vol. 30, pp. 711-719.
21. Soltani, N., Latifi, AM., Alnajar, N., Dezfulian, M., Shokarvi, S., Heydari, M., et al., Biochemical and physiological characterization of three *Microalgae* spp. as candidates for food supplement. *J Appl Biotechnol Rep*, 2016, Vol. 3, pp. 377-381.
22. Moazami, N., Ranjbar, R., Ashori, A., Tangestani, M., Nejad, A.S., Biomass and lipid productivities of marine microalgae isolated from the Persian Gulf and the Qeshm Island. *Biomass Bioenerg*, 2011, Vol. 35, pp. 1935-1939.
23. Mahajan, G., Kamat, M., γ-Linolenic acid production from Spirulina platensis. *Appl Microbiol Biotechnol*, 1995, Vol. 43, pp. 466-469.
24. Ronda, S.R., Lele, S., Culture Conditions stimulating high γ-Linolenic Acid accumulation by *Spirulina* platensis. *Braz J Microbiol*, 2008, Vol. 39, pp. 693-697.
25. Monteiro, M.P.C., Luchese, R.H., Absher, T.M., Effect of three different types of culture conditions on *Spirulina maxima* growth. *Braz Arch Biol Technol*, 2010, Vol. 53, pp. 369-373.
26. Moreira, S.L., Reactor design for a family production of *Spirulina* spp. and parameters determination for a *Spirulina* spp. culture. University of Porto, Master thesis, 2013,
27. Sotiroudis, T.G., Sotiroudis, G.T., Health aspects of *Spirulina* (Arthrospira) microalga food supplement. *J Serb Chem Soc*, 2013, Vol. 78, pp. 395-405.
28. Roughan, P.G., Spirulina: A source of dietary gamma-linolenic acid? *J Sci Food Agric*, 1989, Vol. 47, pp. 85-93.
29. Kozlenko, R., Henson, R., Latest scientific research on Spirulina: Effects on the AIDS virus, cancer and the immune system, 1998, inspiredliving.com
30. Ghaeni, M., Roomiani, L., Review for Application and Medicine Effects of Spirulina, Microalgae. *J Adv Agric Technol,* 2016, Vol. 3, pp. 1-6.
31. Ravi, M., De, S.L., Azharuddin, S., Paul, S.F., The beneficial effects of Spirulina focusing on its immunomodulatory and antioxidant properties. *Nutr Diet Suppl*, 2010, Vol. 2, pp. 73-83.
32. Fazilati, M., Latifi, A.M., Salavati, H., Choopani, A., Antioxidant Properties of Spirulina. *Journal of Applied Biotechnology Reports*, 2016, Vol. 3, pp. 345-351.
33. Desai, K., Sivakami, S., Spirulina: the wonder food of the 21st centry. *Asia Pacific Biotech News*, 2004, Vol. 8, pp. 1298-1302.
34. Zeweil, H., Abaza, I.M., Zahran, S.M., Ahmed, M.H., AboulEla, H.M., Saad, A.A., Effect of *Spirulina* platensis as dietary supplement on some biological traits for chickens under heat stress condition. *Asian J Biomed Pharm Sci*, 2016, Vol. 6, pp. 8-14.
35. Sulibursk a, J., Szulińska, M., Tinkov, A., Bogdański, P., Effect of *Spirulina maxima* supplementation on Calcium, Magnesium, Iron, and Zinc status in obese patients with treated hypertension. *Biol Trace Elem Res*, 2016, Vol. 173, pp. 1-6.
36. Deng, R., Chow, T.J., Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. *Cardiovasc Ther*, 2010, Vol. 28, pp. e33-e45.
37. Hirahashi, T., Matsumoto, M., Hazeki, K., Saeki, Y., Ui, M., Seya, T., Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of Spirulina platensis. *Int Immunopharmacol*, 2002, Vol. 2, pp. 423-434.

38. Kent, M., Welladsen, H.M., Mangott, A., Li, Y., Nutritional evaluation of Australian microalgae as potential human health supplements. *PloS one*, 2015, Vol. 10, pp. e0118985.
39. Gutiérrez-Salmeán, G., Fabila-Castillo, L., Chamorro-Cevallos, G., Aspectos nutricionales y toxicológicos de *Spirulina* (arthrospira). *Nutricion Hospitalaria*, 2015, Vol. 32, pp. 34-40.
40. Konícková, R., Vanková, K., Vaníková, J., Vánová, K., Muchová, L., Subhanová, I., et al., Anti-cancer effects of blue-green alga *Spirulina platensis*, a natural source of bilirubin-like tetrapyrrolic compounds. *Ann Hepatol*, 2014, Vol. 13, pp. 273-283.
41. Golmakani, M.T., Rezaei, K., Mazidi, S., Razavi, S.H., γ-Linolenic acid production by *Arthrospira platensis* using different carbon sources. *Eur J Lipid Sci Technol*, 2012, Vol. 114, pp. 306-314.
42. Colla, L.M., Bertolin, T.E., Costa, J.A.V., Fatty acids profile of *Spirulina platensis* grown under different temperatures and nitrogen concentrations. *Zeitschrift für Naturforschung C*, 2004, Vol. 59, pp. 55-59.
43. Mühling, M., Belay, A., Whitton, B.A., Variation in fatty acid composition of *Arthrospira* (*Spirulina*) strains. *J Appl Phycol*, 2005, Vol. 17, pp. 137-146.
44. Hirano, M., Mori, H., Miura, Y., Matsunaga, N., Nakamura, N., Matsunaga, T., γ-Linolenic acid production by microalgae. *Applied biochemistry and biotechnology*, 1990, Vol. 24, pp. 183-191.
45. Bhakar, R., Brahmdutt, B., Pabbi, S., Total lipid accumulation and fatty acid profiles of microalga *Spirulina* under different nitrogen and phosphorus concentrations. *Egypt J Biol*, 2014, Vol. 16, pp. 57-62.
46. van Rijn, J., Shilo, M., Nitrogen limitation in natural populations of cyanobacteria (*Spirulina* and *Oscillatoria* spp.) and its effect on macromolecular synthesis. *Appl Environ Microbiol*, 1986, Vol. 52, pp. 340-344.
47. Ronda, S.R., Bokka, C.S., Ketineni, C., Rijal, B., Allu, P.R., Aeration effect on *Spirulina platensis* growth and γ-linolenic acid production. *Braz J Microbiol*, 2012, Vol. 43, pp. 12-20.
48. Sajilata, M., Singhal, R., Kamat, M., Fractionation of lipids and purification of γ-linolenic acid (GLA) from *Spirulina platensis. Food Chem*, 2008, Vol. 109, pp. 580-586.
49. Jubie, S., Dhanabal, S., Chaitanya, M., Isolation of methyl gamma linolenate from *Spirulina platensis* using flash chromatography and its apoptosis inducing effect. *BMC complement Altern Med*, 2015, Vol. 15, pp. 263.
50. Jubie, S., Dhanabal, P., Azam, M.A., Muruganantham, N., Kalirajan, R., Elango, K., Synthesis and characterization of some novel fatty acid analogues: A preliminary investigation on their activity against human lung carcinoma cell line. *Lipids Health Dis*, 2013, Vol. 12, pp. 45.
51. Yao, C.H., Be, J.W., Zer, R.Y., Cheng, C.W., Koo, M., Optimization of a continuous preparation method of *Arthrospira platensis* γ-linolenic acid by supercritical carbon dioxide technology using response surface methodology. *Sains Malaysiana*, 2015, Vol. 44, pp. 1739-1744.