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LETTER

Chlorella vulgaris as a Source of Essential Fatty Acids and Micronutrients: A Brief Commentary

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Abstract: Polyunsaturated fatty acids (PUFAs) comprise about 35-40% of the total lipid content from green algae *Chlorella*, reaching up to 24% linoleic acid and 27% α-linolenic acid in *C. vulgaris*. Also, microalgae nutrient composition may be modulated by changes in the culture medium, increasing fatty acid and microelement concentrations in the algae biomass. PUFAs, such as α-linolenic (n-3) and linoleic (n-6) acids, as well as its derivatives, are considered essential for dietary consumption, and their ability to regulate body chemistry has been recently explored in depth. A balanced fatty acid consumption is shown to counteract the negative effects of western diets, such as chronic inflammation and glucose intolerance. In this brief commentary, technological and practical uses of *C. vulgaris* are explored as means to improve dietary quality and, ultimately, human health.

Keywords: *Chlorella*, *C. vulgaris*, Polyunsaturated fatty acids, Food disorders, Obesity, Oxidative stress.

1. INTRODUCTION

1.1. Data Collection and Review Design

Data collection was carried out using indexed electronic databases (e.g. PubMed, LILACS and PubMed Central) and archives from the following university libraries: Central Library of the Federal University of the State of Rio de Janeiro (UNIRIO) and Central Library of the Federal University of Rio de Janeiro (UFRJ). Search for printed and electronic periodic articles was carried out using the following keywords: "polyunsaturated fatty acids", "Chlorella", "*Chlorella vulgaris*", "obesity" and "nutritional disorders", including the terms of lexical proximity and articles written in the English or Portuguese languages.

Due to the large number of initial documents (n > 5498), a software (Zotero Standalone 4.0) was used to organize and select references. Considering the methodological limitations of this commentary, we have adapted our search procedures from Freitas *et al.* (2015) and from the PRISMA (i.e. Preferring Reporting Items for Systematic Reviews and Meta-Analyses) norms and standards, allowing systematic search, optimization of analysis and discussion of the data obtained [1]. Table **1** compiles the selected works to provide an overview of data on *Chlorella* microalgae and its effects on health.

1.2. Obesity and Fatty Acids

Human struggle for gathering food on early social formations resulted in reproductive success, complex societies and the subsequent development of culture, mythology, gender specialization of work and other socially hereditary traits. In the last 10.000 years, our species has dominated virtually all habitable land on earth, and two centuries of intense scientific/technologic development ensured the control of food production, storage and transport, in a way that

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most individuals from developed and developing countries have access to their daily essential nutrient needs.

Despite the benefits of social integration, urban conglomerates now regularly exceed the consumption of basic nutrient needs through a process known as overfeeding, as a consequence of both cultural and behavioral adaptations to modern life and high viability of processed food. More than 1.1 billion people worldwide are overweight, and approximately 1/3 of them have reached obesity [2]. Western countries have the highest obesity indexes, with ~ 36% adults and ~ 17% children/adolescent classified as overweight/obese in the USA [3 - 5]. Also, ~ 20% of adult population from Americas and Caribe are, at least, overweight [2]. In 2014, an estimative from the World Health Organization pointed out that 39% of adults (aged 18 y.o. or older, 38% of men and 40% of women) were overweight, which is nearly two-fold the value estimated in 1980 [6].

Table 1. Experimental works evaluating *Chlorella vulgaris* as a potential dietary resource.

Study	Purpose	Results	Conclusion	Model
Janczyk *et al.*, 2007	To investigate the nutritional value of three *C. vulgaris* products.	All products showed similar protein efficiency ratio and N-balance, with changes in protein digestibility and biological value.	Protein digestibility and biological value of *C. vulgaris* may be enhanced by ultrasonic treatment and reduced by electroporation.	Wistar rats
Queiroz *et al.*, 2016	To evaluate the effect of *C. vulgaris* on the peripheral and central responses to forced swimming stress in rats.	*C. vulgaris* reduced stress-related HPA[1] activation and stress-associated hyperglycemia.	*C. vulgaris* treatment diminished the impact of central and peripheral stressors.	Sprague–Dawley rats
Bae *et al.*, 2013	To observe the suppressive effect of a hot water extract of *C. vulgaris* on histamine-mediated allergic responses.	*Chlorella* prevented histamine release from mast cells and inhibited serum IgE overproduction by ovalbumin-immunized BALB/c mice.	*C. vulgaris* hot water extract may act as an antiallergic dietary agent.	Balb/c mice
Panahi *et al.*, 2013	To evaluate the effect of *C. vulgaris* on the burden of oxidative stress in Iranian smokers.	Six-week *Chlorella* treatment increased serum antioxidant and reduced malondialdehyde levels.	*C. vulgaris* extract significantly improves antioxidant status and attenuates lipid peroxidation in chronic cigarette smokers.	Human
Grammes *et al.*, 2013	To investigate the potential of different microbial ingredients (including *C. vulgaris*) to alleviate SBMIE[2] in Atlantic salmon.	*Chlorella*-treated fish showed healthy intestine at histopathological examination and similar to control in metabolism-associated gene expression.	*C. vulgaris* was highly effective to counteract SBMIE[2] in Atlantic salmon model.	Atlantic Salmon
Kwak *et al.*, 2012	To observe the effect of *C. vulgaris* supplementation on immune/inflammation response in healthy humans.	Eight-week supplementation with *Chlorella* increased serum concentrations of interferon-γ and interleukin-1β. NK cell activity was also augmented.	Data suggest a beneficial immunostimulatory effect of short-term *C. vulgaris* supplementation in healthy subjects.	Human
Sibi, 2015	To study the response of *Chlorella* against *Propionibacterium acnes* through microdilution and *in vitro* with human peripheral blood mononuclear cells.	Chlorella species (including *C. vulgaris*) inhibited lipase activity, influenced ROS and TNF-α production. *C. vulgaris* showed a MIC[3] value of 10 μg/ml.	*Chlorella* species has significant inhibitory activity on *P. acnes*, and modulate the inflammatory response to the pathogen.	*Propionibacterium acnes*
Ebrahimi-Mameghani *et al.*, 2014	To investigate the effect of *C. vulgaris* supplementation on liver enzymes, serum glucose and lipid profile in patients with NAFLD[4].	*C. vulgaris* improved weight, liver enzymes (i.e. ALP) and fasting blood sugar status.	C. vulgaris seems to improve fasting blood sugar and lipid profile in human subjects.	Human
Vecina *et al.*, 2014	To evaluate the prophylactic effect of *C. vulgaris* on body weight, lipid profile, blood glucose and insulin signaling in liver, skeletal muscle and adipose tissue of diet-induced obese mice.	*C. vulgaris* treatment increases the phosphorylation of IR, IRS-1 and Akt and prevents diet-induced high triglyceride, cholesterol and free fatty acid levels.	*Chlorella* modulates the deleterious effects of an experimental high-fat diet in mice.	Balb/c mice

[1]Hypothalamic–pituitary–adrenal. [2]Soybean meal-induced enteropathy. [3]Minimum inhibitory concentration. [4]Non-alcoholic fatty liver disease.

An acknowledged component of chronic obesity is inflammation, with recent evidence suggesting that perturbations in gut microbiota and permeability are the main triggers for the development of obesity-associated inflammation [7]. In this context, the balance between n-3/n-6 polyunsaturated fatty acids (PUFAs) is a key control mechanism in the production of inflammatory/anti-inflammatory mediators. While arachidonic acid (n-6 derivative) gives rise to proinflammatory eicosanoids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are n-3 derivatives, function as substrates for the synthesis of resolvins, autacoids with high anti-inflammatory and tissue-protective properties [8 - 9]. Dietary n-3/n-6 ratios of 1:6 to 1:4 have been proposed to ensure lifelong health and cardiovascular safety, and experimental studies even suggest the benefits of 1:1 ratios to improve obesity-linked inflammation and insulin resistance in rats [10].

Equilibrium between the dietary amounts of PUFAs is not easily achieved in urban societies, mainly due to the low availability of *in natura* animal or vegetable sources of n-3 fatty acids. Consumption of fish is highly associated with long-term cardiovascular health, and can optimize the availability of long-chain fatty acids in the blood of human subjects. Also, authors suggest that supplementation with fish oil may improve gestational health status (reviewed in [11]. While supplementation with fish oil is a good source for replenishing n-3 needs, there is still a demand for non-expensive, cultivable food to be included in western diets.

Chlorella unicellular green algae species, mainly *C. vulgaris*, are easy to be cultivated and behave metabolically according to the nutrients provided in the medium. Biomass obtained from *Chlorella* can be used in the industry, in food preparations, or in nutritional supplements, and data show promising results from the use of each of it [12 - 14].

One cannot, however, infer that only microalgae consumption is enough to provide sufficient dietetic PUFA levels. Known plant sources, for example, may be capable of supporting membrane turnover and renewal in health adults with modest DHA/EPA demands, but no long-term prevention of affections, such as cardiovascular events, should be claimed [11]. Fish is still the main and ideal source of PUFAs in the human diet, and the results indicating otherwise have been shown as misleading and/or inconclusive [15]. Here, *C. vulgaris* is presented as a possible complementary PUFAs source to optimize n-3/n-6 composition of the diet.

1.3. *Chlorella* is Rich in Polyunsaturated Fatty Acids

George and Mildred Burr (1929) were the first to demonstrate the essentiality of unsaturated fatty acids, specially PUFAs, when the signs of deficiency were prevented or cured by providing dietetic linoleic acid (n-6), even if compared to butter or coconut oil [16]. As previously pointed, besides being essential, PUFAs must be provided in an adequate proportion (i.e. n-3/n-6 ~1:6) to ensure life-long health status [10]. Unfortunately, current western fatty acid intake follows a trend towards higher n-6 to n-3 proportion, and epidemiologic studies indicate a consumption of ~1:16 (n-3/n-6) by these populations. While ratios of up to 1:6 or lower suggest beneficial long-term effects, 1:10 or greater proportions are indicative of future adverse consequences [17].

Chlorella have high concentrations of polyunsaturated fatty acids, and almost 1:1 proportion of n-3/n-6 PUFAs [18]. Also, analysis of fatty acid composition obtained from *C. vulgaris* indicates that, among the 19 different fatty acids found, 5 were saturated and 14 were C14 to C24 unsaturated fatty acids [19]. Bewicke and Potter (1993), in a book entitled "*Chlorella*: The Emerald Food", highlight the beneficial possibilities of including *Chlorella* in the western diets, these are: high growth rate, high protein content, resistance to climatic variations, high nutritive value and digestibility, palatability, economical production and others[20]. Recent authors, for instance, suggest the use of *Chlorella* species as biofactories for n-3 fatty acids [21, 22].

Long-chain n-3 and n-6 fatty acids comprise 35-40% of the total lipid content in algae from *Chlorella* genus, reaching up to 24% linoleic acid and 27% α-linolenic acid in *C. vulgaris* [18], which are an accessible source for these essential fatty acids. The possibility of modulation in lipid concentrations through changes in culture medium has stimulated research since the mid-twentieth century [23], either for the synthesis of biomass or to satisfy animal/human nutritional demand.

Several groups have explored methods for cultivation of lipid-rich microalgae, aiming mainly to increase biofuel productivity[24]. Some methods, however, may also show high potential application in food industry[25]. Liu, Wang and Zhou (2008) explored the effects of variable iron chloride ($FeCl_3$) chelate concentrations in augmenting total *C. vulgaris* lipid content. Data showed that 1.2×10^{-5} mol/L^{-1} $FeCl_3$ may increase fatty acid concentrations up to 56.6% of the total biomass [26].

Lv *et al.* (2010) observed that sensible variations in cultivation settings could significantly raise (2.5-fold compared to previous results) lipid content in *C. vulgaris*[27]. A growth protocol using nutrient-rich medium, followed by sudden/acute nutrient depletion, changes in air availability and light intensity induced a final 53% lipid content in total biomass from *C. vulgaris* cultures [28]. Other groups suggest that incubation under high CO_2 levels, nitrogen depletion and reduced incubation time are efficient strategies to increase total lipid mass in these cultivations [29].

Satisfactory fatty acid levels in *Chlorella* may be achieved through stimulus with several stressors, however, to generate a selective increase in PUFAs rather than monounsaturated (MUFAs) and saturated (SAFAs) fatty acids is a more demanding work. Limiting PO_4^{3-}, for example, may result in higher SAFA, MUFA and 18:2n-6 (linoleic acid) content [30]; Also, in a previous review of PUFA sources, authors highlighted *Chlorella* (*C. minutissima*) to be effective for arachidonic acid (AA) and EPA production, but not DHA [31].

Immobilization of *C. vulgaris* with the plant growth promoting bacteria (PGPB) *Azospirillum brasilense* resulted in a large increase of the lipid content from *C. vulgaris*, with approximately 95-98% of fatty acids with 16-18 carbon chains at the final *Chlorella* mass [32]; increasing CO_2 concentration to 2.6% (v/v of culture environment), in turn, promoted a 6-fold positive change in lipid production, with higher intracellular acetyl-CoA content, which is pivotal for fatty acid synthesis [33]. Finding adequate methods for selectively increasing SAFA, MUFA or PUFA content in *C. vulgaris* and other *Chlorella* species may favor the standardization of these microalgae in the food industry.

1.4. Dietary Use and Effects of *Chlorella* on Health

Beside its nutritional value, *C. vulgaris* was also shown to modulate immune mechanisms and to counteract the growth of cancerous cells. When provided as food for senescent humans or model animals, *C. vulgaris* protected both from the development of age-associated diseases, specially hypertension and hyperlipidemia[25], and the general response to stress. Sprague-Dawley rats supplemented (by gavage) with *C. vulgaris* (50 or 200mg/Kg/b.w.), for instance, showed diminished peripheral and central responses to forced swimming stress tests, as seen through smaller corticotropin-releasing factor and c-fos expression levels [34].

Bae *et al.* (2013) investigated the effects of *C. vulgaris* aqueous extracts on *in vitro* immuno-allergic responses using rat peritoneal mast cells, and *in vivo* through evaluation of plasma markers. Data showed that the aqueous extract is capable of suppressing histamine release via modulation of T helper 1 (Th1) activity, thus attenuating allergenic responses in these animals [35]. In addition, *C. vulgaris* dry extracts may modulate oxidative damage in chronically stressed individuals. A study providing 3600 mg/day (six weeks) dry *C. vulgaris* extract to non-comorbidity bearing smokers showed significant decrease in lipid peroxidation and optimized antioxidant status of participants[36]. Other *Chlorella* species (*C. pyrenoidosa*) were also shown to be beneficial for the treatment of signs and symptoms, specifically of fibromyalgia, hypertension, and ulcerative colitis. In this study, patients were supplemented daily with 10 g of *Chlorella* in tablets plus 100 mL of a liquid *Chlorella* extract for 2 to 3 months in a double-blind, placebo-controlled, randomized clinical trial [37].

When supplemented in the diet of Atlantic salmon (*Salmo salar* L.), *C. vulgaris* attenuated *in vivo* gut inflammatory symptoms [38], suggesting applicability in the treatment of human gastrointestinal diseases, such as Crohn's disease and hypersensitivity to prolamins (e.g. wheat gliadin, commonly known as gluten). In a randomized, double-blind clinical trial, Kwak *et al.* (2012) showed that supplementation with *C. vulgaris* can optimize innate immune response, stimulating the activity of Natural Killer cells and raising the concentration of interleukins associated to defense against pathogens [39]. In addition, detectable methylcobalamin (i.e. vitamin B12) [40] and high phosphorus [41] levels were found in *C. vulgaris* samples, again pointing towards the relevant nutritional value of *Chlorella* microalgae.

In a recent *in vitro* study, Sibi (2015) evaluated the antimicrobial activity of lipidic extracts from *Chlorella* microalgae (including *C. vulgaris*) against *Propionibacterium acnes* strains. Data showed that *Chlorella* promotes inhibitory effects over *P. acnes* through attenuation of lipase activity. Also, *Chlorella* extracts modulated oxidative and inflammatory responses from human peripheral blood mononuclear cells stimulated by heat-killed *P. acnes* [19]. Sun *et al.* (2014) proposed the use of selenium-enriched *C. vulgaris* cultures as means to induce bioaccumulation, producing antioxidant-rich biomass with applications in agriculture and human diet. Data showed that *C. vulgaris* is satisfactorily tolerant to selenium accumulation in culture [42].

As indicated by previous studies, *Chlorella* may be used as a dietary antioxidant [36, 42]. In a recent work, patients (n=30) with non-alcoholic liver steatosis were supplemented with 400 mg/day vitamin E and 1200 mg/day *C. vulgaris* extract. The combination was shown to be effective in reducing body mass, activity of liver enzymes, fasting glucose and controlling lipid profile [43]. These data corroborate with the previous results from Vecina *et al.* (2014), where rodents supplemented with *C. vulgaris* for twelve weeks showed tolerance to high-fat diets, reduced triglyceride, cholesterol and free fatty acid levels. Authors suggest that *C. vulgaris* is capable of modulating signaling pathways associated to insulin [14].

Also, data show that *C. vulgaris* may be incorporated to food products, such as pasta, thus enhancing both nutritional and sensorial quality without affecting processing [44]. *Chlorella* may also be added to cookies [45], yellow layer cake [46], imitation processed cheese [47], and others, without modifying sensorial/nutritional properties. Some *Chlorella* substitutions may significantly cheapen the food price, when compared with the original preparation. Increasing nutritional value of food with low cost is relevant, and may be decisive for permanence in the market.

1.5. Toxicity of *Chlorella*

No studies directly linking *Chlorella* ingestion to toxicity or chronic health risks were found. It suggests, however, that the risk of intake by susceptible populations was also not evaluated, implying that pregnant, immunosuppressed, infants and older individuals should avoid using any phytotherapic drug and/or supplement without previous medical prescription, as it is recommended for any other plant extract without complete evidence for safety/effectivity.

Chlorella, however, may be exposed to contamination and bioaccumulation (e.g. Zn^{2+} and Cd^{2+}) like that of plant crops [48], once cultivable algae is highly susceptible to changes in nutrient composition. Alam *et al.* (2015) showed that *C. vulgaris* may accumulate 80% Zn^{2+} and 60% Cd^{2+} from culture medium, efficiently cleaning contaminated water [49]. *C. vulgaris* was also shown to accumulate arsenic when inoculated in rice crops, thus attenuating metal levels in the plant [50]. When cultivated for feeding purposes, however, it's important to know not only the composition of *Chlorella*, but also the microorganisms potentially cohabitating in the nutritive environment.

Cultivated algae, similar to plants, are relatively permissive to the growth of microorganisms, which may be lately consumed by the human population. Data suggests that algal symbiotic bacteria, especially *Pseudomonas* sp., may grow and develop a mutualistic relationship with *C. vulgaris* (ATCC 13482) under photoautotrophic conditions, and that *Chlorella* may benefit from the presence of these organisms in culture medium, as suggested by the increase in algae cell and chlorophyll concentrations in culture [51].

In a previous literature review, Safi *et al.* (2014) pointed out that, despite numerous health benefits and rich nutrient composition, *C. vulgaris* and other algae are used mainly as nutraceuticals rather than as/in food products due to the lack of a common regulatory legislation capable of stablishing quality and good practice requirements for microalgae cultivation (see [52]). These procedures, when sufficiently regulated by law, may represent an important step in the introduction of safely grown microalgae in the diet of western populations.

CONCLUSION

Chlorella vulgaris is regularly used as food, nutritional supplement, soil nutrient for plants and in the synthesis of biomass. Unfortunately, western countries tend to consume lower amounts of microalgae than Asian nations. One reason for such is the low variety of *Chlorella* food products available in the market. The growth of soy and soy-derivative products, however, may stimulate the industry to apply processed algae into a myriad of formulations, once it has the advantage of being highly susceptible to manipulation in its centesimal composition, specially referring to lipid content.

Application of *C. vulgaris* in the food industry is not new, however, it is usually consumed in larger amounts by countries where western diet is uncommon. Developing palatable *Chlorella*-enriched food may increase total PUFA content in food and ultimately optimize the proportion of essential fatty acids in the diet, ensuring improvements in several aspects of health, such as plasma lipids, insulin sensitivity, immune response and inflammation.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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REFERENCES

[1] Freitas HR, Pereira AS, Ramos TS. The effects of acute/chronic glutamine and glutamine peptide supplementation on the performance and immune function in young active adult athletes. Curr Nutr Food Sci 2015; 11(4): 315-22. [http://dx.doi.org/10.2174/1573401311666150729225554]

[2] Haslam DW, James WP. Obesity. Lancet 2005; 366(9492): 1197-209.
[http://dx.doi.org/10.1016/S0140-6736(05)67483-1] [PMID: 16198769]

[3] Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 2012; 307(5): 491-7.
[http://dx.doi.org/10.1001/jama.2012.39] [PMID: 22253363]

[4] Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. JAMA 2012; 307(5): 483-90.
[http://dx.doi.org/10.1001/jama.2012.40] [PMID: 22253364]

[5] Ogden CL, Carroll MD, Kit BK, Flegal KM, *et al.* Prevalence of obesity among adults: United States, 2011–2012. JAMA 2014; 311(8): 806-14.
[http://dx.doi.org/10.1001/jama.2014.732] [PMID: 24570244]

[6] World Health Organization (WHO). Global status report on noncommunicable diseases 2014. WHO Library Cataloguing-in-Publication Data.

[7] Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol 2015; 3(3): 207-15.
[http://dx.doi.org/10.1016/S2213-8587(14)70134-2] [PMID: 25066177]

[8] Calder PC. Fatty acids and inflammation: the cutting edge between food and pharma. Eur J Pharmacol 2011; 668(September)(Suppl.1): S50-8.
[http://dx.doi.org/10.1016/j.ejphar.2011.05.085] [PMID: 21816146]

[9] Hsiao HM, Sapinoro RE, Thatcher TH, *et al.* A novel anti-inflammatory and pro-resolving role for resolvin D1 in acute cigarette smoke-induced lung inflammation. PLoS One 2013; 8(3): e58258.
[http://dx.doi.org/10.1371/journal.pone.0058258] [PMID: 23484005]

[10] Liu HQ, Qiu Y, Mu Y, *et al.* A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats. Nutr Res 2013; 33(10): 849-58.
[http://dx.doi.org/10.1016/j.nutres.2013.07.004] [PMID: 24074743]

[11] Williams CM, Burdge G. Long-chain n-3 PUFA: plant v. marine sources. Proc Nutr Soc 2006; 65(1): 42-50.
[http://dx.doi.org/10.1079/PNS2005473] [PMID: 16441943]

[12] Xu H, Miao X, Wu Q. High quality biodiesel production from a microalga *Chlorella* protothecoides by heterotrophic growth in fermenters. J Biotechnol 2006; 126(4): 499-507.
[http://dx.doi.org/10.1016/j.jbiotec.2006.05.002] [PMID: 16772097]

[13] Shim JY, Shin HS, Han JG, *et al.* Protective effects of *Chlorella vulgaris* on liver toxicity in cadmium-administered rats. J Med Food 2008; 11(3): 479-85.
[http://dx.doi.org/10.1089/jmf.2007.0075] [PMID: 18800895]

[14] Vecina JF, Oliveira AG, Araujo TG, *et al. Chlorella* modulates insulin signaling pathway and prevents high-fat diet-induced insulin resistance in mice. Life Sci 2014; 95(1): 45-52.
[http://dx.doi.org/10.1016/j.lfs.2013.11.020] [PMID: 24333277]

[15] Nichols PD, Kitessa SM, Abeywardena M. Commentary on a trial comparing krill oil versus fish oil. Lipids Health Dis 2014; 13: 2.
[http://dx.doi.org/10.1186/1476-511X-13-2] [PMID: 24383554]

[16] Smith W, Mukhopadhyay R. Essential fatty acids: the work of George and Mildred Burr. J Biol Chem 2012; 287(42): 35439-41.
[http://dx.doi.org/10.1074/jbc.O112.000005] [PMID: 23066112]

[17] Alarcón G, Roco J, Medina A, Van Nieuwenhove C, Medina M, Jerez S. Stearoyl-CoA desaturase indexes and n-6/n-3 fatty acids ratio as biomarkers of cardiometabolic risk factors in normal-weight rabbits fed high fat diets. J Biomed Sci 2016; 23: 13.
[http://dx.doi.org/10.1186/s12929-016-0235-6] [PMID: 26792598]

[18] Petkov G, Garcia G. Which are fatty acids of the green alga *Chlorella*? Biochem Syst Ecol 2007; 35: 281-5.
[http://dx.doi.org/10.1016/j.bse.2006.10.017]

[19] Sibi G. Inhibition of lipase and inflammatory mediators by *Chlorella* lipid extracts for antiacne treatment. J Adv Pharm Technol Res 2015; 6(1): 7-12.
[http://dx.doi.org/10.4103/2231-4040.150364] [PMID: 25709963]

[20] Bewicke D, Potter BA. 1993.

[21] Adarme-Vega TC, Lim DK, Timmins M, Vernen F, Li Y, Schenk PM. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Fact 2012; 11: 96.
[http://dx.doi.org/10.1186/1475-2859-11-96] [PMID: 22830315]

[22] Ferreira SP, Holz JC, Lisboa CR, Costa JA. Fatty acid profile of *Chlorella* biomass obtained by fed batch heterotrophic cultivation. Int Food Res J 2017; 24(1): 284-91.

[23] Harris RV, James AT. The fatty acid metabolism of *Chlorella vulgaris*. Biochim Biophys Acta 1965; 106(3): 465-73.
[http://dx.doi.org/10.1016/0005-2760(65)90063-9] [PMID: 5881329]

[24] Feng Y, Li C, Zhang D. Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium. Bioresour Technol 2011; 102(1): 101-5.
[http://dx.doi.org/10.1016/j.biortech.2010.06.016] [PMID: 20620053]

[25] Janczyk P, Franke H, Souffrant WB. Nutritional value of *Chlorella vulgaris*: Effects of ultrasonication and electroporation on digestibility in rats. Anim Feed Sci Technol 2007; 132: 163-9.
[http://dx.doi.org/10.1016/j.anifeedsci.2006.03.007]

[26] Liu ZY, Wang GC, Zhou BC. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. Bioresour Technol 2008; 99(11): 4717-22.
[http://dx.doi.org/10.1016/j.biortech.2007.09.073] [PMID: 17993270]

[27] Lv JM, Cheng LH, Xu XH, Zhang L, Chen HL. Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions. Bioresour Technol 2010; 101(17): 6797-804.
[http://dx.doi.org/10.1016/j.biortech.2010.03.120] [PMID: 20456951]

[28] Mujtaba G, Choi W, Lee CG, Lee K. Lipid production by *Chlorella vulgaris* after a shift from nutrient-rich to nitrogen starvation conditions. Bioresour Technol 2012; 123: 279-83.
[http://dx.doi.org/10.1016/j.biortech.2012.07.057] [PMID: 22940330]

[29] Widjaja A, Chien C, Ju Y. Study of increasing production from fresh water microalgae *Chlorella vulgaris*. J Taiwan Inst Chem Eng 2009; 40: 2013-4.
[http://dx.doi.org/10.1016/j.jtice.2008.07.007]

[30] Chia MA, Lombardi AT, Melão MdaG, Parrish CC. Lipid composition of *Chlorella vulgaris* (Trebouxiophyceae) as a function of different cadmium and phosphate concentrations. Aquat Toxicol 2013; 128-129: 171-82.
[http://dx.doi.org/10.1016/j.aquatox.2012.12.004] [PMID: 23306106]

[31] Abedi E, Sahari MA. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci Nutr 2014; 2(5): 443-63.
[http://dx.doi.org/10.1002/fsn3.121] [PMID: 25473503]

[32] Leyva LA, Bashan Y, Mendoza A, de-Bashan LE. Accumulation of fatty acids in *Chlorella vulgaris* under heterotrophic conditions in relation to activity of acetyl-CoAcarboxylase, temperature, and co-immobilization with Azospirillum brasilense [corrected]. Naturwissenschaften 2014; 101(10): 819-30.
[http://dx.doi.org/10.1007/s00114-014-1223-x] [PMID: 25129521]

[33] Jose S, Suraishkumar GK. High carbon (CO2) supply leads to elevated intracellular acetyl CoA levels and increased lipid accumulation in *Chlorella vulgaris*. Algal Res 2016; 19: 307-15.
[http://dx.doi.org/10.1016/j.algal.2016.08.011]

[34] Souza Queiroz J, Marín Blasco I, Gagliano H, *et al. Chlorella vulgaris* reduces the impact of stress on hypothalamic-pituitary-adrenal axis and brain c-fos expression. Psychoneuroendocrinology 2016; 65: 1-8.
[http://dx.doi.org/10.1016/j.psyneuen.2015.12.002] [PMID: 26685709]

[35] Bae MJ, Shin HS, Chai OH, Han JG, Shon DH. Inhibitory effect of unicellular green algae (*Chlorella vulgaris*) water extract on allergic immune response. J Sci Food Agric 2013; 93(12): 3133-6.
[http://dx.doi.org/10.1002/jsfa.6114] [PMID: 23426977]

[36] Panahi Y, Mostafazadeh B, Abrishami A, *et al.* Investigation of the effects of *Chlorella vulgaris* supplementation on the modulation of oxidative stress in apparently healthy smokers. Clin Lab 2013; 59(5-6): 579-87.
[PMID: 23865357]

[37] Merchant RE, Andre CA. A review of recent clinical trials of the nutritional supplement *Chlorella pyrenoidosa* in the treatment of fibromyalgia, hypertension, and ulcerative colitis. Altern Ther Health Med 2001; 7(3): 79-91.
[PMID: 11347287]

[38] Grammes F, Reveco FE, Romarheim OH, Landsverk T, Mydland LT, Øverland M. *Candida utilis* and *Chlorella vulgaris* counteract intestinal inflammation in Atlantic salmon (Salmo salar L.). PLoS One 2013; 8(12): e83213.
[http://dx.doi.org/10.1371/journal.pone.0083213] [PMID: 24386162]

[39] Kwak JH, Baek SH, Woo Y, *et al.* Beneficial immunostimulatory effect of short-term *Chlorella supplementation*: enhancement of natural killer cell activity and early inflammatory response (randomized, double-blinded, placebo-controlled trial). Nutr J 2012; 11: 53.
[http://dx.doi.org/10.1186/1475-2891-11-53] [PMID: 22849818]

[40] Kumudha A, Selvakumar S, Dilshad P, Vaidyanathan G, Thakur MS, Sarada R. Methylcobalamin--a form of vitamin B12 identified and characterised in *Chlorella vulgaris*. Food Chem 2015; 170: 316-20.
[http://dx.doi.org/10.1016/j.foodchem.2014.08.035] [PMID: 25306351]

[41] Tokusoglu O, Uunal MK. Biomass nutrient profiles of three microalgae: Spirulina platensis, *Chlorella vulgaris*, and *Isochrisis galbana*. J Food Sci 68: 4.

[42] Sun X, Zhong Y, Huang Z, Yang Y. Selenium accumulation in unicellular green alga *Chlorella vulgaris* and its effects on antioxidant enzymes and content of photosynthetic pigments. PLoS One 2014; 9(11): e112270.
[http://dx.doi.org/10.1371/journal.pone.0112270] [PMID: 25375113]

[43] Ebrahimi-Mameghani M, Aliashrafi S, Javadzadeh Y, AsghariJafarabadi M. The effect of *Chlorella vulgaris* supplementation on liver enzymes, serum glucose and lipid profile in patients with non-alcoholic fatty liver disease. Health Promot Perspect 2014; 4(1): 107-15.
[PMID: 25097844]

[44] Fradique M, Batista AP, Nunes MC, Gouveia L, Bandarra NM, Raymundo A. Incorporation of Chlorella vulgaris and Spirulina maxima biomass in pasta products 2010.

[45] Bang BH, Kim KP, Jeong EJ. Quality characteristics of cookies that contain different amounts of *Chlorella* powder. Korean J Food Preserv 2013; 20(6): 798-804.
[http://dx.doi.org/10.11002/kjfp.2013.20.6.798]

[46] Kim KJ, Chung HC. Quality characteristics of yellow layer cake containing different amounts of *chlorella* powder. Korean J Food Cookery Sci 2010; 26(6): 860-5.
[http://dx.doi.org/10.5851/kosfa.2010.30.5.860]

[47] Shalaby SM, Yasin NM. Quality characteristics of croissant stuffed with imitation processed cheese containing microalgae *Chlorella vulgaris* biomass. World J Dairy Food Sci 2013; 8(1): 58-66.

[48] Roy M, McDonald LM. Metal uptake in plants and health risk assessments in metal-contaminated smelter soils. Land Degrad Dev 2015; 26: 785-92.
[http://dx.doi.org/10.1002/ldr.2237]

[49] Alam MA, Wan C, Zhao XQ, Chen LJ, Chang JS, Bai FW. Enhanced removal of Zn(2+) or Cd(2+) by the flocculating *Chlorella vulgaris* JSC-7. J Hazard Mater 2015; 289: 38-45.
[http://dx.doi.org/10.1016/j.jhazmat.2015.02.012] [PMID: 25704433]

[50] Upadhyay AK, Singh NK, Singh R, Rai UN. Amelioration of arsenic toxicity in rice: Comparative effect of inoculation of *Chlorella vulgaris* and Nannochloropsis sp. on growth, biochemical changes and arsenic uptake. Ecotoxicol Environ Saf 2016; 124: 68-73.
[http://dx.doi.org/10.1016/j.ecoenv.2015.10.002] [PMID: 26473328]

[51] Guo Z, Tong YW. The interactions between *Chlorella vulgaris* and algal symbiotic bacteria under photoautotrophic and photoheterotrophic conditions. J Appl Phycol 2014; 26: 1483.
[http://dx.doi.org/10.1007/s10811-013-0186-1]

[52] Safi C, Zebib B, Merah O, Pontalier PY, Vaca-Garcia C. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. Renew Sustain Energy Rev 2014; 35: 265-78.
[http://dx.doi.org/10.1016/j.rser.2014.04.007]