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Metabolites of interest for food technology produced by microalgae from the Northeast Brazil[1]

Metabólitos de interesse à tecnologia de alimentos produzidos por microalgas do Nordeste do Brasil

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ABSTRACT - There is an increasing demand for bioprospection focusing on microalgae isolated from the northeastern region of Brazil with potential importance for food industries. To attend that need, we evaluated the characteristics of 12 regional species of microalgae grown under controlled cultivation conditions (temperature = 24 ± 1 ºC, illumination 150 µmol photons m^{-2} s^{-1}, photoperiod of 12 h) in terms of their nutritional quality and lipid profiles. Significant differences in growth characteristics and chemical compositions were observed among the species investigated. High carbohydrate contents (> 25 g 100 g^{-1}) were recorded in various strains of *Chlorococcum* and the marine microalga *Amphidinium carterae*; high protein contents (> 35 g 100 g^{-1}) were observed in *Scenedesmus acuminatus* and *Pediastrum tetras*; and high lipid contents (> 25 g 100 g^{-1}) in *A. carterae* and some strains of *Chlorococcum* sp. (cf. *hypnosporum*). *Chlamydomonas* sp. demonstrated the greatest production of carotenoids (64.92 mg g^{-1}), chlorophyll-a (234.74 mg g^{-1}), and chlorophyll-b (59.34 mg g^{-1}). The lipid profiles of *Chlorella* cf. *minutissima*, four strains of *Chlorococcum* sp. (cf. *hypnosporum*), *P. tetras*, *Planktothrix isothrix*, and S. *acuminatus* indicated the presence of palmitic, oleic (ω-9), linoleic (ω-6) and α-linolenic (ω-3) acids, with more than 50% omegas in the total composition of their fatty acids. In terms of chemical nutrients, the microalgae cited were found to be potential sources of omegas, carotenoids, and chlorophylls that could be used in food industries.

Key words: Polyunsaturated fatty acids. Carotenoids. Omegas. Microalgae cultivation.

RESUMO - Existe uma demanda por pesquisas prospectivas de microalgas isoladas da região Nordeste do Brasil que possam ser potencialmente importantes à indústria de alimentos. Nesta pesquisa foram avaliadas as características da cinética de crescimento sob condições controladas de cultivo (temperatura = 24 ± 1 ºC, iluminação 150 µmol fótons m^{-2} s^{-1}, fotoperíodo de 12 h), a qualidade nutricional e o perfil lipídico de doze espécies regionais de microalgas visando suprir esta lacuna. Diferenças nas características de crescimento e na composição química foram observadas nas espécies pesquisadas, com maiores teores de carboidratos (> 25 g 100 g^{-1}) registrados em várias cepas de *Chlorococcum* e na microalga marinha *Amphidinium carterae*, de proteína (> 35 g 100 g^{-1}) em *Scenedesmus acuminatus* e *Pediastrum tetras* e de lipídios (> 25 g 100 g^{-1}) em *A. carterae* e em algumas cepas de *Chlorococcum* sp. (cf. *hypnosporum*). A *Chlamydomonas* sp. apresentou os maiores teores de carotenoides (64,92 mg g^{-1}), clorofila-a (234,74 mg g^{-1}) e clorofila-b (59,34 mg g^{-1}). O perfil lipídico evidenciou a presença dos ácidos palmítico, oleico (ω-9), linoleico (ω-6) e α-linolênico (ω-3), com mais de 50% de ômegas em sua composição total de ácidos graxos, em *Chlorella* cf. *minutissima*, em quatro cepas de *Chlorococcum* sp. (cf. *hypnosporum*), *P. tetras*, *Planktothrix isothrix* e *S. acuminatus*. No que concerne aos nutrientes químicos foi observado que as microalgas citadas são fontes potenciais de produção de ômegas, carotenoides e clorofilas para serem utilizados na indústria de alimentos.

Palavras-chave: Ácidos graxos poli-insaturados. Carotenoides. Ômegas. Cultivo de microalga.

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INTRODUCTION

Recent research has analyzed the chemical compositions of microalgae and certified their positive contributions to human health and possible uses as food resources (ANDRADE *et al*., 2018; HAYES *et al*., 2018; SATHASIVAM; KI, 2018). Theoretically, microalgae are capable of producing more lipids than any other conventional crop, and numerous species can synthesize considerable quantities of essential fatty acids (EFA), especially omega-3 (ω-3) and 6 (ω-6) (the two most abundant), as well as α-linolenic acid (ALA, C18:3 ω-3) and linoleic acid (AL, C18:2 ω-6) (BELLOU *et al*., 2016; HO *et al*., 2014) – both precursors in the human body to long chain (≥ C20) polyunsaturated fatty acids (PUFA) (RINCÓN-CERVERA *et al*., 2016). Those microorganisms could be used as sustainable sources of EFA for human consumption or for use in animal rations as replacements for fish oil, which is now their principal source (RYCKEBOSCH *et al*., 2014).

Western diets are currently excessive in terms of ω-6, but deficient in ω-3, resulting in a imbalance in the ω-6:ω-3 ratio that can interfere in the conversion of ω-3 ALA into eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). That lack of conversion can result in increased levels of arachidonic acid (AA, C20:4 ω-6) in the phospholipid membranes that, over time, result in the excessive production of pro-inflammatory eicosanoids and the consequent hardening and contraction of blood vessels, increasing pain transmission, immunosuppression and, the pathogenesis of cardiovascular, inflammatory, and autoimmune diseases, as well as cancer – as increasing levels of ω-3 exert a suppressor effect (RUBIO-RODRÍGUEZ *et al*., 2010; SUBASH-BABU; ALSHATWI, 2018). There is not yet a consensus, however, in respect to optimal ω-6: ω-3 ratios, nor sufficient evidence to define the maximum tolerable dose, although some researchers suggest that the ratios between those acids should lie between 4-5:1, but never above 10:1 (CANDELA; LÓPEZ; KOHEN, 2011; WARNER *et al*., 2017).

The present work sought to characterize and compare the lipid profiles of 12 regional strains of microalgae isolated from marine and Different freshwater environments in northeastern Brazil to determine if they produce significant levels of omegas ω-3, ω-6, and ω-9 and could serve as alternative sources of compounds of interest to the food industry. The nutritional qualities of those algal strains were also investigated in terms of their percentage contents of carbohydrates, proteins, lipids, carotenoid pigments and chlorophyll-a and b, as well as their growth characteristics in mono-specific cultures under controlled conditions.

MATERIALS AND METHODS

Biomass production and growth characteristics

Twelve regional strains of microalgae maintained that the Microalgae Collection of the Laboratory of Reef Environments and Biotechnology with Microalgae (LARBIM/UFPB) were investigated, including 11 freshwaters and one marine species. All of the strains were isolated from distinct aquatic environments in northeastern Brazil: nine from Paraíba State (PB), one from Bahia State (BA), one from Pernambuco State (PE), and one from Rio Grande do Norte State (RN) (Table 1).

The species were cultivated in triplicate in flat bottomed 6 L flasks containing 5 L of Conway media (WALNE, 1966) for the cultivation of the marine microalga, or 5 L of Zarrouk medium (ZARROUK, 1966) for cultivating *Chamydomonas*, *Pediastrum*, and *Scenedesmus*, or WC medium (GUILLARD; LORENZEN, 1972) for cultivating the other freshwater strains. The microalgae were grown in culture chambers (24 ± 1 ºC) under a light intensity of approximately 150 µmol photons m^{-2} s^{-1}, furnished by 40 W fluorescent lamps, under a 12 h photoperiod, with continuous forced air (0.1 L min^{-1}) injection.

Culture growth was accompanied by measuring "in vivo" fluorescence (Turner Design Fluorometer) and by cell counts using Fuchs–Rosenthal or Sedgewick-Rafter chambers (for filamentous forms) as viewed under a Leica binocular microscope. Growth curves were prepared for each species, allowing calculations of their growth velocity (*k*), expressed as the number of cell divisions per day (STEIN 1973), the duration (in days) of their log phase, maximum cell density (MCD), culture time, final biomass yield, and biomass productivity per day. Upon reaching their respective stationary phases, the cultures were interrupted and the biomasses produced were concentrated by centrifuging (at 18 ºC), frozen at -30 °C, and subsequently lyophilized. The dry biomasses were weighed using an analytical balance and maintained under refrigeration (9 ºC) until analyzed.

Chemical analyses

Determinations (in triplicate) were made of: proteins, following Lowry *et al*. (1951); carbohydrates, following Kochert (1978); total lipids, following Folch, Lees and Stanley (1957); total carotenoids, following Strickland and Parsons (1968); and chlorophyll-a and b, following Jeffrey and Humphrey (1975). The fatty acid profiles of the species were determined using gas chromatography, following Menezes *et al*. (2013), and quantified by normalization of the areas of the methyl esters and expressing them as percentage areas (%).

Table 1 - List of the microalgae investigated in the present project, citing their respective taxonomic groups and origins

Microalgae	Taxonomic groups	Origen
Marine species		
Amphidinium carterae M18C	Dinoflagellate	Praia do Cabo Branco, João Pessoa-PB. Extraído da esponja Cynachrella sp
Freshwater species		
Chlamydomonas sp. D132WC	Chlorophyceae	Vale do Capão, Cachoeira da Purificação, Chapada Diamantina-BA
Chlorella cf. *minutissima* D101Z	Chlorophyceae	ETE Mangabeira Calha Parshal, João Pessoa-PB
Chlorococcum sp. (cf. *hypnosporum*) D28Z	Chlorophyceae	Açude do Cais, Cuité-PB
Chlorococcum sp. (cf. *hypnosporum*) D37Z	Chlorophyceae	Açude do Cais, Cuité-PB
Chlorococcum sp. (cf. *hypnosporum*) D65Z	Chlorophyceae	Lagoa do Hotel Eco Resort do Cabo, Cabo de Santo Agostinho-PE
Chlorococcum sp. (cf. *hypnosporum*) D76Z	Chlorophyceae	Rio Quinturaré, Frei Martinho-PB
Chlorococcum sp. D106Z	Chlorophyceae	Tanque dos porcos, Frei Martinho-PB
Pediastrum tetras D121WC	Chlorophyceae	Prainha, Frei Martinho-PB
Planktothrix isothrix D39Z	Cyanobacteria	Açude de Acauã, Itatuba-PB
Scenedesmus acuminatus D115WC	Chlorophyceae	Bebedouro das ovelhas, Frei Martinho-PB
Synechococcus nidulans D112Z	Cyanobacteria	Açude Quixaba, Caicó-RN

Statistical analyses

All of the data obtained from the analyses were submitted to statistical treatments using *Statistica* 7.0 software, at a 5% level of significance. The homoscedasticity of the variances of all of the variables analyzed were confirmed using the Levene test. The differences among the variables analyzed among the species were compared using one-way ANOVA and the Tukey HSD *a posteriori* test.

RESULTS AND DISCUSSION

Kinetic growth characteristics

The kinetic growth characteristics of the microalgae species investigated here are listed in Table 2. The statistical analyses verified significant differences in terms of their maximum cell density values (MCD) ($F = 363,7311$; $gl = 11$; $p<0.01$; Table 2), final yields ($F = 907,6998$; $gl = 11$; $p<0.01$; Table 2), and biomass productivity ($F = 1294,2997$; $gl = 11$; $p<0.01$; Table 2). The growth velocities (*k*) did not significantly differ between the different species analyzed ($F = 0.7561$; $gl = 11$; $p = 0.6802$; Table 2).

Not all of the species that demonstrated high *k* values produced large quantities of biomass or rapidly reached their log phase. Similarly, not all of the species with large k values produced the highest cell concentrations (Table 2). Those data indicated that each species responded differently to the culture conditions, and reinforced the importance of determining the unique behaviors of the different strains potentially useful for biotechnological applications under controlled conditions. Significant differences were observed between clones of the same species, such as clones D28Z, D37Z, D65Z and D76Z of *Chlorococcum* sp. (cf. *hypnosporum*) (Table 2), which demonstrated wide variations in their kinetic growth characteristics - reinforcing the idea that various factors (possibly genetic) can affect microalgae growth in addition to the physical and/or chemical conditions in the environment.

The values encountered here corroborated data published by other authors in terms of the biomass productivities of various microalgae. The maximum biomass production of 98.73 mg L^{-1} d^{-1} for the chlorophyte *Chlorococcum* sp. D106Z seen here was greater than that reported by Rodolfi *et al.* (2009) (21.8 mg L^{-1} d^{-1}). Those authors also evaluated *S. acuminatus*, and encountered values greater than those reported here (35.1-53.9 mg L^{-1} d^{-1}). Chiu *et al*. (2008) cultivated *C. minutissima* and obtained a biomass productivity of 143 mg L^{-1} d^{-1}, while Nakanishi *et al*. (2014) reported the productivity of *Chlamydomonas* sp. as 169.1 mg L^{-1} d^{-1}, both values greater than those reported here. The data encountered in the literature for different microalgae confirm the concept that different intrinsic and extrinsic factors can alter the kinetics of microalgae growth.

Chemical analyses

Carbohydrate analyses revealed values varying between 14.50 g 100 g^{-1} of biomass (*S. acuminatus*

Table 2 - Kinetic characteristics of the growth of the 12 microalgae species investigated

Species	k	Log phase (days)	CT (days)	MCD (cells mL^{-1} 10^5)	Final yields (mg L^{-1})	Biomass productivity (mg L^{-1} day^{-1})
M18C	0.83 a ± 0.23	6	19	23.92 d ± 5.66	435 h ± 5.90	22.89 h ± 0.31
D132WC	0.72 a ± 0.45	5	19	43.48 d ± 7.12	536 f ± 9.45	28.21 g ± 0.49
D101Z	0.57 a ± 0.36	10	26	46.73 d ± 8.55	1140 a ± 32.5	43.85 d ± 1.25
D28Z	0.54 a ± 0.12	5	19	68.07 d ± 5.90	756 d ± 3.68	39.79 e ± 0.19
D37Z	0.50 a ± 0.16	3	11	20.25 d ± 0.39	538 f ± 10.36	48.91 c ± 0.94
D65Z	0.48 a ± 0.11	2	17	142.80 c ± 10.10	890 c ± 5.20	52.35 b ± 0.30
D76Z	0.71 a ± 0.22	6	15	25.90 d ± 2.03	640 e ± 12.21	42.67 d ± 0.81
D106Z	0.50 a ± 0.16	3	11	407.99 b ± 19.09	1086 b ± 25.63	98.73 a ± 2.33
D121WC	0.63 a ± 0.17	5	21	7.54 e ± 0.68	486 g ± 7.96	23.14 h ± 0.38
D39Z	0.64 a ± 0.15	5	21	38.20 d ± 3.86	1110 ab ± 21.3	52.86 b ± 1.01
D115WC	0.58 a ± 0.19	5	13	20.13 d ± 0.57	452 gh ± 3.78	34.77 f ± 0.29
D112Z	0.69 a ± 0.28	5	13	788.17 a ± 68.59	632 e ± 12.3	48.62 c ± 0.95
p- value	0.6802			6.72E-24	1,22E-28	1,75E-30

K = growth velocities; CT = cultivatione time, MCD = maximum cell density., M18C = *Amphidinium carterae* M18C, D132WC = *Chlamydomonas* sp. D132WC, D101Z = *Chlorella* cf. *minutissima* D101Z, D28Z = *Chlorococcum* sp. (cf. *hypnosporum*) D28Z, D37Z = *Chlorococcum* sp. (cf. *hypnosporum*) D37Z, D65Z = *Chlorococcum* sp. (cf. *hypnosporum*) D65Z, D76Z = *Chlorococcum* sp. (cf. *hypnosporum*) D76Z, D106Z = *Chlorococcum* sp. D106Z, D121WC = *Pediastrum tetras* D121WC, D39Z = *Planktothrix isothrix* D39Z, D115WC = *Scenedesmus acuminatus* D115WC, D112Z = *Synechococcus nidulans* D112Z; *k*, CT, MCD, yields and productivity values expressed as means with standard errors. Means followed by the same letters in the same column do not statistically differ (ANOVA and Tukey Test, p≥0.05)

D115WC) to 53.81 g 100 g^{-1} (*Chlorococcum* sp. D106Z), with expressive values (greater than 25 g 100 g^{-1}) noted for *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z, D37Z, D65Z and D76Z and for the microalga *A. carterae* M18C (F = 250.4351; gl = 11; p<0.01; Table 3).

In terms of carbohydrates, Kiran *et al.* (2015) reported values inferior to those encountered here with *Chlorococcum* sp. when exposed to different culture media nitrogen concentrations, and they obtained yields of 18 g 100 g^{-1} in culture medium containing 100 mg of sodium nitrate per liter (equivalent to the nitrogen concentration in the Zarrouk medium used here). The yields obtained in the present work for that microalga were greater, varying from 26.32 to 53.81 g 100 g^{-1}. The carbohydrate contents of microalgae are important not only in terms of producing supplements and rations for animals, but also for the production of biofuels through fermentation (CHEW *et al*., 2017).

The species studied here showed statistically different protein concentrations (F=73,1832; gl=11; p<0.01; Table 3). The species *S. acuminatus* D115WC (37.73 g 100 g^{-1}) and *P. tetras* D121WC (35.40 g 100 g^{-1}) had the highest protein concentrations, while *P. isothrix* D39Z (22.99 g 100 g^{-1}) and *Chlorococcum* sp. D106Z (24.84 g 100 g^{-1}) had the lowest. Expressive values (greater than 30 g 100 g^{-1}) were encountered in the marine microalga *A. carterae* M18C and in the freshwater chlorophytes *Chlamydomonas* sp. D132WC and *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z and D65Z. It is important to note that carbohydrate and protein contents were significantly different between the D28Z and D37Z clones of *Chlorococcum* sp. (cf. *hypnosporum*) that had been isolated from the same locality, showing that different lineages of the same species can demonstrate significant differences in their metabolisms.

Lipid analyses demonstrated differences between the species investigated (F = 76,4348; gl = 11; p<0.01, Table 3), with the highest lipid percentages being found in *A. carterae* M18C and *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z, D37Z, and D65Z. Those results were similar to those described by Mahapatra and Ramachandra (2013) for *Chlorococcum* sp. (30.55 g 100 g^{-1}), by Ho *et al.* (2014) for *Chlamydomonas* sp. (15.3 g 100 g^{-1}), and by Lemahieu *et al*. (2013) for *Chlorella* (14.7 g 100 g^{-1}). It is important to note, however, that culture conditions and the phase of development at harvesting can be manipulated to direct microalgae metabolism to produce certain desired metabolites. Nitrogen depletion, for example, will force microalgae metabolism to diminish protein or peptide concentrations but increase the percentages of energy-rich compounds such as carbohydrates and lipids, or polysaccharides and fatty acids (HO *et al*., 2014).

Table 3 - Carbohydrate, protein, and lipid contents in the biomasses of the 12 species of microalgae investigated

Species	Carbohydrate (g 100 g^{-1})	Protein (g 100 g^{-1})	Lipid (g 100 g^{-1})
M18C	29.31 c ± 0.87	31.60 c ± 1.14	29.78 a ± 0.95
D132WC	15.93 e ± 1.84	34.55 b ± 1.41	15.48 ef ± 0.50
D101Z	16.13 e ± 1.40	25.95 de ± 0.22	18.26 de ± 2.46
D28Z	27.62 c ± 0.41	34.01 bc ± 0.18	26.34 ab ± 1.83
D37Z	33.59 b ± 0.79	28.00 d ± 0.69	25.27 abc ± 1.19
D65Z	26.32 c ± 1.71	32.72 bc ± 0.93	26.05 ab ± 2.46
D76Z	33.15 b ± 1.66	27.36 de ± 0.70	22.91 bcd ± 2.04
D106Z	53.81 a ± 0.59	24.84 ef ± 0.52	5.99 h ± 0.01
D121WC	21.93 d ± 1.67	35.40 ab ± 0.72	12.96 fg ± 1.00
D39Z	17.44 e ± 0.46	22.99 f ± 0.50	21.02 cd ± 1.35
D115WC	14.50 e ± 1.24	37.73 a ± 1.76	6.97 h ± 1.99
D112Z	17.77 e ± 0.80	27.34 de ± 1.27	9.48 gh ± 1.50
p- value	5.67E-22	1.04E-15	6.34E-16

M18C = *Amphidinium carterae* M18C, D132WC = *Chlamydomonas* sp. D132WC, D101Z = *Chlorella* cf. *minutissima* D101Z, D28Z = *Chlorococcum* sp. (cf. *hypnosporum*) D28Z, D37Z = *Chlorococcum* sp. (cf. *hypnosporum*) D37Z, D65Z = *Chlorococcum* sp. (cf. *hypnosporum*) D65Z, D76Z = *Chlorococcum* sp. (cf. *hypnosporum*) D76Z, D106Z = *Chlorococcum* sp. D106Z, D121WC = *Pediastrum tetras* D121WC, D39Z = *Planktothrix isothrix* D39Z, D115WC = *Scenedesmus acuminatus* D115WC, D112Z = *Synechococcus nidulans* D112Z; Values expressed as means with standard errors. Means followed by the same letters in the same column do not statistically differ (ANOVA and Tukey Test, $p \geq 0.05$).

Significant differences (F=146,0379; gl=11; p<0.01; Table 4) were also observed between the 12 species in terms of their productions of carotenoids, with minimum and maximum amounts of those compounds being produced by *Chlorococcum* sp. D106Z (1.57 mg g^{-1}) and *Chlamydomonas* sp. D132WC (64.92 mg g^{-1}) respectively.

In terms of chlorophyll-a and b production, *Chlorococcum* sp D106Z demonstrated significantly lower concentrations (3.61 mg g^{-1} of chlorophyll-a and 1.18 mg g^{-1} of chlorophyll-b) than *Chlamydomonas* sp. D132WC (234.74 and 59.34 mg g^{-1} respectively) (chlorophyll-a: F = 191,8190; gl = 11; p<0.01 and chlorophyll-b: F = 77,0232; gl = 8; p<0.01; Table 4).

According to Bouman *et al.* (2018), the photosynthetic pigments present in microalgae reflect chromatic adjustments needed to maximize energy capture under distinct solar irradiation conditions. From a human health point of view, carotenoids (including both carotenes and xanthophylls) act as antioxidants, providing protection against oxidative stress (SATHASIVAM; KI, 2018). Some xanthophylls, such as violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and lutein occur both in microalgae and higher plants, although microalgae also produce different types of xanthophylls, such as loroxanthin, astaxanthin, and canthaxanthin (synthesized by green algae), and diatoxanthin, diadinoxanthin, and fucoxanthin (synthesized by brown algae or diatoms) (BARREDO, 2012). Chlorophylls likewise have beneficial effects for human health due to their anticancer properties and anti-inflammatory and anti-oxidant activities, and can help prevent arteriosclerosis as well as atherothrombotic cardiovascular diseases (PEMMARAJU *et al*., 2018).

Fatty acid compositions

The fatty acid profiles of the microalgae examined here are presented in Tables 5 and 6. Fatty acids with carbon chains varying from C8 to C24, both saturated and unsaturated (with 1 to 6 unsaturated bonds), were identified. The most frequent fatty acids were: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1, ω-9), linoleic (AL, C18:2 ω-6), α-linolenic (ALA, C18:3 ω-3), and stearidonic (STD, C18:4 ω-3). Among those, palmitic acid stood out as the principal saturated fatty acid (SFA), with the microalgae examined producing that fatty acid at concentrations varying from 11.3% (*Chlorococcum* sp. (cf. *hypnosporum*) D65Z) to 38.0% (*S. nidulans* D112Z) of all fatty acid methyl esters (FAME) produced. The same pattern of palmitic acid predominance was reported by Nakanishi *et al*. (2014) for *Chlamydomonas* sp., by Ambrozova *et al*. (2014) for eight microalgae, and by Campos *et al*. (2010) for 10 species of marine microalgae.

The unsaturated fatty acids (USFA) present in all of the microalgae studied were oleic (ω-9), AL (ω-6), and ALA (ω-3), with percentages varying from 2% (*A. carterae*

Table 4 - Carotenoid and chlorophyll-a and b contents in the biomasses of the 12 microalgae species investigated

Species	Carotenoid (mg g^{-1})	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})
M18C	17.64 cd ± 4.40	102.57 b ± 6.52	ND
D132WC	64.92 a ± 3.94	234.74 a ± 23.22	59.34 a ± 7.76
D101Z	13.47 de ± 1.88	42.53 cd ± 5.14	15.89 c ± 2.13
D28Z	21.02 c ± 1.57	56.84 c ± 3.17	22.18 c ± 4.93
D37Z	12.26 de ± 1.88	37.99 cd ± 5.07	14.92 c ± 2.22
D65Z	13.30 de ± 0.89	33.51 d ± 2.57	15.53 c ± 1.07
D76Z	10.40 e ± 2.13	30.31 d ± 5.36	13.03 cd ± 2.56
D106Z	1.57 f ± 0.19	3.61 f ± 0.27	1.18 e ± 0.37
D121WC	10.48 e ± 2.91	23.96 de ± 1.84	4.65 de ± 0.64
D39Z	8.68 e ± 1.39	29.89 d ± 1.51	ND
D115WC	34.79 b ± 2.64	97.71 b ± 5.17	34.61 b ± 2.42
D112Z	13.99 de ± 1.11	25.79 de ± 4.11	ND
p- value	3.32E-19	1.33E-20	2,43345E-12

ND = not determined, M18C = *Amphidinium carterae* M18C, D132WC = *Chlamydomonas* sp. D132WC, D101Z = *Chlorella* cf. *minutissima* D101Z, D28Z = *Chlorococcum* sp. (cf. *hypnosporum*) D28Z, D37Z = *Chlorococcum* sp. (cf. *hypnosporum*) D37Z, D65Z = *Chlorococcum* sp. (cf. *hypnosporum*) D65Z, D76Z = *Chlorococcum* sp. (cf. *hypnosporum*) D76Z, D106Z = *Chlorococcum* sp. D106Z, D121WC = *Pediastrum tetras* D121WC, D39Z = *Planktothrix isothrix* D39Z, D115WC = *Scenedesmus acuminatus* D115WC, D112Z = *Synechococcus nidulans* D112Z; Values expressed as means with standard errors. Means followed by the same letters in the same column do not statistically differ (ANOVA and Tukey Test, $p \geq 0.05$)

M18C) to 32.5% (*P. tetras* D121WC), 0.3% (*A. carterae* M18C) to 30% (*P. isothrix* D39Z), and 1.7% (*P. isothrix* D39Z) to 34% (*Chlorococcum* sp. (cf. *hypnosporum*) D28Z) of the total FAME produced respectively. The species that produced the highest levels of oleic acid were *Chlorococcum* sp. (cf. *hypnosporum*) strains D37Z and D76Z, *C. minutissima* D101Z, *S. acuminatus* D115WC, *P. tetras* D121WC, and *Chlamydomonas* sp. D132WC. In terms of AL, the highest levels were observed in *P. isothrix* D39Z, *Chlorococcum* sp. (cf. *hypnosporum*) D65Z, *Chlorococcum* sp. D106Z, and *S. nidulans* D112Z. In terms of ALA, the species that demonstrated the highest concentrations were *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z, D37Z, D65Z, and D76Z, *C. minutissima* D101Z, and *Chlorococcum* sp. D106Z (Tables 5 and 6). Humans do not synthesize ALA or AL as they do not produce the enzymes Δ-12 and Δ-15 desaturase, and those acids (precursors of ω-3 and ω-6 respectively) must be obtained in our diet (LEE *et al*., 2016).

Radmann and Costa (2008) analyzed *S. nidulans* and reported low percentages of AL (2.71%) and AA (0.49%), and slightly higher percentages of ALA (7.61%). Evaluations of *Chlamydomonas* sp. cultivated at different nitrate concentrations by Ho *et al.* (2014) showed percentages of oleic acid varying from 9.1-26.6%, of AL varying from 21.7-25.3%, and of ALA varying from 14.4-5.4%. Campos, Barbarino and Lourenço (2010) reported 4.7% AL, 17.5% ALA, and 0.8% EPA in *C. minutissima*. Mahapatra and Ramachandra (2013) reported 1.59%, 14.3% and 7.07% of oleic acid, AL, and ALA in *Chlorococcum* sp respectively; in that same report, those same fatty acids varied between 5.2-22.5%, 8.6-30%, and 1.7-28.3%, respectively, in all of the strains of *Chlorococcum* sp.

The microalgae examined in the present work did not demonstrate good ω-6/ω-3 ratios (Table 5 and 6) when compared to the suggested healthy ratio of 4-5:1 (CANDELA; LÓPEZ; KOHEN, 2011; WARNER *et al*., 2017); even microalgae that demonstrated high concentrations of ω-6 had inadequate ratios, as observed in *Chlamydomonas* sp. D132WC (1.05), *P. isothrix* D39Z (28.24), and *S. nidulans* D112Z (2.44). The same low ω-6/ω-3 ratios were observed by Ryckebosch *et al*. (2014), varying from 0.053 to 2.0 among nine different species examined, although the ratio seen in fish oil is 0.071, indicating that even the current principal source of omegas has a low ratio of those compounds.

PUFA/SFA ratios can be used to rapidly evaluate the fatty acid profiles of the microalgae analyzed. According to Ambrozova *et al*. (2014), the larger that value, the greater will be the potential benefits to human health. The highest ratios were observed here in the species *C. minutissima* D101Z; *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z, D37Z, D65Z and D76Z, *Chlorococcum* sp. D106Z, and *P. isothrix* D39Z (Table 5). Ambrozova *et al*. (2014) analyzed the PUFA/SFA

Table 5 - Fatty acid profiles of the microalgae species cultivated (percentage values in relation to the total fatty acid methyl esters)

Fatty acid (%)	Species					
	M18C	D132WC	D101Z	D28Z	D37Z	D65Z
Caprylic, C8:0	0,20					
Pelargonium, C9:0	0,10					
Capric, C10:0			1,30			
Hendecanoic, C11:0						
Lauric, C12:0	14,36	0,80		2,00		
Lauroleic, C12:1	0,30					
Tridecyl, C13:0	0,10					
Myristic, C14:0	1,12	1,00	2,60		2,30	0,50
Myristoleic, C14:1	0,80	0,20	1,30			1,00
Pentadecylic, C15:0	0,12					
9-pentadecenoic, C15:1			0,30			
Palmitic, C16:0	36,20	29,10	16,80	20,00	16,20	11,30
Palmitoleic, C16:1	0,30	6,20	7,40		6,60	6,80
11-hexadecenoic, C16:1	1,30					
7,10-hexadecadienoic, C16:2		1,50	1,40	3,90	0,90	1,70
6,9,12-hexadecatrienoic, C16:3						
7,10,13-hexadecatrienoic, C16:3, ω-3		1,30		21,00		5,00
Margaric, C17:0	0,20		3,80			0,90
Heptadecaenoic, C17:1		5,60	8,70			5,60
Stearic, C18:0	4,60	5,00	1,00		0,50	18,10
7-octadecenoic, C18:1	1,80					
Oleic, C18:1, ω-9	2,00	20,10	23,10	3,30	17,50	5,20
Vaccenic, C18:1	9,30				0,20	1,70
Linoleic (AL), C18:2, ω-6	0,30	6,50	11,20	9,40	11,50	20,00
Gamma-linolenic, C18:3, ω-6		8,80	0,30			1,20
Alpha-linolenic (ALA), C18:3, ω-3	2,20	3,80	20,30	34,00	28,00	20,70
Stearidonic (STD), C18:4, ω-3	4,50	9,50	0,20	1,20	1,20	
Arachidic, C20:0	3,00					
Gadoleic, C20:1	0,40					
Ecoisatrienoic, C20:3, ω-9	0,90					
Arachidonic (AA), C20:4, ω-6			0,30			0,30
Eicosapentanoic (EPA), C20:5, ω-3	8,20					
Behenic, C22:0	0,50					
Docosahexaenoic (DHA), C22:6, ω-3	6,60					
Lignoceric, C24:0	0,60					
DUFA profile					3,10	
PUFA profile				5,50	12,00	
∑ ω-3	17,00	14,60	20,50	56,20	28,00	25,70
∑ ω-6	0,30	15,30	11,80	9,40	11,50	21,50
∑ ω-9	2,90	20,10	23,10	3,30	17,50	5,20
ω-6/ω-3	0,02	1,05	0,58	0,17	0,41	0,84
PUFA/SFA	0,37	0,87	1,32	3,41	2,99	1,59

M18C = *Amphidinium carterae* M18C, D132WC = *Chlamydomonas* sp. D132WC, D101Z = *Chlorella* cf. *minutissima* D101Z, D28Z = *Chlorococcum* sp. (cf. *hypnosporum*) D28Z, D37Z = *Chlorococcum s*p. (cf. *hypnosporum*) D37Z, D65Z = *Chlorococcum* sp. (cf. *hypnosporum*) D65Z

Table 6 - Fatty acid profiles of the microalgae species cultivated (percentage values in relation to the total fatty acid methyl esters)

Fatty acid (%)	Species					
		D76Z	D106Z	D121WC	D39Z	D115WC	D112Z
Caprylic, C8:0						0,30	
Pelargonium, C9:0							
Capric, C10:0							
Hendecanoic, C11:0			0,10				
Lauric, C12:0		1,50	0,40		0,20	0,30	
Lauroleic, C12:1							
Tridecyl, C13:0			0,50				
Myristic, C14:0	2,10	2,10	1,80		0,50	1,30	
Myristoleic, C14:1					0,20	1,00	
Pentadecylic, C15:0			0,20				
9-pentadecenoic, C15:1			0,60			0,30	
Palmitic, C16:0	15,40	17,40	29,70	35,00	31,50	38,00	
Palmitoleic, C16:1	7,50	1,30	1,00	6,00	1,40	17,60	
11-hexadecenoic, C16:1							
7,10-hexadecadienoic, C16:2	0,40	2,20	0,30		1,70		
6,9,12-hexadecatrienoic, C16:3		18,30	4,20				
7,10,13-hexadecatrienoic, C16:3, ω-3	0,90	1,10			1,60	1,00	
Margaric, C17:0		11,10	1,50				
Heptadecaenoic, C17:1					2,20		
Stearic, C18:0	0,80	0,70	2,80	1,90	9,80	1,10	
7-octadecenoic, C18:1							
Oleic, C18:1, ω-9	22,50	6,20	32,50	7,70	31,90	9,20	
Vaccenic, C18:1		0,80	1,40			1,00	
Linoleic (AL), C18:2, ω-6	8,60	15,30	8,20	30,00	10,90	19,30	
Gamma-linolenic, C18:3, ω-6		1,30	0,30	18,00	0,20		
Alpha-linolenic (ALA), C18:3, ω-3	28,30	19,50	11,40	1,70	7,10	6,70	
Stearidonic (STD), C18:4, ω-3	0,40	1,20	1,80		0,80	1,00	
Arachidic, C20:0			0,70				
Gadoleic, C20:1							
Ecoisatrienoic, C20:3, ω-9							
Arachidonic (AA), C20:4, ω-6	0,50					1,90	
Eicosapentanoic (EPA), C20:5, ω-3			0,60				
Behenic, C22:0							
Docosahexaenoic (DHA), C22:6, ω-3							
Lignoceric, C24:0							
DUFA profile							
PUFA profile	12,70						
∑ ω-3	29,20	21,80	13,80	1,70	9,50	8,70	
∑ ω-6	9,10	16,60	8,50	48,00	11,10	21,20	
∑ ω-9	22,50	6,20	32,50	7,70	31,90	9,20	
ω-6/ω-3	0,31	0,76	0,62	28,24	1,17	2,44	
PUFA/SFA	2,83	1,80	0,71	1,35	0,53	0,73	

D76Z = *Chlorococcum* sp. (cf. *hypnosporum*) D76Z, D106Z = *Chlorococcum* sp. D106Z, D121WC = *Pediastrum tetras* D121WC, D39Z = *Planktothrix isothrix* D39Z, D115WC = *Scenedesmus acuminatus* D115WC, D112Z = *Synechococcus nidulans* D112Z

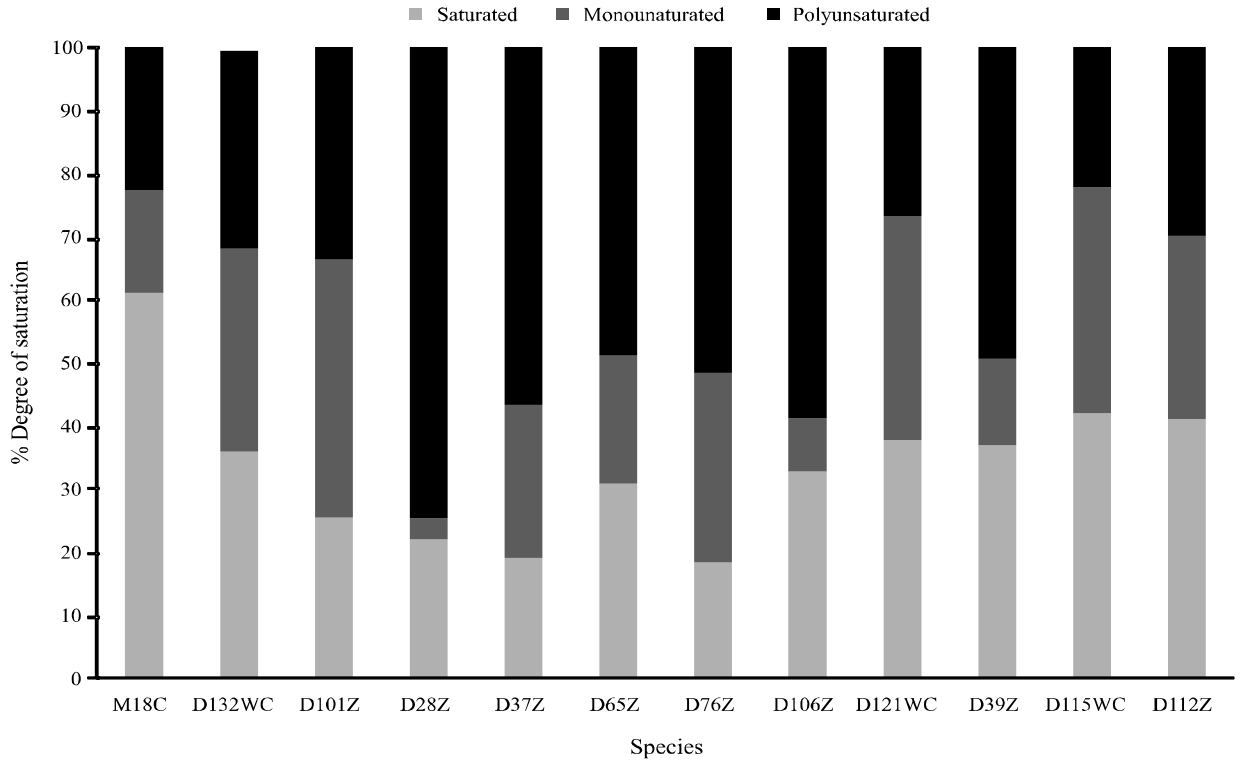
fatty acid ratios of numerous microalgae and observed variations between 0.46 and 2.13. Campos, Barbarino and Lourenço (2010) reported a ratio of 0.88 for *C. minutissima*; Mahapatra and Ramachandra (2013) reported a value of 0.32 for *Chlorococcum* sp.; Radmann and Costa (2008) reported a high value for *S. nidulans* (2.66); and Ho *et al*. (2014) reported PUFA/SFA ratios between 1.0-1.21 for *Chlamydomonas* sp.

Based on the results obtained with other species of microalgae, it is evident that differences in fatty acid profiles can be found even within the same species, indicating the existence of intraspecific differences presumably due to the fact that the environments from which they were isolated molded their metabolic natures. The ability of those same microalgae to survive under different and extreme conditions, however, demonstrates their diversity and the unique lipid profiles of those organisms (PALIWAL *et al*., 2017).

Higher concentrations of saturated and monounsaturated fatty acids and generally lower percentages of polyunsaturated fatty acids where found in the microalgae examined here (Figure 1); only *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z (75%), D37Z (56.9%), and D76Z (51.8%) and *Chlorococcum* sp. D106Z (58.9%) showed high concentrations of PUFA.

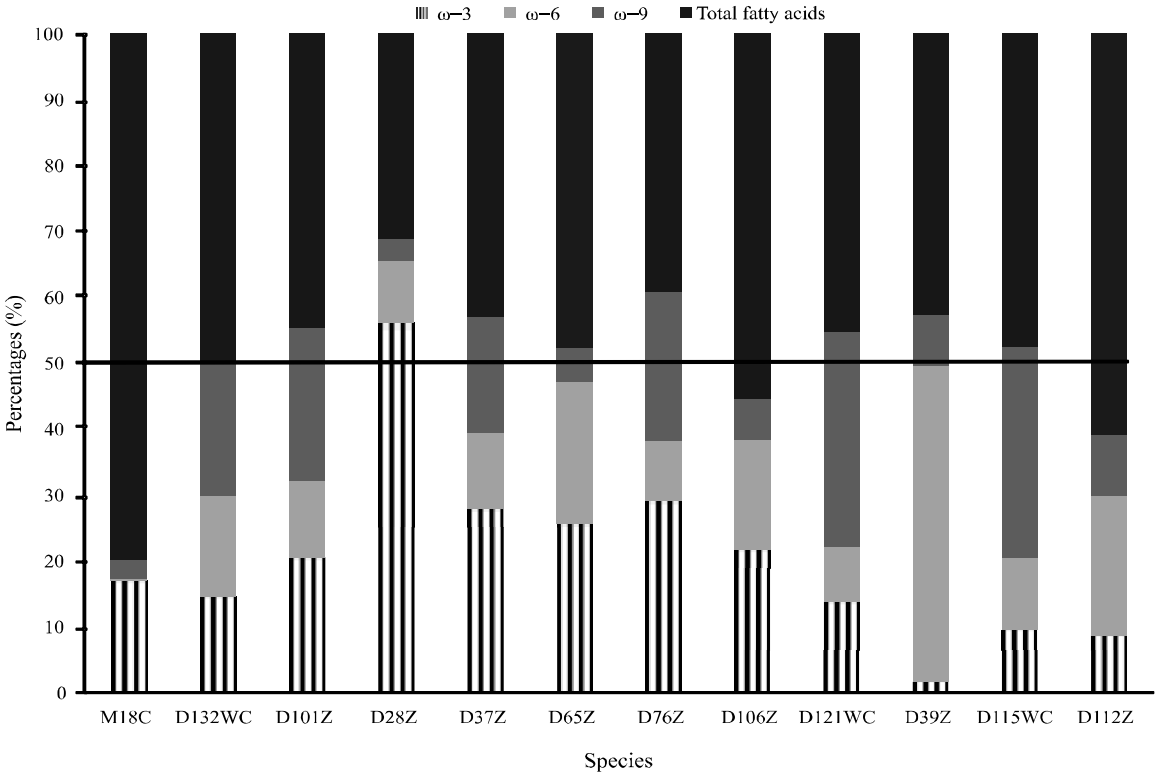
In terms of the percentages of the omegas ω-3, ω-6, and ω-9 (Figure 2), the microalgae *C. minutissima* D101Z, *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z, D37Z, D65Z and D76Z, *P. tetras* D121WC, *P. isothrix* D39Z, and *S. acuminatus* D115WC demonstrated more than 50% omegas in the composition of their FAME, with *Chlamydomonas* sp D132WC showing exactly 50% - indicating a very high potential for utilization in the food industry. Microalgae oils can also be sources of other nutritionally interesting compounds, such as carotenoids, phytosterols, and antioxidants, thus increasing the functionality of microalgae oils as compared to fish oil (RYCHEBOSCH *et al*., 2014) and aggregating value, with carotenoids acting as antioxidants to preserve the relatively unstable PUFA and thus increasing lipidic stability. Fish oil is currently considered the principal source of EFA, although fish have only limited capacities to synthesize PUFA and

Figure 1 - Percentage composition by degree of saturation of the microalgae species examined



M18C = *Amphidinium carterae* M18C, D132WC = *Chlamydomonas* sp. D132WC, D101Z = *Chlorella* cf. *minutissima* D101Z, D28Z = *Chlorococcum* sp. (cf. *hypnosporum*) D28Z, D37Z = *Chlorococcum* sp. (cf. *hypnosporum*) D37Z, D65Z = *Chlorococcum* sp. (cf. *hypnosporum*) D65Z, D76Z = *Chlorococcum* sp. (cf. *hypnosporum*) D76Z, D106Z = *Chlorococcum* sp. D106Z, D121WC = *Pediastrum tetras* D121WC, D39Z = *Planktothrix isothrix* D39Z, D115WC = *Scenedesmus acuminatus* D115WC, D112Z = *Synechococcus nidulans* D112Z

Figure 2 - Percentage of omega ω-3, ω-6, and ω-9 fatty acids based on the total percentages of fatty acids in the microalgae examined



M18C = *Amphidinium carterae* M18C, D132WC = *Chlamydomonas* sp. D132WC, D101Z = *Chlorella* cf. *minutissima* D101Z, D28Z = *Chlorococcum* sp. (cf. *hypnosporum*) D28Z, D37Z = *Chlorococcum* sp. (cf. *hypnosporum*) D37Z, D65Z = *Chlorococcum* sp. (cf. *hypnosporum*) D65Z, D76Z = *Chlorococcum* sp. (cf. *hypnosporum*) D76Z, D106Z = *Chlorococcum* sp. D106Z, D121WC = *Pediastrum tetras* D121WC, D39Z = *Planktothrix isothrix* D39Z, D115WC = *Scenedesmus acuminatus* D115WC, D112Z = *Synechococcus nidulans* D112Z

most of those compounds are bio-accumulated through the food chain (in which microalgae have a principal role) (SAWYER *et al*., 2016). As such, the possibility of using microalgae to obtain EFA appears quite promising.

CONCLUSIONS

1. Interspecific variations seen in the growth characteristics and chemical compositions of 12 microalgae indicated that each displayed a different response to standard culture conditions;

2. The species *Chlorella* cf. *minutissima* D101Z, *Chlorococcum* sp. (cf. *hypnosporum*) strains D28Z, D37Z, D65Z, and D76Z, *Pediastrum tetras* D121WC, *Planktothrix isothrix* D39Z, and *Scenedesmus acuminatus* D115WC demonstrated elevated percentages of PUFA, including ω-3, ω-6, and ω-9, as well as carotenoids and chlorophylls - nutrients vital to human health - making those species potential alternative sources of metabolites for food industries.

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