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Metabolites produced by microalgae from northeastern Brazil with potential food industry uses

Metabólitos produzidos por microalgas do nordeste brasileiro com potenciais usos na indústria alimentícia

Metabolitos producidos por microalgas del noreste de Brasil con usos potenciales en la industria alimentaria

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Abstract

The production potential of metabolites of interest to the food industry was evaluated in 17 microalgae species isolated from natural sources in northeastern Brazil. The species were cultivated to their stationary phase under controlled conditions, when the experiments were interrupted and the dry biomass harvested. We observed differences in their growth parameters, productivity, and the biochemical compositions of their biomasses, with high levels of protein productivity in *Monoraphidium litorale* D296WC (48.96%), *Kirchneriella concorta* D498WC (42.49%), *Monoraphidium griffithi* D499WC (48.37%), *Chlamydomonas sp.* D530WC (44.80%), and *Cosmarium sp cf. depressum* D578WC (49.32). The greatest carbohydrate productivities were observed in *Xanthonema sp.* D464WC (34.15%), *K. concorta* D498WC (38.95%), and *Scenedesmus acuminatus* D514WC (36.54%). The three different extraction techniques of microalgae lipids all gave slightly different results, with the method utilizing phospho-vanillin being considered the most rapid and it requires only small quantities of biomass. Unsaturated fatty acids (oleic, linoleic, and linolenic) were encountered at high levels in most of the species, especially α-linolenic acid (ω3), which reached concentrations above 30% in *Golenkinia radiata* (D325WC). Due to their high productivity, rapid

growth, and the large numbers of important dietary metabolites they produce, the species *Monoraphidium litorale* (D296WC), *Xanthonema sp.* (D464WC) and *Monoraphidium griffithi* (D499WC) show significant potential for utilization by the food industry as sources of proteins, lipids, and carbohydrates.
Keywords: Experimental cultivation; Biomass; Chemical composition.

Resumo

O potencial de produção de metabólitos de interesse para à indústria alimentícia foi avaliado em 17 espécies de microalgas isoladas de fontes naturais no nordeste do Brasil. As espécies foram cultivadas até a fase estacionária sob condições controladas, quando os experimentos foram interrompidos e a biomassa seca colhida. Observamos diferenças em seus parâmetros de crescimento, produtividade e composição bioquímica de suas biomassas, com altos níveis de produtividade protéica em Monoraphidium litorale D296WC (48,96%), Kirchneriella concorta D498WC (42,49%), Monoraphidium griffithi D499WC (48,37%), Chlamydomonas sp. D530WC (44,80%) e Cosmarium sp cf. depressão D578WC (49,32). As maiores produtividades de carboidratos foram observadas em Xanthonema sp. D464WC (34,15%), K. concorta D498WC (38,95%) e Scenedesmus acuminatus D514WC (36,54%). As três diferentes técnicas de extração de lipídios de microalgas deram resultados ligeiramente diferentes, sendo o método que utiliza a fosfovanilina considerado o mais rápido e requer apenas pequenas quantidades de biomassa. Os ácidos graxos insaturados (oleico, linoleico e linolênico) foram encontrados em níveis elevados na maioria das espécies, especialmente o ácido α-linolênico (ω3), que atingiu concentrações acima de 30% em Golenkinia radiata (D325WC). Devido à sua alta produtividade, rápido crescimento e grande número de importantes metabólitos alimentares que produzem, as espécies Monoraphidium litorale D296WC, Xanthonema sp. D464WC e Monoraphidium griffithi D499WC apresentam potencial significativo para utilização pela indústria alimentícia como fontes de proteínas, lipídios e carboidratos.
Palavras-chave: Cultivo experimental; Biomassa; Composição química.

Resumen

Se evaluó el potencial de producción de metabolitos de interés para la industria alimentaria en 17 especies de microalgas aisladas de fuentes naturales en el noreste de Brasil. Las especies se cultivaron hasta la fase estacionaria en condiciones controladas, momento en el que se interrumpieron los experimentos y se recolectó la biomasa seca. Se observaron diferencias en sus parámetros de crecimiento, productividad y composición bioquímica de su biomasa, con altos niveles de productividad proteica en *Monoraphidium costatae* D296WC (48,96 %), *Kirchneriella concorta* D498WC (42,49 %), *Monoraphidium griffithi* D499WC (48,37 %), *Chlamydomonas* sp. D530WC (44,80%) y *Cosmarium* sp *cf.* depresión D578WC (49.32). Los mayores rendimientos de carbohidratos se observaron en *Xanthonema* sp. D464WC (34,15%), *K. concorta* D498WC (38,95%) y *Scenedesmus acuminatus* D514WC (36,54%). Las tres técnicas diferentes para extraer lípidos de microalgas dieron resultados ligeramente diferentes, siendo el método que usa fosfovainillina el más rápido y que requiere solo pequeñas cantidades de biomasa. Los ácidos grasos insaturados (oleico, linoleico y linolénico) se encontraron en niveles elevados en la mayoría de las especies, especialmente el ácido α-linolénico (ω3), que alcanzó concentraciones superiores al 30% en *Golenkinia radiata* (D325WC). Debido a su alta productividad, rápido crecimiento y gran cantidad de importantes metabolitos alimentarios que producen, las especies *Monoraphidium littorale* D296WC, *Xanthonema* sp. D464WC y *Monoraphidium griffithi* D499WC tienen un potencial significativo para su uso en la industria alimentaria como fuentes de proteínas, lípidos y carbohidratos.
Palabras clave: Cultivo experimental; Biomassa; Composición química.

1. Introduction

Interest in the technological applications of microalgae has grown considerably throughout the world. Microalgae are potential sources of new products and represent one of the principal technological and economic frontiers with a real potential for satisfying commercial demands in the areas of food resources, energy, fine chemicals, cosmetics, and pharmaceuticals.

Large-scale microalgae production has already proven to be economically viable for expanding markets. The engineering technologies used in microalgae bioprocesses have advanced considerably in recent years, generating numerous consumer products. Biofuels (including bio-diesel, bio-ethanol, bio-hydrogen, and bio-methane) have been the principal areas of concentration until recently, although many species of microalgae produce other organic compounds with high economic value, such as vitamins (A and E), pigments, proteins, lipids, carbohydrates, diverse polysaccharides, esters, phycobilins, and fatty acids such as the omegas (ω-3, ω-6, ω-9). Pharmaceutical and fine chemical products have also been the focus of

numerous investigations, including the production of bioactive compounds such as antioxidants, antibiotics and toxins, as well as cosmetics (Suastes-Rivas et al., 2020).

Microalgae are easily cultivated, and many species demonstrate high photosynthetic efficiencies and high levels of biomass and triglyceride production (Calixto et al., 2016b). Within that context, interest in those microorganisms is not limited to the production of biodiesel as they demonstrate a promising future as sustainable sources of cooking oils. Some species can produce up to 100 times more oil per unit area than oleaginous plants such as soybeans or oil palms (Ras et al., 2011).

Findings like those demonstrate that the use of microalgae in the food industry has enormous potential. Some species have already been incorporated into the production of products such as bread, spaghetti, gums, drinks, and yogurts that are commercialized in a number of countries (Toker et al., 2018a), while other microalgae products demonstrate great potential for use as coloring agents, antioxidants, emulsion agents, and gels, making them valuable future sources of compounds for human and animal consumption.

The term microalgae comprises a large variety of photosynthetic unicellular organisms from distinct taxonomic groups that do not necessarily have close phylogenetic relationships. They are present in rivers, streams, lakes, estuaries, and the open sea, and can be found in humid sites on rocks and soil, as well as in arid, semiarid, and desert sites, in phytotelmata, hydrothermal basins, and growing on ice (Suastes-Rivas et al., 2020).

Estimated that there are approximately 44,000 species considered to be microalgae, most of which are diatoms –but only a relatively small number in that total have been investigated in terms of the contexts cited above. Some authors even estimate that the total diversity of microalgae may exceed 200,000 species, indicating that extremely large numbers of unknown species have not be considered for biotechnological exploitation. Additionally, it is important to note that differences in natural or artificial cultivation conditions, and the culture media itself can provoke considerable variations in the chemical components produced (Verspreet et al., 2021), indicating that a great deal of basic research must still be undertaken that could guarantee the sustainable large-scale production of desired chemicals from microalgae.

In this way, the importance of bioprospecting for microalgae native to areas that are still poorly studied, such as the Northeast region of Brazil, is highlighted. The present project, therefore, aims to determine the chemical compositions of monospecific cultures of regional microalgae cultivated in synthetic medium, characterized the fatty acids they produced and compared lipid extraction methods to discover species that have commercial potential, especially for the food industry.

2. Methodology

2.1 Study species and culture conditions

The microalgae studied here were obtained from culture collections held at the Laboratory of Reef Environments and Biotechnology of Microalgae at the Federal University of Paraíba (LARBIM/UFPB), Brazil. Those microalgae had been maintained in WC medium in culture chambers (25± 1 ºC) under an illumination of 12 hours per day (approximately 150 µmol photons m^{-2} s^{-1}) provided by 40 W florescent lamps (Guillard & Lorenzen 1972).

The species studied were identified to the lowest taxonomic level possible based on diacritical criteria focusing on the morphological characteristics of their cells, traditional phycological manuals, reference articles, and internet searches. The algae had been previously isolated from collections made in streams, rivers, animal watering tanks, reservoirs, carciniculture and fish breeding tanks, and even from drinking water filters, in the Brazilian states of Paraíba (13 species), Piauí (01), and Ceará (02), in September, November, and December/2014, and in January/2015 (Table 1).

Table 1. List of the microalgae studied and their origins. Numbers in parenthesis refer to the reference numbers in the culture bank at LARBIM/UFPB.

SPECIES	PROCEDENCIA	
Coelastrum cambricum D294WC	Riacho da Bica, Parque Arruda Câmara, João Pessoa	PB
Monoraphidium litorale D296WC	Tributário do Rio Boa Água, Conde	PB
Coelastrum astreoideum D316WC	Riacho da Bica, Parque Arruda Câmara, João Pessoa-	PB
Golenkinia radiata D325WC	Ilha do Rodeadouro, Rio São Francisco	PE
Oocystis solitaria D338WC	Bebedouro das galinhas, Bayeux	PB
Chlorella sp. D359WC	Filtro de água potável, João Pessoa	PB
Coenocystis sp. D392WC	Tributário do Rio Jacarapé, João Pessoa	PB
Xanthonema sp. D464WC	Viveiro de carcinicultura pós-despesca, Aldeia Tramataia	PB
Scenedesmus ecornis D477WC	Fonte dos Milagres, Parque Nacional de Sete Cidades	PI
Kirchneriella concorta D498WC	Açude fazenda Panati, Taperoá	PB
Monoraphidium griffithi D499WC	Açude fazenda Panati, Taperoá	PB
Scenedesmus dimorphus D514WC	Açude Ipark, Fortaleza	CE
Monoraphidium contortum D525WC	Barragem de Gramame, Conde	PB
Chlamydomonas sp. D530WC	Viveiro de carcinicultura pós-despesca, Aldeia Tramataia	PB
Kirchneriella cornuta D544WC	Tributário do Rio Jacarapé, João Pessoa	PB
Scenedesmus ecornis D545WC	Lagoa azul, Parque Nacional de Jericoacara	CE
Cosmarium sp. cf. *depressum* D578WC	Tanque de criação de Peixe, Alhandra	PB

PB= Paraíba State; PE= Pernambuco State; PI = Piauí State; CE= Ceará State. **Source:** Authors.

The study species were cultivated in triplicate in 6 L flasks containing 5 L of media, with constant aeration provided by small membrane compressors (Resun AOC2) under the same light and temperature conditions encountered when they were isolated. The basic cultivation media utilized was WC (Guillard & Lorenzen, 1972), prepared with sterile distilled water. Microalgae growth was accompanied by analysis of their *in vivo* fluorescence using a Turner 10005R fluorometer, and by cell counts in Fuchs–Rosenthal chambers, to determine the growth curve of each species. The experiments were interrupted at the beginning of the stationary phase; the biomasses produced were then concentrated in a refrigerated centrifuge at 18 ºC (2607 x g for 20 min), frozen in an ultra freezer (-30 °C), lyophilized, and subsequently weighed. The growth velocity (*k*) of each species, which represents the daily duplication rate of its cells, was calculated for the exponential phase using the Fogg & Thake (1987) formula. We also calculated the total biomass yield and biomass productivity (expressed in milligrams per liter per day of cultivation).

2.2 Chemical analyses of the biomasses and the analytical methods used

Protein contents were determined using the methodology described by (Lowry et al., 1951)), using bovine albumin as the standard; carbohydrates were determined using the Kochert (1978) method, with glucose as the standard; lipids were determined using the phospho-vanillin spectrophotometric method (using 1 mg of the lyophilized biomass) (Mishra et al., 2014) as well as the gravimetric methods of (Folch et al., 1957) and (Bligh & Dyer, 1959) (using, in both cases, 50 mg of the dry biomass).

The methyl esters of the fatty acids were prepared by direct transesterification of the biomasses of each species following Menezes et al., (2013), adapted to micro-scales. The analyses were performed by gas chromatography in a GCMS-QP2010 gas chromatograph (Shimadzu, Kioto, Japan) equipped with a Durabound DB-23 column (30 m x 0.25 mm x 0.25 μm). The injector and detector temperatures were fixed at 230 °C and the column temperature was 90 °C. The temperature gradients of the column where from 90 to 150 °C (10 °C min^{-1}), 150 to 200 °C (5 °C min^{-1}), and 200 to 230 °C (3 °C min^{-1}) with a running time of 34 minutes, using helium as the carrier gas at 187.2 mL/min.

2.3 Statistical treatments

The data obtained were submitted to statistical treatments using Statistica 7.0 software, comparing the means by the one-way ANOVA test (post-hoc Tukey-HSD). The Shapiro-Wilk test was used to examine the normality of the data, and Levene's homoscedasticity test was used to analyze variations in the parameters. The value $p<0.05$ was used to identify significant differences between the means of the parameters analyzed.

3. Results and Discussion

3.1 Microalgae growth and biomass production

The species cultivated demonstrated distinct growth patterns, as indicated by their growth velocity values and final biomass productions (Table 2). The mean values of the growth constant (*k*) values were significantly different among the different species ($F = 565.82$; Gl, 16, $p < 0.05$; Table 2), varying from 0.31 divisions/day in *Coelastrum cambricum* (D294WC) to 0.93 in *Scenedesmus dimorphus* (D514WC). The largest biomass productions were obtained with the species *Cosmarium* sp. cf. *depressum* (D578WC) and *Kirchneriella concorta* (D498WC), and the smallest production with *Coenocystis sp*. (D392WC).

Some of the species demonstrated biomass productivities similar to, or greater than, that recorded for strain D514WC (*Scenedesmus dimorphus*), even with lower growth velocities (ex. D294WC, D296WC, D464WC, D498WC, D499WC, D530WC, D545WC, and D578WC). It is notable that the species with the lowest growth rate (D294WC, *Coelastrum cambricum*) demonstrated a higher final yield and higher biomass productivity than strain D514WC.

Table 2 – Growth velocities and biomass productions of the 17 microalgae species studied. Values expressed as means and standard deviations.

SPECIES	*k*	Yield biomass (mg/L)	Productivity Biomass (mg/L/dia)
Coelastrum cambricum D294WC	$0,31^{j} \pm 0,09$	$790^{f} \pm 0,57$	65,83
Monoraphidium litorale D296WC	$0,64^{f} \pm 0,14$	$860^{c} \pm 1,73$	71,67
Coelastrum astreoideum D316WC	$0,56^{g} \pm 0,43$	$570^{m} \pm 1,52$	40,71
Golenkinia radiata D325WC	$0,37^{i} \pm 0,11$	$418^{n} \pm 2,64$	34,83
Oocystis solitaria D338WC	$0,45^{h} \pm 0,14$	$245^{p} \pm 1,00$	20,42
Chlorella sp. D359WC	$0,66^{f} \pm 0,41$	$370^{o} \pm 2,00$	26,43
Coenocystis sp. D392WC	$0,65^{d} \pm 0,07$	$190^{q} \pm 0,57$	15,83
Xanthonema sp. D464WC	$0,75^{cd} \pm 0,45$	$745^{g} \pm 1,53$	62,08
Scenedesmus ecornis D477WC	$0,46^{h} \pm 0,09$	$687^{j} \pm 2,08$	57,25
Kirchneriella concorta D498WC	$0,83^{b} \pm 0,33$	$921^{b} \pm 0,43$	76,75
Monoraphidium griffithi D499WC	$0,75^{c} \pm 0,15$	$840^{d} \pm 0,45$	70,00
Scenedesmus dimorphus D514WC	$0,93^{a} \pm 0,22$	$720^{i} \pm 1,52$	60,00
Monoraphidium contortum D525WC	$0,55^{g} \pm 0,12$	$640^{l} \pm 2,51$	53,33
Chlamydomonas sp. D530WC	$0,64^{f} \pm 0,13$	$810^{e} \pm 1,15$	67,50
Kirchneriella cornuta D544WC	$0,78^{c} \pm 0,26$	$430^{m} \pm 1,73$	30,71
Scenedesmus ecornis D545WC	$0,65^{f} \pm 0,12$	$730^{h} \pm 1,15$	60,83
Cosmarium sp cf. depressum D578WC	$0,61^{f} \pm 0,27$	$1210^{a} \pm 1,004$	100,83

Means followed by the same letters in the same column do not differ statistically (ANOVA and Tukey test, $p \geq 0.05$). Source: Authors.

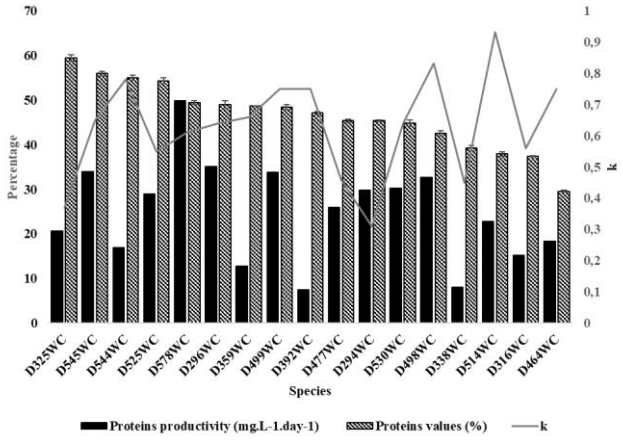
Those data indicate that each species responds differently to identical culture conditions, showing differences in their metabolism and cell physiologies, and consequently, in the products expected from their cultivated biomasses. Understanding those growth behaviors will be especially important in terms of those species demonstrating potential for biotechnological applications, principally under large-scale culturing conditions.

3.2 Biochemical compositions of the biomasses of the cultivated species

3.2.1 Proteins

Four of the microalgae species cultivated demonstrated protein contents superior to 50% of their biomasses: G*olenkinia radiata* (D325WC; 59.37%), *Monoraphidium contortum* (D525WC; 54.30% ± 0.43); K*irchneriella cornuta* (D544WC; 54.93% ± 0.53); and *Scenedesmus ecornis* (D545WC; 55.89% ± 0.55) (Figure 1). It could be observed, however, that the highest quantities of proteins produced were not necessarily related to high productivity. The species *Monoraphidium litorale* D296WC, *Kirchneriella concorta* D498WC, *Monoraphidium griffithi* D499WC, *Chlamydomonas sp.* D530WC, and *Cosmarium sp cf. depressum* D578WC demonstrated high growth rates and high protein productivities, suggesting their selection for commercial production purposes.

Figure 1. Protein contents of the 17 microalgae species studied.



Source: Authors.

High protein production levels have been recorded in various species of microalgae Verspreet et al., (2021) recorded a protein content of 45-46% in the biomass of *C. Vulgaris* and *N. gaditana*. (Calixto et al., 2016b) using different media such as biocomposts from leftover compost from fruit and vegetable foods (HB), from residues of the industrial processing of sugarcane into sucroseand and alcohol (VB), and biocompost prepared from chicken dung (BCE), obtained the highest levels of protein in VB and HB media with *Chlorella sp*., and in BCE with *Chlamydomonas* sp. and *Lagerheimia longiseta*. The protein levels of this last strain reached more than 60% in the HB medium, while the other strains presented values close to 50% in the VB medium. The other media showed levels of protein production between 30 and 55%.

Matos et al., (2015) cultivated *Chlorella* sp. in an alternative medium based on desalinization residues, and obtained protein contents of 46.80% – a value close to that recorded for *Chlorella* sp. (strain D359WC) in the present study (48.62%). Those results indicate that alternative media may represent solutions for reducing production costs.

Biomass protein compositions were different even among otherwise identical species isolated from different locations ($F=655.3447$; gl 16; $p<0.05$, Figure 1), as could be seen with *Scenedesmus ecornis* (strains: D477WC and D545WC). Those

results suggest that pre-adaptations to specific habitats will influence the chemical characteristics of a species, possibly due to the absence (or presence) of important micronutrients and trace metals in those environments.

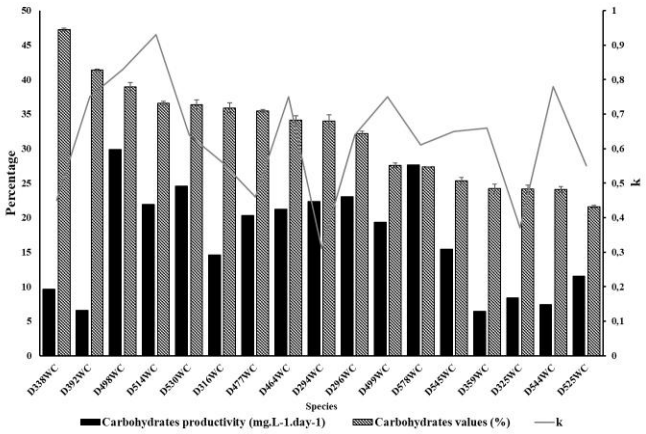
The microalgae *Monoraphidium litorale* D296WC, *Kirchneriella concorta* D498WC, *Monoraphidium griffithi* D499WC, *Chlamydomonas sp.* D530WC, and *Cosmarium sp cf. depressum* D578WC demonstrated protein contents higher than some conventional animal and plant sources, with higher protein productions than chickens (24%), fish (24%), cattle (22%), or peanuts (26%) (Moorhead & Capelli, 2011).

Those data show that many of the species evaluated in the present study could potentially be used in the food industry as alternative protein sources, although it will be necessary to select and evaluate efficient extraction processes. The use of enzymes or ultrasound have been studied to identify efficient but less aggressive techniques for rupturing the cell walls that can avoid permanent damage to the microalgae and therefore facilitate greater protein production and extraction levels (Verspreet et al., 2021).

3.2.2 Carbohydrates

Carbohydrate concentrations varied from 24.1% ± 0.65 g.100g^{-1} in *Monoraphidium contortum* (D525WC) to 47.24% ± 0.20 g.100g^{-1} in *Oocystis solitaria* (D502WC.) ($F = 638.54$; gl 16, $p < 0.05$; Figure 2). All of the species cultivated demonstrated carbohydrate concentrations >20%, with some of them having values near to, or higher than, those recorded in the literature for microalgae such as *P. purpureum* (51,2%), *C. vulgaris* (16%) (Verspreet et al., 2021). Calixto et al., 2016b) reported carbohydrate concentrations near 60 % in the chlorophytes *Chlorella sp.* and *Lagerheimia longiseta* in control medium, and values near or greater than 60% when alternative medium were used with hortifruiticulture bio-composts.

Figure 2. Carbohydrate concentrations, carbohydrate productivity, and the growth velocities of 17 species of microalgae.



Source: Authors.

Schulze et al., (2017) compared the carbohydrate levels of 46 species of microalgae, and reported 24.5% in *Coelastrum* sp. and 26.9% in *Scenedesmus acuminatus*, values below those reported for the same species tested in the present study [*Coelastrum* sp. (D294WC) (33.97%) and *Scenedesmus acuminatus* (D514WC) (36.54%)].

The species *Xanthonema sp.* D464WC (34.15%), *Kirchneriella concorta* D498WC (38.95%), and *Scenedesmus acuminatus* D514WC (36.54%) demonstrated the highest carbohydrate productions, together with the highest rates of productivity and cell growth, therefore representing promising sources of carbohydrates for large-scale harvesting.

3.2.3 Lipids

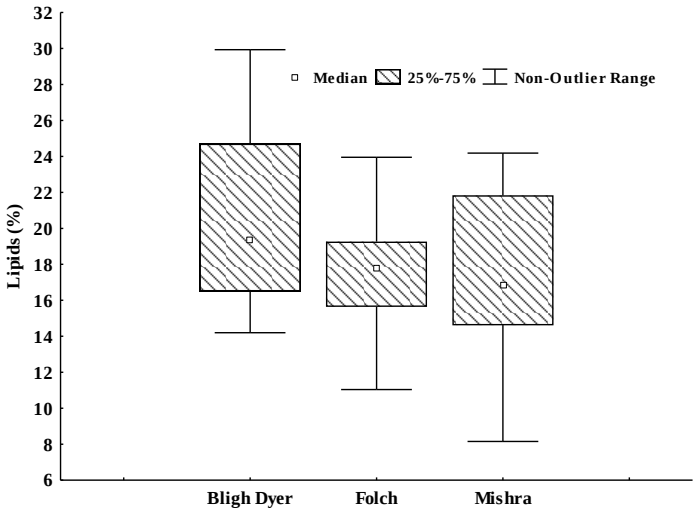
We evaluated three different techniques for extracting lipids, as alterations in lipid fraction compositions are usually observed according to the polarity of the extraction solvent. All three techniques demonstrated differences in terms of the percentages of lipids extracted from each microalga. The methods proposed by Bligh & Dyer (1959) and Mishra et al., (2014) showed the greatest extraction efficiencies in the largest numbers of species, although the species that demonstrated the highest lipid contents using the Folch et al., (1957) technique likewise yielded high concentrations with the first two methods (Table 3; Figure 3).

Table 3 – Lipid levels determined by the three extraction methods used determined in the 17 species of microalgae studied.

Espécies	Lipídios (%) Bligh & Dyer (1959)	Lipídios (%) Folch et al., (1957)	Lipídios (%) Mishra et. al., (2014)
D294WC	24,64^{c} ±0,47	19,58^{de} ±0,40	21,91^{b} ±0,21
D296WC	24,38^{c} ±0,45	23,50^{c} ±0,39	23,91^{a} ±0,30
D316WC	24,63^{c} ±0,10	16,49^{fg} ±0,33	17,54^{c} ±0,44
D325WC	14,65^{g} ±0,40	18,66^{de} ±0,20	16,66^{c} ±0,25
D338WC	17,95^{e} ±0,03	11,34^{i} ±0,43	8,98^{f} ±0,82
D359WC	22,50^{d} ±0,43	14,53^{h} ±0,35	16,49^{c} ±0,74
D392WC	16,61^{f} ±0,36	17,62^{ef} ±0,25	13,92^{de} ±0,13
D464WC	29,57^{a} ±0,34	28,30^{b} ±0,28	23,45^{ab} ±0,42
D477WC	15,60^{fg}±0,35	15,56^{g} ±0,29	14,69^{d} ±0,52
D498WC	17,58^{ef} ±0,29	15,46^{h} ±0,17	16,80^{c} ±0,73
D499WC	27,27^{b} ±0,30	29,54^{a} ±0,46	22,02^{b} ±0,14
D514WC	21,50^{d} ±0,44	17,39^{fg} ±0,22	20,99^{b} ±0,61
D525WC	26,38^{b} ±0,40	19,43^{de} ±0,36	22,19^{b} ±0,52
D530WC	14,49^{g} ±0,28	15,88^{g} ±0,07	14,35 ±0,62
D544WC	16,58^{f} ±0,21	15,51^{gh} ±0,39	13,03^{e} ±0,81
D545WC	19,43^{e} ±0,30	18,55^{e} ±0,37	16,78^{c} ±0,57
D578WC	16,62^{f} ±0,21	18,51^{e} ±0,33	16,59^{c} ±0,35

Values expressed as mean and standard deviation. In lines in capital letters, means followed by equal letters do not differ statistically (ANOVA e Teste de Tukey, $p < 0,05$). Source: Authors.

Figura 3. Comparison of lipid extraction methods in the 17 microalgae species studied.



Source: Authors.

The three lipid extraction techniques used demonstrated significant differences in lipid productions among the different species tested (F= 599.5040; gl = 16; p<0.05, Folch et al., (1959); F = 602.71; gl = 16, p<0.05, Bligh & Dyer, 1959; F = 185.57, gl = 16; p<0.05, Mishra et al., (2014) (Table 3). The technique of Bligh & Dyer (1959) yielded values between 14.49% ±0.28 for D530WC and 29.57% ±0.34 for D464WC (*Xanthonema sp.*); the method of Folch et al., (1957) gave values between 11.34 ±0.43 in D338WC and 29.54% ±0.46 in D499WC (*Monoraphidium griffithi*); the Mishra et. al., (2014) method gave values between 8.98% ±0.82 in D338WC and 23.91% ±0.30 in D296WC.

Results similar to those of the present study were reported by (Calixto et al, 2016b) in *Chlamydomonas* sp. (21.4%); Verspreet et al., (2021) too reported results similar to those of the present study in *P. purpureum* (13,2%), *C. vulgaris* (21,8%), and *C. nivalis* (22,2 %).

The lipid contents of microalgae biomasses generally vary between 20 and 50% of their dry weight, although values between 1 and 70% have been reported by some workers (Cuellar-Bermudez et al., 2015). Under stress conditions, or by otherwise altering cultivation conditions, however, many species can be induced to synthesize and accumulate high concentrations of fatty acids, reaching values minimum 68,70% of their dry weight (Verspreet et al., 2021).

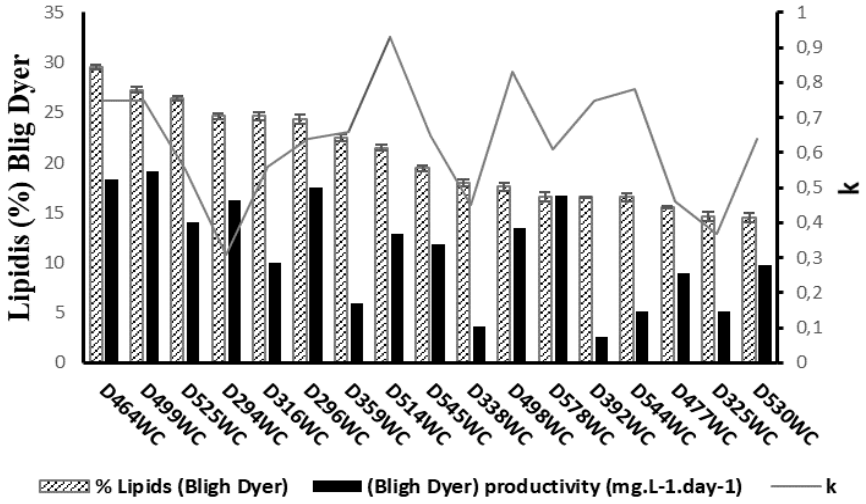
No significant differences were observed (F = 602.71; gl 16, p < 0.05) between some species of the same genus, such as D294WC (*Coelastrum cambricum)* and D316WC (*Coelastrum astreoideum*), which demonstrated lipid contents above 24% when analyzed using the Bligh & Dyer, (1959) methodology; however, when analyzed using the techniques proposed by Folch et al., (1957) and Mishra et al., (2014) those species differed significantly (F=599.50; F=185.57, gl 16, p < 0.05; Table 3), with values below 24%.

The differences in the lipid contents determined by the three different techniques are related to the types and quantities of materials extracted by the solvents utilized. The use of polar solvents to disrupt the cell wall, as occurs in the Folch et al., (1957) and Bligh & Dyer, (1959) methods, may also solubilize the neutral lipids, pigments, and other compounds of intermediate polarity Ramluckan et al., (2014). Additionally, the purification steps are quite distinct between the three techniques, which could be crucial in determining final lipid concentrations. The technique described by Mishra et al., (2014) tends to be more selective, as it involves the colorimetric identification of the lipids based on a standard curve determine using vegetable oil.

The significant differences observed between the three techniques may be related to the different types of microalgae cell walls and their different intracellular contents – so that there is no single method adequate for uniformly quantifying their lipids. The methodologies described by Bligh & Dyer, (1959) and Mishra et al., (2014) are less labor-intensive, however, especially that of Mishra et al., (2014), which also requires smaller quantities of biomass.

Even after determining the best lipid extraction techniques, it was still necessary to identify which species demonstrate the greatest lipid productivity associated with the highest growth rates. The methods of Bligh & Dyer, (1959) and Mishra et al., (2014) were found here to be the most adequate for the microalgae studied, and the species D464WC (*Xanthonema sp.*), D499WC (*Monoraphidium griffithi*), and D296WC (*Monoraphidium litorale*) demonstrated the greatest lipid productions, associated with high productivity and rapid growth (Figure 4).

Figure 4. Growth velocity (k) and lipids (percentage and productivity) analyzed by the methodologies of Blig Dyer, (1959) and Mishra et al., (2014) in the 17 species of microalgae studied.



Source: Authors.

3.2.4 Fatty acids

The 17 species analyzed demonstrated variations in their fatty acids in terms of different chain lengths (generally between 12 and 24 carbon atoms) and different levels of unsaturation (Table 4). The most frequent fatty acids were: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1, 237 ω-9), linoleic (C18:2 ω-6), and α-linolenic (C18:3 ω-3).

Palmitic acid (C16:0) was the predominant fatty acid in most of the species, with values between 18.03% in *Kirchneriella concorta* (D498WC*)* and 38.15% in *Coelastrum cambricum* (D316WC*)*, and was the principal unsaturated fatty acid (UFA) present in all of the microalgae cultivated in the present study. Stearic acid (C18:0), which is predominant in many oleaginous sources, was encountered in only small quantities in the microalgae studied here, varying between 1.81% in *Golenkinia radiata* (D325WC*)* to 3.84% in *Coelastrum astreoideum* (D316WC). Calixto et al., (2016b) reported only extremely small concentrations of that fatty acid in the microalgae they evaluated, with the exception of *Scenedesmus acuminatus*, which produced 9.8%. Stearic acid contents can be significantly elevated in some microalgae, however, such as *Cyanobacterium aponinum* (29.4%) and *Phormidium* sp. (47.6%) (both cyanobacteria) which were studied by (Karatay & Dönmez, 2011).

The unsaturated fatty acids oleic, linoleic, and linolenic were encountered in high concentrations in most of the species examined here, especially α-linolenic acid (ω3), with concentrations above 30% in *Golenkinia radiata* (D325WC). The species showing the highest concentrations of oleic acid were *Chlorela* sp. (D359WC) and *Scenedesmus ecornis* (D477WC), with 26.03% and 26.36% respectively. The greatest concentrations of linoleic acid (ω6) were encountered in *Golenkinia radiata* (D325WC) and *Xanthonema* sp. (D464WC), with 19.92% and 19.17% respectively.

Humans cannot synthesize the essential ω3 and ω6 fatty acids, and they must be obtained in one's diet. Studies suggest that humans improve their quality of life with diets containing 1:1 portions of ω-6 and ω-3. Western diets, however, show unbalanced proportions, sometimes reaching 15:1 or even 16.7:1 which can lead to a number of diverse chronic inflammations, cardiovascular diseases, cancer, and obesity (Verspreet et al., 2021). Only the species *Xanthonema* sp. (D464WC) and *Monoraphidium contortum* (D525WC) demonstrated reasonable portions of ω-6/ω-3 (1.37 and 7.35 respectively).

In terms of the degrees of saturation of the fatty acids encountered in the 17 species analyzed (Table 4), we observed greater concentrations of saturated (SFA) and mono-unsaturated fatty acids (MUFA) and lower concentrations of polyunsaturated fatty acids (PUFA). Similar results were reported by (EL Arroussi et al., 2017).

PUFA are vital components of human nutrition and are known to have numerous beneficial effects on human health. A healthy diet of PUFA, including ω3 and ω6 fatty acids, can modulate inflammatory processes and other cellular functions. Although many of the species analyzed here exhibited high levels of SFA, some species such as *Golenkinia radiata* D325WC and *Kirchneriella cornuta* D544WC also demonstrated high concentrations of PUFA

Ambrozova et al., (2014) observed that high PUFA/SFA ratios can result in significant health benefits, and those ratios can be used for rapid evaluations of fatty acid profiles when analyzing samples. Those authors reported PUFA/SFA proportions in microalgae that varied from 0.46 to 2.13. Ho et al., (2014) reported smaller ratios, varying from 1.0-1.21 in *Chlamydomonas sp*. The highest ratios in the present study were observed with D325WC *Golenkinia radiata* and D294WC *Coelastrum cambricum* (2.04% and 1.68% respectively).

According to the British Health Ministry, the PUFA/SFA lipidic profile ratios in food sources should be above 0.4 to avoid illnesses associated with the over-consumption of saturated fats (Ambrozova et al., 2014). All of the species analyzed in the present study were compatible with that recommendation. As such, the results of the present research offer excellent potential alternatives for the food industry in terms of microalgae rich in PUFA.

Table 4. Composition of fatty acids by degree of saturation of the seventeen species of microalgae studied.

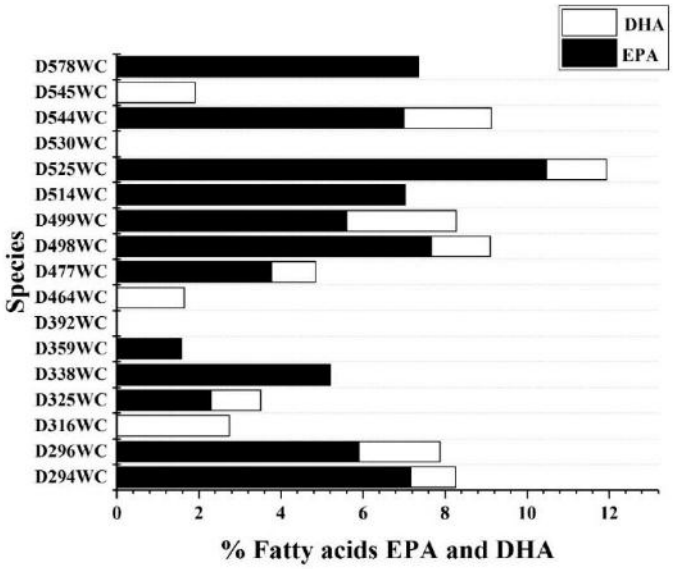
% Ácidos graxos	Espécies																
	D294WC	D296WC	D316WC	D325WC	D338WC	D359WC	D392WC	D464WC	D477WC	D498WC	D499WC	D514WC	D525WC	D530WC	D544WC	D545WC	D578WC
C6:0										7,67							
C12:0		1,02	0,96		0,49	0,50		0,60	0,36	0,70	0,62	0,34	0,68	0,55	1,03	0,65	0,90
C13:0	0,40	1,10	1,61	0,52	0,43	0,43	1,99			0,60		0,37		2,10	0,70		1,18
C14:0	1,54	2,93	3,74	1,34	1,73	1,73	3,75	2,10	1,46	1,84	2,65	1,25	1,37	4,12	2,34	6,33	2,66
C14:1	1,09	2,18	3,31	0,61	0,40	0,40	3,52	0,79	1,05	1,30	2,42	1,00		4,17	1,73	1,28	2,77
C15:0		0,62	1,06	0,46			0,85		0,39	0,60		0,36		0,63	0,94	0,93	0,88
C15:1		0,88		0,42				0,86					0,59		0,80	0,57	0,69
C16:0	20,30	22,22	38,15	20,20	29,17	29,17	35,23	29,59	27,75	18,03	29,15	24,30	20,90	35,00	21,66	31,22	33,24
C16:1	0,95	1,80	2,23	0,85	1,58	1,60	1,87	1,18	1,04	1,10	2,11	1,03	1,01	2,26	2,29	10,33	2,13
C17:0					0,55	0,55	1,46	0,90	0,67	0,90	1,02	0,45	0,67	1,13	1,09		
C17:1				0,69				0,80	3,58			2,77		2,26		0,95	
C18:0	1,84	3,18	3,84	1,81	2,71	2,70	3,32	3,55	2,78	2,38	2,35	2,30	2,20	2,75	2,90	3,25	2,75
C18:1n9t	3,24	1,86	5,80	2,13	1,85	1,85	7,22	1,85	2,01	1,74	2,64	2,49	2,30	11,34	2,26		1,73
C18:1n9c	23,52	14,29	7,07	5,63	26,03	26,03	5,32	10,14	26,36	19,12	10,08	21,15	23,28	2,82	8,79	1,12	1,94
C18:2n6t	0,64				0,66	0,66		0,95					0,85	0,94		1,06	
C18:2n6c	7,35	8,44	3,81	19,92	10,88	10,87	8,52	19,17	6,97	6,01	6,27	10,54	5,37	3,84	6,21	12,07	3,72
C18:3n6	0,64	1,01			1,61	1,61	4,27	1,28	1,13	0,94		1,03		1,62		17,59	1,74
C18:3n3	23,29	18,60	9,92	34,54	13,99	13,98	11,68	13,96	15,03	16,84	18,87	18,81	19,53	16,97	28,85	1,64	26,20
C20:0				1,57	1,50	1,50	2,95	2,07	1,46	1,93	2,54		1,85	2,20			2,41
C20:1	0,92	1,40	2,15	1,01	1,00	1,00			0,84	1,14	1,45	0,81	1,26	1,45	1,72		
C20:2	1,29	2,03	3,29									1,08				1,90	
C20:3n3		1,86	2,61					1,40		1,12	1,54						
C20:3n6			2,81					1,79			1,95				1,91		1,96
C20:4n6	1,06	1,60	2,44	1,09	1,09	1,09	1,82	1,41	0,91	1,14	1,61	0,77	1,26	2,24	2,16		1,57
C20:5n3	7,16	5,91		2,30	1,57	1,57			3,77	7,67	5,60	7,03	10,47		6,99		7,35
C21:0			2,46	0,93			1,78			1,09					1,74		
C22:0		2,60		1,65	1,72	1,71		2,10	1,36	2,21	2,49	1,32	1,96			3,58	2,36
C22:1n9				1,13			2,17	1,87		1,35	1,96		1,68			1,71	
C22:2	1,07									1,15		0,80			1,73	1,90	

C22:6 n-3	1,1	1,97	2,74	1,20				1,64	1,08	1,43	2,68		1,46		2,14	1,91	
C23:0	1,12				1,04	1,05	2,28						1,31	1,59			1,81
C24:1	1,47	2,5															
AGS	25,21	33,67	51,82	28,48	39,34	39,34	53,61	40,91	36,23	37,95	40,82	30,69	30,94	50,09	32,41	45,96	48,20
AGMI	31,19	24,91	20,56	12,47	30,86	30,88	20,10	17,49	34,88	25,75	20,66	29,25	30,12	24,30	17,59	15,97	9,26
AGPI	43,60	41,42	27,62	59,05	29,80	29,78	26,29	41,60	28,89	36,30	38,52	40,06	38,94	25,61	50,00	38,07	42,54
AGMI/AGS	1,24	0,74	0,40	0,44	0,78	0,78	0,37	0,43	0,96	0,68	0,51	0,95	0,97	0,49	0,54	0,35	0,19
AGPI/AGS	1,73	1,23	0,53	2,07	0,76	0,76	0,49	1,02	0,80	0,96	0,94	1,31	1,26	0,51	1,54	0,83	0,88
ω-6/ω-3	0,32	0,45	0,38	0,58	0,78	0,78	0,73	1,37	0,46	0,36	0,33	0,56	0,27	0,23	0,22	7,35	0,14

C6:0 = C12:0 = Lauric; C13:0 = tridecylic acid; C14:0 = myristic acid; C14:1 = myristoleic acid; C15:0 = pentadecanoic acid; C15:1 = 9-pentadecenoic; C16:0 = palmitic acid; C16:1 ϖ7 = palmitoleic acid; C17:0 = heptadecanoic acid; C17:1 = cis-10-heptadecanoic acid; C18:0 = stearic acid; C18:1 ϖ9 = oleic acid C18:2 ϖ6 = linoleic acid; C18:3 ϖ3 = α-linolenic acid; C20:0 = arachidic acid; C24:0 = arachidonic acid. SFA= Saturated Fatty Acids, MUFA = Monounsaturated Fatty Acids, PUFA= Polyunsaturated Fatty Acids. Source: Authors.

Both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were encountered in some of the microalgae species analyzed here (Figure 5). They were not, however, detected in *Chlamydomonas sp* (D530WC) or *Coenocystis sp.* (D392WC). The highest EPA concentrations (10.47%) were observed in *Monoraphidium contortum* (D525WC). Other species demonstrated EPA concentrations above 7%, such as: *Coelastrum cambricum* (D294WC), *Kirchneriella concorta* (D498WC), *Scenedesmus dimorphus* (D514WC), and *Cosmarium* sp. *cf. depressum* (D578WC). DHA values varied from 1.08% in *Scenedesmus ecornis* D477WC to 2.74% in *Coelastrum astreoideum* D316WC. Ryckebosch et al., (2014) studied nine species of microalgae and encountered EPA levels between 2.8 mg/g^{-1} (*Isochrysis T-iso*) and 193 $mg.g^{-1}$ (*Nannochloropsis oculata*), and DHA levels between 0.8 mg/g^{-1} (*Tetraselmis suecica*) and 46 mg/g^{-1} (*Isochrysis T-iso*).

Figure 5. Percentages of EPA and DHA fatty acids in the 17 microalgae species.



Source: Authors.

Terrestrial vascular plants can provide us with large quantities of fatty acids but they do not produce EPA and DHA. As such, microalgae represent one of the principal sources of those two longchain PUFA in the biosphere. Some species have been reported to produce considerable quantities of EPA and DHA, such as *Rhodomonas salina* (EPA= 20.7 and DHA= 13.3 $mg\ g^{-1}$ C) (Chen et al., 2011).

EPA and DHA use by the food industry requires certain innovations, and the goals of a number of studies have been to produce appropriate carriers that will not degrade fatty acid compositions. Toker et al., (2018a) obtained good results with white chocolate fortified with EPA/DHA, indicating it as a promising carrier of heat-sensitive bioactive substances. In another study, Toker et al., (2018b) placed microcapsules of EPA/DHA (from both fish and microalgae sources) into dark chocolate, and likewise obtained positive results. Those initiatives demonstrate the possibility of more widespread use of microalgae in the food industry in the near future.

4. Conclusion

Variations in the chemical compositions and growth parameters of the microalgae species studied here indicated their differing responses to culture conditions. The species *Monoraphidium litorale* (D296WC), *Xanthonema sp.* (D464WC), and *Monoraphidium griffithi* (D499WC) were identified as good sources of lipids, proteins, and carbohydrates, and produced high

percentages of polyunsaturated nutraceuticals. We were therefore able to demonstrate the real possibility of encountering, within native regional populations of microalgae, numerous species that produce high levels of compounds of great interest to the food industry and the importance of regional bio-prospecting.

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