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Microwave-Assisted Extraction of Fatty Acids from Cultured and Commercial Phytoplankton Species

Jéssica Aparecida Moretto [1], Alexander Ossanes de Souza [1], Lucas Moraes Berneira [2], Luiz Gustavo G. Brigagão [3], Claudio Martin Pereira de Pereira [2], Attilio Converti [4,*] and Ernani Pinto [1,5]

[1] Center of Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Piracicaba 13416-000, Brazil; jessica.moretto@usp.br (J.A.M.); alexandersouza@usp.br (A.O.d.S.); ernani@usp.br (E.P.)
[2] Lipidomic and Bioorganic Laboratory, Center of Chemical, Food and Pharmaceutical Sciences, Forensic Chemistry, Federal University of Pelotas, Pelotas 96010-610, Brazil; lucas.berneira@hotmail.com (L.M.B.); claudio.martin@ufpel.edu.br (C.M.P.d.P.)
[3] Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo (USP), Av. Prof. Lineu Prestes 580, Bl. 17, São Paulo 05508-900, Brazil; gustavogb@usp.br
[4] Department of Civil, Chemical and Environmental Engineering, Pole of Chemical Engineering, University of Genoa, Via Opera Pia 15, 16145 Genoa, Italy
[5] Food Research Center (FoRC–CEPID), University of São Paulo (USP), São Paulo 05508-220, Brazil
* Correspondence: converti@unige.it

Abstract: (1) Background: The extraction of fatty acids from microalgae and cyanobacteria is mostly performed with organic solvents and laborious procedures. Microwave-assisted extraction (MAE) can be a more effective and environmentally friendly process than traditional extraction (TE), which uses a large volume of solvent and conduction heating. Freshwater phytoplankton inhabits diverse aquatic environments and is a promising source of fatty acids and green precursors in the synthesis of biofuel, including cyanobacterial biomass. Therefore, the aim of this study was to investigate the potential of MAE to extract fatty acids from a *Chlorella* sp. microalga and two cyanobacteria, namely, *Arthrospira* sp. and *Sphaerospermopsis torques-reginae*, for biodiesel production. For this purpose, the lipid content and fatty acid profile of these strains were compared after treating biomass with the two extraction methods. (2) Methods: MAE and TE were used as extraction procedures; gas chromatography–mass spectrometry was used to assess the fatty acid profiles, and X-ray spectroscopy was used to analyze biomass. (3) Results: Although the fatty acid profile of the oil obtained by TE showed higher concentrations of fatty acids, the MAE method was able to extract more types of fatty acids. The variety of fatty acids extracted by the MAE, especially those with unsaturated chains, allowed for better quality biodiesel, presenting advantages over previous methods and studies. According to the analyses, essential fatty acids 16:0, 16:1, and 18:2 were found to be abundant in both cyanobacterial strains and in microalga, showing potential for biofuel production. Additionally, metal composition was determined as its content may indicate potential pro-oxidant influence in biofuel production. (4) Conclusions: MAE is a useful and green strategy to extract fatty acids from freshwater phytoplankton. Cyanobacteria can also be a beneficial source of fatty acids for biodiesel synthesis.

Keywords: microwave-assisted extraction; cyanobacteria; microalgae; lipids

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1. Introduction

The increasing energy demand, the expected shortage of conventional fossil fuels, and the recognized effects on climate global emergency has led to the development of novel energy sources, including biodiesel [1,2]. Biofuel production from phytoplankton biomass is an extremely promising and important issue due to the relevance it has gained with the increase in petroleum prices and its environmental advantages [3,4].

In this sense, microalgae and cyanobacteria are widespread photosynthetic organisms with high oil content. The advantage of using these organisms is that they can grow quickly

in freshwater, seawater, or wastewater. Moreover, their cultivation contributes to reducing the presence of greenhouse gases in the atmosphere as they convert approximately 2 tons of inorganic CO_2 into 1 ton of biomass [5–7]. Therefore, microalgae and cyanobacteria can be a promising alternative for biodiesel production as well as conventional raw materials [1,8–10].

Current methods regarding biodiesel synthesis from microalgae and cyanobacteria biomass are time-consuming and require large amounts of organic solvents as procedures rely on an initial step of oil extraction followed by transesterification to synthesize fatty acid alkyl esters [2,3]. In this sense, "green" techniques such as ultrasound-assisted transesterification and microwave irradiation have been studied to overcome the disadvantages associated with conventional biofuel synthesis [7,11].

Among ecofriendly methodologies, the application of microwave irradiation has a great potential for biodiesel production, as the extraction and transesterification can occur in a single step in situ. This combination not only reduces the synthesis time, solvent amount, and production cost eliminating complex and unneeded steps of the procedure [1,12], but also assists the biodiesel production from microbial biomass through cell wall disruption, rapid and homogeneous heat generation, and pressure, thereby enabling an efficient oil extraction [8,10,13].

Despite the numerous advantages in the use of microorganisms to produce biodiesel, the literature lacks studies involving common freshwater cyanobacteria for this purpose. Therefore, the aim of this study was to investigate the potential of MAE to extract fatty acids from a *Chlorella* sp. microalga and two cyanobacteria, namely, *Arthrospira* sp. and *Sphaerospermopsis torques-reginae*, for biodiesel obtention. Furthermore, two different fatty acid and total lipid extraction methods, i.e., traditional extraction and microwave-assisted extraction, were compared.

2. Materials and Methods

2.1. Chemicals and Standards

Methanol, *n*-hexane, and potassium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). A C_4C_{24} FAME Mix standard was acquired from Supelco (Bellefonte, PA, USA).

2.2. Cultures and Commercial Samples

Samples of *Arthrospira* sp. (batch: 2018092611) and *Chlorella* sp. (batch: 21461095890) were acquired at commercial grade from Rofimex (Dionísio Cerqueira, Brazil). *Sphaerospermopsis torques-reginae* was isolated from a reservoir in the state of Pernambuco, Brazil, and cultivated in the laboratory. This strain was cultivated in ASM-1 medium at 22 µmol photons m^{-2} s^{-1} using a 12:12 h (light/dark) photoperiod at 25 °C (±1 °C) [14–16].

2.3. Traditional Extraction (TE) of Lipids and Fatty Acids

The biomass of each strain was lyophilized, and 30 mg of each dry biomass was resuspended in 1 mL of ultrapure water (Milli-Q, Merck Millipore, Billerica, MA, USA). Total lipids were extracted using a modified method of Bligh and Dyer [17–19].

The oil resulting from the extraction process was derivatized, and the fatty acids were analyzed as methyl-esters (FAMEs). The transesterification was carried out by adding 1.8 mL of a methanolic solution of 5% HCl [20]. In addition, 200 µL of chloroform containing nonadecanoic acid was added. Nonadecanoic acid is not produced by cyanobacteria, so it was used as an internal standard.

The reaction was carried out at 100 °C for 15 min. After this step, 1 mL of hexane + 1 mL of ultrapure water were added to the mixture. Then, the tubes were shaken for 1 min, and the aqueous and organic phases were separated by centrifugation at 5000 rpm for 15 min. The reactions were performed in triplicate. The organic phase was transferred to an amber vial for analysis by gas chromatography coupled with mass spectrometry (GC–MS).

2.4. Gas Chromatography–Mass Spectrometry

The extraction triplicates of each sample were analyzed by a Gas Chromatograph-Mass Spectrometer, model GCMS QP2010 (Shimadzu, Kyoto, Japan), using helium as the carrier gas and an RTX-5MS capillary column as the stationary phase. Temperatures of the injection port and detector were 220 and 240 °C, respectively. GLC temperature program: 50–130 °C/20 °C min^{-1}, 130–220 °C/5 °C min^{-1} (10 min hold), 33 min run time. Biodiesel constituents were identified using the C_4C_{24} FAME Mix standard and a NIST-17 spectral library. Semi-quantification of samples was made by area normalization, and results were expressed as percentage [18,20,21].

2.5. Microwave-Assisted In Situ Extraction and Transesterification

Briefly, 500 mg of the samples was mixed with 10 mL of a 2% (w/v) methanolic solution of potassium hydroxide. The mixture was introduced in an open vessel microwave digestion system (CEM, Charlotte, NC, USA) and subjected to 100 W for 5 min under constant stirring, according to Pacheco et al. [22]. Afterward, the material was transferred to a separatory funnel, and the biodiesel was extracted using 10 mL of *n*-hexane. Finally, samples were dried under reduced pressure. All analyses were performed in triplicate (n = 3) [19,21]. In this procedure, the extraction and transesterification processes took place simultaneously.

2.6. X-ray Spectroscopy

Cyanobacterial and microalgal biomasses were analyzed via X-ray spectroscopy, model EDX-720 (Shimadzu, Kyoto, Japan), using an X-ray beam energy of 20 to 40 keV [23,24].

2.7. Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's test. The results, expressed as mean ± standard deviation, were considered statistically significant with $p \leq 0.05$.

3. Results

3.1. Lipids and Fatty Acids

The composition of lipids from microalgae and cyanobacteria can vary according to the fatty acids that compose them, the species, and even the lipid extraction method. Therefore, to prove good efficiency and possible advantages of microwave-assisted extraction (MAE), the traditional extraction (TE) was also carried out for comparative purposes. The lipid content of *Chlorella* sp., *Arthrospira* sp., and *S. torques-reginae* ranged between 11% and 23%.

As shown in Figure 1, and according to percentage obtained from GC–MS analysis of triplicate extractions and statistical analyses, TE was more efficient than MAE in extracting total lipids from *Chlorella* sp. and *S. torques-reginae*, but there was no statistical difference between the results of these methods for *Arthrospira* sp. As expected, the microalga *Chlorella* sp. had a higher concentration of lipids than the cyanobacteria. Nevertheless, it is possible to observe that the use of cyanobacterial biomass is also a clean and sustainable alternative to produce biodiesel.

Given the abundance of *Sphaerospermopsis* sp. in bloom episodes and the satisfactory percentage of lipids detected in *S. torques-reginae* to produce biodiesel, this strain was also chosen to compare the MAE and TE methods for fatty acid extraction.

Several fatty acids were identified in the *S. torques-reginae* extract obtained by TE, most of them being saturated fatty acids (SAFAs) such as 16:0 and 18:0, while polyunsaturated fatty acids (PUFAs) such as 18:2 and 18:3 were present in intermediate contents, and monounsaturated fatty acids (MUFAs) such as 16:1 and 18:1 in the lowest ones (Table 1).

Analyzing the fatty acid profile of the strains was essential, as it determined the quality of the biofuel. Although the fatty acid profile of the oil obtained by TE showed higher concentrations of fatty acids, the MAE method was able to extract more types of fatty acids.

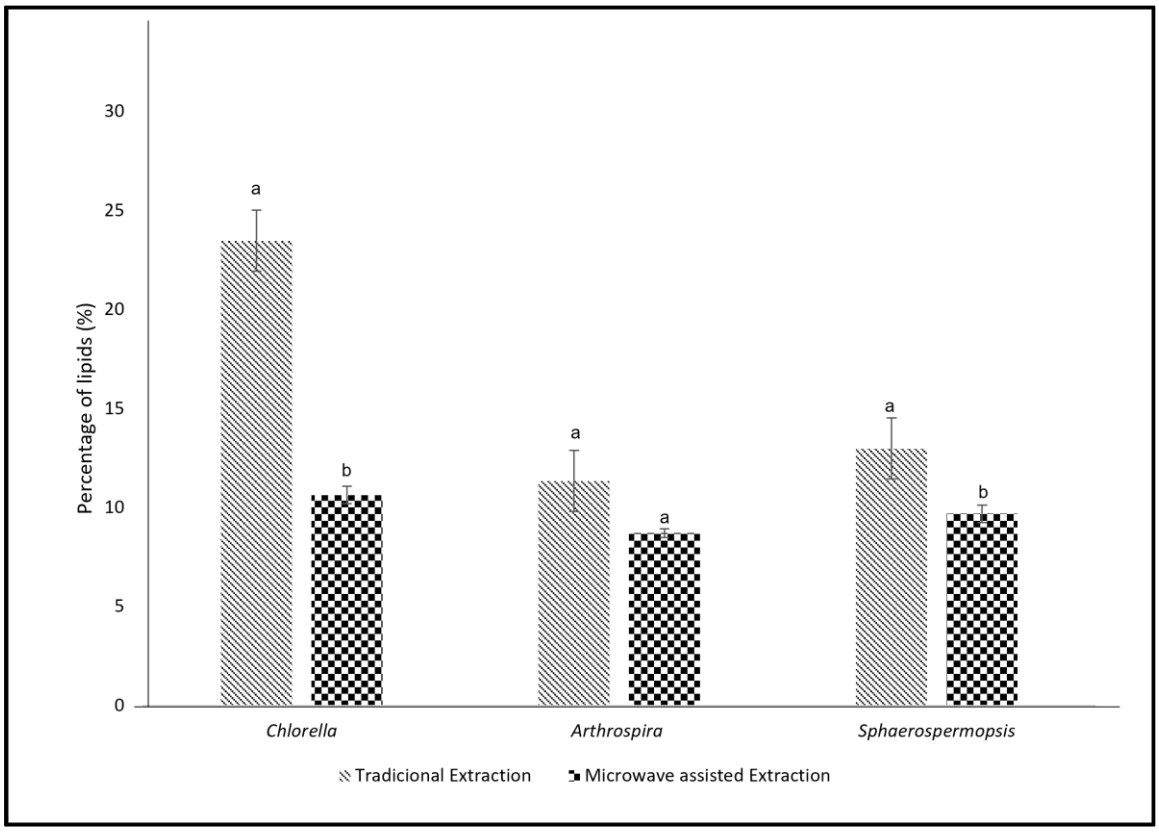

Figure 1. Comparison of lipid recovery yields from *Chlorella* sp., *Arthrospira* sp., and *Sphaerospermopsis torques-reginae* using traditional extraction and microwave-assisted extraction obtained by GC–MS (n = 3). Different letters indicate significant differences in the results ($p \leq 0.05$).

Table 1. Average composition (% of area) of fatty acids obtained by traditional and microwave-assisted extractions of cyanobacterial and microalgal biomass (n = 3).

		***Arthrospira* sp.**	***Chlorella* sp.**	***S. torques***	
Fatty Acid		**MAE**	**MAE**	**MAE**	**TE**
C10:0	Capric acid	0.11 ± 0.01^{a}	0.20 ± 0.01^{b}	n.a.	n.a.
C14:0	Myristic acid	0.93 ± 0.00^{a}	1.46 ± 0.18^{b}	2.44 ± 0.10^{c}	n.a.
C15:0	Pentadecanoic acid	0.44 ± 0.00^{a}	0.88 ± 0.06^{b}	n.a.	n.a.
C16:0	Palmitic acid	37.41 ± 0.29^{a}	18.30 ± 0.29^{b}	34.85 ± 0.09^{c}	54.36 ± 0.10
C16:1	Palmitoleic acid	10.77 ± 0.24^{a}	11.86 ± 0.23^{b}	8.92 ± 0.46^{c}	11.17 ± 0.11
C17:0	Heptadecanoic acid	0.76 ± 0.02^{a}	0.51 ± 0.04^{b}	n.a.	n.a.
C17:1	Heptadecaenoic acid	0.78 ± 0.01^{a}	1.86 ± 0.13^{b}	n.a.	n.a.
C18:0	Stearic acid	$2.13 \pm 0.04^{a,b}$	1.55 ± 0.07^{c}	2.87 ± 0.29^{b}	12.59 ± 0.19
C18:1	Oleic acid	1.11 ± 0.04^{a}	1.33 ± 0.04^{b}	7.49 ± 0.15^{c}	2.55 ± 0.09
C18:2	Linoleic acid	25.83 ± 0.17^{a}	31.93 ± 0.47^{b}	33.94 ± 0.26^{c}	5.89 ± 0.11
C18:4	Stearidonic acid	0.19 ± 0.01^{a}	0.82 ± 0.14^{b}	n.a.	n.a.
C20:4	Arachidonic acid	0.19 ± 0.01^{a}	0.62 ± 0.00^{b}	n.a.	n.a.
C21:5	Tetracosanolpentaenoic acid	0.74 ± 0.00^{a}	26.82 ± 1.19^{b}	n.a.	n.a.
Phytol		18.40 ± 0.16^{a}	28.01 ± 2.38^{b}	8.83 ± 0.28^{c}	n.a.
∑SFAs		41.79 ± 0.35^{a}	22.91 ± 0.40^{b}	40.13 ± 0.28^{c}	66.95 ± 0.16
∑MUFAs		12.66 ± 0.18^{a}	15.05 ± 0.40^{a}	16.20 ± 0.47^{b}	13.72 ± 0.38
∑PUFAs		26.96 ± 0.18^{a}	26.96 ± 0.18^{a}	35.74 ± 0.25^{b}	5.89 ± 0.26

Note: Results expressed as average ± standard deviation. n.a.—non-available, SFAs—saturated fatty acid acids, MUFAs—monounsaturated fatty acids, PUFAs—polyunsaturated fatty acids, MAE—microwave-assisted extraction, TE—traditional extraction. Different letters (a–c) indicate significant differences in the results ($p \leq 0.05$).

Table 1 shows that the biofuel would be composed of 14 FAs ranging from C10 to C21 with SFAs, MUFAs, and PUFAs accounting for 6, 3, and 5 of the detected compounds. In general, the most abundant FAs in samples were palmitic (C16:0), linoleic (C18:2), and palmitoleic (C16:1) acids, which had significant differences in concentration among the samples. Other FAs found in noticeable concentrations were myristic (C14:0), stearic (C18:0), and oleic (C18:1) acids.

Comparison among the classes of FAs revealed that PUFAs were predominant, constituting from 35.74 ± 0.25% (*S. torques-reginae*) to 26.96 ± 0.18% (*Chlorella* sp.) of samples, with almost coincident percentages of n3 and n6 PUFAs. On the other hand, SFAs, mainly in the forms of C14:0 and C16:0, accounted for 41.79 ± 0.35% (*Arthrospira* sp.) to 22.91 ± 0.40% (*Chlorella* sp.). MUFAs corresponded the smallest fraction among the classes, ranging from 16.20 ± 0.47% (*S. torques-reginae*) to 12.66 ± 0.18% (*Arthrospira* sp.).

All the strains showed a good correlation between saturated and unsaturated fatty acids, with a profile similar to that described for some other species of cyanobacteria and microalgae [25–27]. These results may open the way for the use of *S. torques-reginae* and other cyanobacteria to produce sustainable biodiesel.

3.2. X-ray Spectroscopy

As is known, X-ray spectroscopy analysis allows the elemental characterization of different matrices with minimal sample manipulation, reducing possible contamination or use of reagents. This analysis is essential to evaluate the elements present in a raw material, since the presence of some elemental ions can harm the biodiesel production process.

X-ray spectroscopy analysis (Table 2) showed that samples were composed of a wide variety of elements. Elements found in the highest concentrations were calcium (22.07–35.79%), potassium (21.44–37.80%), and phosphorus (10.30–15.35%), while those found in intermediate concentrations were sulfur (11.25–12.59%), iron (6.25–11.04%), and chlorine (4–11%). Other elements were found in negligible amounts, accounting for a total concentration of only 0.56%.

Table 2. Metal composition (%) of cyanobacterial and microalgal biomass.

Metal	Sample		
	***Chlorella* sp.**	***Arthrospira* sp.**	***S. torques-reginae***
Calcium	35.79	22.07	31.25
Potassium	21.44	37.80	26.42
Phosphorus	15.35	10.30	12.03
Sulfur	11.25	12.69	11.25
Iron	11.04	6.25	8.26
Chlorine	4.54	10.46	10.25
Others	0.56	0.42	0.54

4. Discussion

Microwave technology has enabled the development of faster, safer, and economical methods to extract and convert lipids into biofuel. The rapidly wavering electric field produced by microwaves generates heat due to frictional forces resulting from inter- and intramolecular movements of dielectric/polar material in the cell's molecules [2,3]. Furthermore, intracellular heating generates water vapor that disrupts the cell from the inside. In turn, this can lead to the electroporation effect, i.e., a considerable increase in the electrical conductivity and permeability of cyanobacterial and microalgal cell wall as well as cytoplasmic membrane resulting from an applied external electrical field [10,28].

The opening of cell membrane makes the extraction of intracellular metabolites efficient, because it forces out compounds from the cell matrix, producing good quality extracts [2]. In general, microwave-assisted extraction (MAE) has been considered a potential alternative to conventional extraction methods to produce biodiesel from microorganisms

due to (i) reduced extraction time, (ii) reduced solvent usage, and (iii) improved transesterification yield [10,13,28]. An alternative to improve the efficiency of MAE would be to increase the amount of organic solvent used, but the MAE method had several advantages over the TE, due to the lower use of organic solvents as well as greater ease, speed, and efficiency, in addition to following the principles of green chemistry to produce biodiesel. In addition to the advantages, we observed that the MAE method allowed the release of more FA compounds, increased the concentration of C18:1 and C18:2, and also allowed the in-situ synthesis of biodiesel.

The variety of fatty acids obtained, especially those with unsaturated chains, allows for better quality biodiesel [13,29]. In this sense, this study provided important information on the production of biodiesel from the microalga *Chlorella* sp. and the cyanobacteria *Arthrospira* sp. and *Sphaerospermopsis torques-reginae* using in situ transesterification assisted by microwave irradiation, an environmentally friendly methodology. As expected from the well-known prevalence of triglycerides over phosphoglycerides in eucaryotic cells, the microalga *Chlorella* sp. had a higher concentration of lipids than the cyanobacteria prokaryotic cells. Nevertheless, it is possible to observe that the use of cyanobacterial biomass is also a clean and sustainable alternative for the production of high-quality biodiesel.

Our findings on fatty acid profile from commercially available sources of *Chlorella* sp. and *Arthrospira* sp. as well as *S. torques-reginae* are similar to those of previous studies [28–32]. The observed fatty acid species are the same; however, small differences between the concentrations reported in the literature (Table S1) and those from the current study may be ascribed to different harvest and reaction procedures [10,29,30]. It is worth noting that the fatty acid profile of organisms can be modified not only by varying environmental conditions, but also through genetic means that could further optimize it, enabling their use as a potential biodiesel source [21,24–26,33].

The quality and physicochemical properties of biodiesel is considerably dependent on fatty acid composition used in the transesterification process. In this sense, a high contents of saturated fatty acid (SFA) methyl esters leads to high viscosity biodiesel and, therefore, can cause engine problems and gum formation in vehicles. On the other hand, the presence of long chain fatty acid and polyunsaturated fatty acid (PUFA) methyl esters is associated with low viscosity of the biodiesel [13,33]. However, it is noteworthy that a prevalence of PUFAs can lead to oxidative degradation processes, thus affecting the overall biodiesel quality. In this sense, the quality of the biodiesel produced from the strains investigated in this study would be suitable for use as an engine biofuel, since in the samples, there was a balance between saturated and unsaturated fatty acids similar to that of biodiesel obtained from conventional sources. These results indicate the potential of these microorganisms in biodiesel production [8,34].

X-ray spectroscopy analyses were also performed for elemental characterization of biodiesel since the presence of contaminants also interferes with the quality of burning emissions, performance, engine integrity, safety of transport, and even handling of biodiesel. For instance, the presence of transition metals in the synthesized biodiesel can lead to oxidative instability, as these elements are known to initiate the formation of free radicals that decompose unsaturated fatty acids and, therefore, negatively affect biodiesel physicochemical properties [13,27]. Moreover, this information makes it possible to assess the potential of raw materials to produce biodiesel, since the biodiesel quality must meet the regulations of the National Agency for Gas, Petroleum, Natural Gas and Biofuels (ANP).

On the basis of the results of average composition of fatty acids (Table 1) and of the elemental analysis (Table 2), we found that all the raw materials tested in this study proved to be an alternative for biodiesel production, not compromising the transesterification reaction or the parameters required by ANP [33–36].

As a future perspective, the MAE method can be improved and applied to a greater amount of biomass and can be used to check the presence of other fatty acids in different microorganisms, in addition to expanding the study to other microalgae and cyanobacteria of environmental importance. Moreover, the present study was carried out only on a

laboratory scale; therefore, the next steps would be performing the experiments at an industrial level and evaluating the costs of producing biodiesel from the strains under study.

5. Conclusions

Biodiesel production from the cyanobacteria tested in this study (*Arthrospira* sp. and *Sphaerospermopsis torques-reginae*) was as promising as that from the microalga *Chlorella* sp. Their abundant proliferation in eutrophic environments and the balance between saturated and unsaturated fatty acids in both types of biomass led to an adequate quality of biodiesel. Moreover, the microwaved-assisted extraction method was proven to be an alternative for the extraction and synthesis of biodiesel in one pot, reducing the costly steps found in conventional procedures.

Furthermore, the low amounts of elements in the biomass of the studied strains are expected to imply low risks of affecting the quality of burning emissions, performance, engine integrity, safety of transport, and handling of biodiesel.

The use of microwave, X-ray, and GC techniques allowed for a quick extraction and identification of important lipids in biodiesel obtaining. For all these aspects, this study adds important information about "green techniques" to produce biofuels and aims to improve biotechnological applications of cyanobacteria and microalgae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12052407/s1, Table S1: Composition of fatty acids (% area) reported in the literature.

Author Contributions: All authors contributed to the study conceptualization and design. Material preparation, data collection, and analyses were performed by L.G.G.B., J.A.M., L.M.B. and A.O.d.S.; J.A.M. and L.M.B. wrote the first draft of the manuscript, and all authors commented on previous versions of the document and revised it; E.P., A.C. and C.M.P.d.P. were responsible for funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Patil, P.D.; Gude, V.G.; Mannarswamy, A.; Cooke, P.; Munson-McGee, S.; Nirmalakhandan, N.; Lammers, P.; Deng, S. Optimization of Microwave-Assisted Transesterification of Dry Algal Biomass Using Response Surface Methodology. *Bioresour. Technol.* **2011**, *102*, 1399–1405. [CrossRef] [PubMed]
2. Velasquez-Orta, S.B.; Lee, J.G.M.; Harvey, A. Alkaline in Situ Transesterification of *Chlorella vulgaris*. *Fuel* **2012**, *94*, 544–550. [CrossRef]
3. Tsigie, Y.A.; Huynh, L.H.; Ismadji, S.; Engida, A.M.; Ju, Y.-H. In Situ Biodiesel Production from Wet *Chlorella vulgaris* under Subcritical Condition. *Chem. Eng. J.* **2012**, *213*, 104–108. [CrossRef]
4. Huang, D.; Zhou, H.; Lin, L. Biodiesel: An Alternative to Conventional Fuel. *Energy Procedia* **2012**, *16*, 1874–1885. [CrossRef]

5. Kapoore, R.V.; Butler, T.O.; Pandhal, J.; Vaidyanathan, S. Microwave-Assisted Extraction for Microalgae: From Biofuels to Biorefinery. *Biology* **2018**, *7*, 18. [CrossRef]
6. Mata, T.M.; Martins, A.A.; Caetano, N.S. Microalgae for Biodiesel Production and Other Applications: A Review. *Renew. Sustain. Energy Rev.* **2010**, *14*, 217–232. [CrossRef]
7. Delucchi, M.A. A Lifecycle Emissions Model(lem): Lifecycle Emissions from Transportation Fuels, Motor Vehicles, Transportation Modes, Electricity Use, Heating and Cooking Fuels, and Materials. 2003, p. 445. Available online: https://escholarship.org/uc/item/9vr8s1bb#main (accessed on 24 October 2021).
8. Li, Y.; Horsman, M.; Wu, N.; Lan, C.Q.; Dubois-Calero, N. Biofuels from Microalgae. *Biotechnol. Prog.* **2008**, *24*, 815–820. [CrossRef]
9. Wang, B.; Li, Y.; Wu, N.; Lan, C.Q. CO_2 Bio-Mitigation Using Microalgae. *Appl. Microbiol. Biotechnol.* **2008**, *79*, 707–718. [CrossRef]
10. Martínez, N.; Callejas, N.; Morais, E.G.; Vieira Costa, J.A.; Jachmanián, I.; Vieitez, I. Obtaining Biodiesel from Microalgae Oil Using Ultrasound-Assisted in-Situ Alkaline Transesterification. *Fuel* **2017**, *202*, 512–519. [CrossRef]
11. Li, Y.; Horsman, M.; Wang, B.; Wu, N.; Lan, C.Q. Effects of Nitrogen Sources on Cell Growth and Lipid Accumulation of Green Alga *Neochloris oleoabundans*. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 629–636. [CrossRef]
12. Chee Loong, T.; Idris, A. Rapid Alkali Catalyzed Transesterification of Microalgae Lipids to Biodiesel Using Simultaneous Cooling and Microwave Heating and Its Optimization. *Bioresour. Technol.* **2014**, *174*, 311–315. [CrossRef] [PubMed]
13. Berneira, L.M.; Rockembach, C.T.; da Silva, C.C.; de Freitas, S.C.; Rosa, B.N.; Pinto, E.; Anjos, F.M.; dos Santos, M.A.Z.; de Pereira, C.M.P. Employment of Thermal Analysis Applied to the Oxidative Stability Evaluation of Biodiesel Using Chalcone Analogues. *J. Therm. Anal. Calorim.* **2021**, *146*, 1473–1482. [CrossRef]
14. Bortoli, S.; Oliveira-Silva, D.; Krüger, T.; Dörr, F.A.; Colepicolo, P.; Volmer, D.A.; Pinto, E. Growth and Microcystin Production of a Brazilian *Microcystis aeruginosa* Strain (LTPNA 02) under Different Nutrient Conditions. *Revista Brasileira de Farmacognosia* **2014**, *24*, 389–398. [CrossRef]
15. Fernandes, K.A.; Pinto, E.; Ferraz, H.G.; Vereau, F. Availability of Guanitoxin in Water Samples Containing *Sphaerospermopsis torques-reginae* Cells Submitted to Dissolution Tests. *Pharmaceuticals* **2020**, *13*, 402. [CrossRef] [PubMed]
16. Shirai, M.; Matumaru, K.; Ohotake, A.; Takamura, Y.; Aida, T.; Nakano, M. Development of a Solid Medium for Growth and Isolation of Axenic *Microcystis* Strains (Cyanobacteria). *Appl. Environ. Microbiol.* **1989**, *55*, 2569–2571. [CrossRef] [PubMed]
17. Bligh, E.G.; Dyer, W.J. A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef]
18. Kumari, N.; Singh, R.K. Bio-Diesel Production from Airborne Algae. *Environ. Chall.* **2021**, *5*, 100210. [CrossRef]
19. Cancela, A.; Maceiras, R.; Urrejola, S.; Sanchez, A. Microwave-Assisted Transesterification of Macroalgae. *Energies* **2012**, *5*, 862–871. [CrossRef]
20. Ichihara, K.; Fukubayashi, Y. Preparation of Fatty Acid Methyl Esters for gas-Liquid Chromatography. *J. Lipid Res.* **2010**, *51*, 635–640. [CrossRef]
21. Fazril, I.; Shamsuddin, A.H.; Nomanbhay, S.; Kusomo, F.; Hanif, M.; Ahmad Zamri, M.F.M.; Akhiar, A.; Ismail, M.F. Microwave-Assisted in Situ Transesterification of Wet Microalgae for the Production of Biodiesel: Progress Review. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *476*, 012078. [CrossRef]
22. Pacheco, B.S.; da Silva, C.C.; de Freitas, S.C.; Berneira, L.M.; da Silva, V.L.; Winkel, K.; Ferreira, L.B.; de Pereira, C.M.P. Employment of Alternative Raw Materials for Biodiesel Synthesis. In *Increased Biodiesel Efficiency: Alternatives for Production, Stabilization, Characterization and Use of Coproduct*; Trindade, M., Ed.; Springer International Publishing: Cham, Switzerland, 2018; pp. 33–55; ISBN 978-3-319-73552-8.
23. Parial, D.; Patra, H.K.; Roychoudhury, P.; Dasgupta, A.K.; Pal, R. Gold Nanorod Production by Cyanobacteria—A Green Chemistry Approach. *J. Appl. Phycol.* **2012**, *24*, 55–60. [CrossRef]
24. Wang, B.; Li, Y.; Zheng, J.; Hu, Y.; Wang, X.; Hu, B. Efficient Removal of U(VI) from Aqueous Solutions Using the Magnetic Biochar Derived from the Biomass of a Bloom-Forming Cyanobacterium (*Microcystis aeruginosa*). *Chemosphere* **2020**, *254*, 126898. [CrossRef] [PubMed]
25. Xu, H.; Miao, X.; Wu, Q. High Quality Biodiesel Production from a Microalga *Chlorella protothecoides* by Heterotrophic Growth in Fermenters. *J. Biotechnol.* **2006**, *126*, 499–507. [CrossRef] [PubMed]
26. Romano, I.; Bellitti, M.R.; Nicolaus, B.; Lama, L.; Manca, M.C.; Pagnotta, E.; Gambacorta, A. Lipid Profile: A Useful Chemotaxonomic Marker for Classification of a New Cyanobacterium in *Spirulina* Genus. *Phytochemistry* **2000**, *54*, 289–294. [CrossRef]
27. Ben-Amotz, A.; Tornabene, T.G.; Thomas, W.H. Chemical Profile of Selected Species of Microalgae with Emphasis on Lipids. *J. Phycol.* **1985**, *21*, 72–81. [CrossRef]
28. da Silva, C.C.; Pacheco, B.S.; de Freitas, S.C.; Berneira, L.M.; dos Santos, M.A.Z.; Pizzuti, L.; de Pereira, C.M.P. Hydroxychalcones: Synthetic Alternatives to Enhance Oxidative Stability of Biodiesel. In *Increased Biodiesel Efficiency: Alternatives for Production, Stabilization, Characterization and Use of Coproduct*; Trindade, M., Ed.; Springer International Publishing: Cham, Switzerland, 2018; pp. 81–110. ISBN 978-3-319-73552-8.
29. Nayak, S.N.; Bhasin, C.P.; Nayak, M.G. A Review on Microwave-Assisted Transesterification Processes Using Various Catalytic and Non-Catalytic Systems. *Renew. Energy* **2019**, *143*, 1366–1387. [CrossRef]
30. Sarsekeyeva, F.; Zayadan, B.K.; Usserbaeva, A.; Bedbenov, V.S.; Sinetova, M.A.; Los, D.A. Cyanofuels: Biofuels from Cyanobacteria. Reality and Perspectives. *Photosynth. Res.* **2015**, *125*, 329–340. [CrossRef]

31. Passos, L.S.; Almeida, É.C.; de Pereira, C.M.P.; Casazza, A.A.; Converti, A.; Pinto, E. Chemical Characterization of *Microcystis aeruginosa* for Feed and Energy Uses. *Energies* **2021**, *14*, 3013. [CrossRef]
32. Gülyurt, M.Ö.; Özçimen, D.; İnan, B. Biodiesel Production from *Chlorella protothecoides* Oil by Microwave-Assisted Transesterification. *Int. J. Mol. Sci.* **2016**, *17*, 579. [CrossRef]
33. Sharma, A.K.; Sahoo, P.K.; Singhal, S.; Joshi, G. Exploration of Upstream and Downstream Process for Microwave Assisted Sustainable Biodiesel Production from Microalgae *Chlorella vulgaris*. *Bioresour. Technol.* **2016**, *216*, 793–800. [CrossRef]
34. Choe, E.; Min, D.B. Comprehensive Food Science and Food Safety Reviews in Mechanisms and Factors for Edible Oil Oxidation. *Compr. Rev. Food Sci. Food Saf.* **2006**, *5*, 169–186. [CrossRef]
35. Agência Nacional do Petróleo, Gás Natural e Biocombustíveis. *Resolução ANP Nº 45 de 25/08/2014*; Agência Nacional do Petróleo, Gás Natural e Biocombustíveis: Brasilia, Brazil, 2014. Available online: https://www.gov.br/anp/pt-br/canais_atendimento/imprensa/noticias-comunicados/anp-publica-resolucao-sobre-especificacao-do-biodiesel (accessed on 2 November 2021).
36. Wu, H.; Miao, X. Biodiesel Quality and Biochemical Changes of Microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* in Response to Nitrate Levels. *Bioresour. Technol.* **2014**, *170*, 421–427. [CrossRef] [PubMed]