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Nannochloropsis gaditana and *Dunaliella salina* as Feedstock for Biodiesel Production: Lipid Production and Biofuel Quality

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Authors' contributions

All the authors contributed equally. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Microalgal lipids have a wide range of applications, from biodiesel manufacture in the energy industry to the production of polyunsaturated fatty acids for the pharmaceutical industry. Microalgal lipid productivity and quality, however, vary greatly depending on cultivation parameters.
Aims: In this study, we investigated the potential of two marine microalgae, *Nannochloropsis gaditana* and *Dunaliella salina*, to be used as feedstock for biodiesel production.
Methodology: A Taguchi L_4 orthogonal array design was applied to understand the effects of sodium acetate (0 or 2 g L^{-1}), sodium bicarbonate (0 or 2 g L^{-1}), and sodium nitrate (25 or 75 mg L^{-1}) concentrations on biomass and lipid productivities. Fatty acid methyl ester (FAME) profiles of microalgal lipids obtained under the best conditions were determined, and FAME results were used to predict biodiesel properties.

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Results: Both carbon sources (sodium acetate and sodium bicarbonate) improved biomass productivity. Lipid productivity was enhanced only by sodium acetate. The highest lipid productivities obtained were 10.25 ± 1.02 and 12.12 ± 0.28 mg L^{-1} day^{-1} for *N. gaditana* and *D. salina*, respectively. Palmitic (C16:0), stearic (C18:1), oleic (C18:1), linoleic (C18:2), lauric (C12:0), and myristic (C14:0) acids were the major components of *D. salina* oil. The major fatty acids in *N. gaditana* oil were C16:0, C18:0, and C18:1.
Conclusion: The great differences in FAME profiles resulted in different biodiesel properties. Biodiesel from *N. gaditana* oil was predicted to have a higher cetane number (73.20) than that derived from *D. salina* oil (59.59). *D. salina* oil biodiesel, however, was predicted to have better properties than *N. gaditana* oil biodiesel, including lower cloud point (0.46°C) and cold filter plugging point (−7.27°C).

Keywords: Nannochloropsis gaditana; dunaliella salina; biodiesel; microalgal lipids.

1. INTRODUCTION

Third-generation biofuels produced from microalgal biomass are a promising alternative to fossil fuels [1]. Microalgae, which are microscopic photosynthetic organisms found in marine and freshwater environments, stand out from other microorganisms in their capacity to accumulate lipids that can be converted into biodiesel while sustaining high growth rates and excellent photosynthetic efficiency [2].

The amount and quality of oil produced by photosynthetic microorganisms are directly related to cultivation conditions, such as the concentration of nutrients and carbon sources in the culture medium, pH, salinity, light intensity, and temperature. The carbon source, for example, is required for biomass growth and lipid production and can significantly alter microalgal productivity and oil fatty acid profile.

Najafabadi et al. [3] studied the effect of various carbon sources on microalgae cultivation and sodium acetate provided the highest oil productivity when compared to the other carbon sources tested (CO_2, sodium bicarbonate, and molasses). A different behavior was found by Zhu et al. [4], who assayed the growth of *Dunaliella salina* in a photobioreactor, demonstrating that the optimal carbon source was bicarbonate. Li et al. [5] also assessed the influence of various carbon sources on the culture of *Chlorella minutissima*, who concluded that glycerin provided the highest lipid productivity, and simultaneously posed an effect over the Fatty Acid Methyl Ester (FAME) profile. Montoya et al. [6] also found differences in the FAME profile when using CO_2 as carbon source. From their studies, inserting 8% v/v of CO_2 in the aeration system increased the oleic acid (C18:1), while the gamma-linolenic acid content was reduced. Selvakumar and Umadevi [7], working with two microalgae species also found differences in the FAME distribution when using different carbon sources. Their results with *Tetraselmis gracilis* showed the highest polyunsaturated fatty acids contents using glucose and the lowest with sucrose. On the other hand, *Platymonas convolutae* gave the opposite behavior, i.e., attaining high polyunsaturated fatty acids contents with sucrose, and low contents with glucose [7]. This suggests that the carbon source can modify the FAME profile, and differs for each microalgae species.

The proportions and physicochemical properties of fatty acid alkyl esters in biodiesel are of great importance, as they determine the quality of the biofuel. Structural characteristics of fatty acids, such as chain length, degree of unsaturation, and branching pattern, play an important role in biodiesel quality parameters, such as cetane number, cold flow properties, and oxidation stability [8,9]. Therefore, to ensure good engine performance, it is necessary to look at the physical and chemical properties of the raw material used for biodiesel production.

High proportions of palmitic acid (C16:0) in the lipid material contribute to the quality of biodiesel in terms of cetane number, a dimensionless parameter related to fuel ignition quality. On the other hand, raw materials with a high content of linolenic acid (C18:3) may result in low-quality biodiesel that is prone to polymerization when subjected to high temperatures and leads to the build-up of carbon deposits in the engine, a property expressed by the iodine value. The European Union limits the content of linolenic acid in biofuel to 12% (w/w) to ensure that iodine value specifications are met [8].

In the present work, *Dunaliella salina* and *Nannochloropsis gaditana* were chosen as model study based on their good reported results in relation to biomass and lipid productivities [10–13]. In addition, the microalgae *Dunaliella salina* is of interest due to its highly tolerance to salinity variations in the cultivation media [14,15]. Thus, the aim of this study was to evaluate these microalgae as feedstocks for biodiesel production; the focus was on cultivation optimization, lipid production and predicting the biofuel quality based on the fatty acids profile.

2. MATERIALS AND METHODS

2.1 Microalgal Strains and Cultivation Conditions

The marine microalgae *Nannochloropsis gaditana* (BMAK 130) and *Dunaliella salina* (BMAK 116) were originated from the Seaweed Culture Collection of the Oceanographic Institute (University of São Paulo, SP, Brazil) and were kindly provided by the Department of Biological Oceanography (University of São Paulo, SP, Brazil). All reagents were of analytical grade.

A Taguchi L_4 orthogonal array design was used to determine the influence of sodium acetate (0 or 2 g L^{-1}, X_1), sodium bicarbonate (0 or 2 g L^{-1}, X_2), and sodium nitrate (25 or 75 mg L^{-1}, X_3) concentrations on lipid and biomass productivities. Microalgal cultures (10% v/v in f/2 medium without silica [16]) were transferred to tank photobioreactors (15 cm wide and 33 cm high; 4 L working volume), sparged with sterile air at 1.4 L min^{-1}, and maintained at 24 ± 1 °C under 150 klux for 7 days. All cultivation experiments were performed in duplicate for error analysis.

Microalgal cells were recovered by flocculation using a 1 mol L^{-1} $FeCl_3$ solution [17]. Biomass productivity (P_B) was calculated by dividing the total amount of dry biomass produced in a cultivation run by the working volume of the photobioreactor (4 L) and the cultivation period (7 days).

Design expert v. 6.0 (Stat-Ease Corporation, USA), Statistica v. 8.0 (Stat Soft Inc., USA), and Minitab v. 18.0 (Minitab Inc., USA) were used for regression and graphical analyses of the data. Lipid productivity (P_L) was the response variable. Design expert v. 6.0 was used for graphical and numerical analyses based on the criterion of desirability.

2.2 Microalgal Oil Extraction

Microalgal oil was extracted from biomass according to a modified Folch method. Extraction was performed using a 3:1 (v/v) chloroform/methanol mixture under sonication for 10 min. This step was repeated three times [18]. Ultrasound induces cavitation in the extraction medium causing cell rupture while the solvent dissolves and retains microalgal lipids in solution [19,20]. The extract was rotary evaporated to remove residual solvent and subsequently dried at 60 °C to constant weight.

2.3 Fatty Acid Methyl Ester Profile Analysis

Fatty acid methyl esters (FAMEs) were synthesized according to [21] and identified by gas chromatography (CG). CG analyses were performed using a PerkinElmer® Clarus 580 chromatograph equipped with a flame ionization detector (250 °C, 40 mL min^{-1} H_2, 400 mL min^{-1} synthetic air). A 30 m capillary column (0.25 mm internal diameter) coated with 5% diphenyl/95% dimethylpolysiloxane (non-polar stationary phase) was used. The oven heating rate was set to 3 °C min^{-1} from 120 to 235 °C and to 1 °C min^{-1} from 235 °C to 255 °C, totaling 60 min of analysis. Nitrogen was used as carrier gas at a flow rate of 5 mL min^{-1}. Supelco® 37 Component FAME MIX (Sigma–Aldrich®), a standard mixture of 37 FAMEs (C4–C24), was used as external standard.

2.4 Biodiesel Properties Predicted from FAME Profiles

Biodiesel properties of microalgal oils produced under the best cultivation conditions (2 g L^{-1} sodium acetate and 75 mg L^{-1} nitrate) were estimated on the basis of FAME profiles using Biodiesel Analyzer© [22,23].

3. RESULTS AND DISCUSSION

The Taguchi experimental design matrix as well as the P_B (biomass productivity) and P_L (lipid productivity) results for both microalgae are presented in Table 1 and graphically in Fig. 1. Sodium acetate and sodium bicarbonate concentrations were set at 0 or 2 g L^{-1}. Nitrate concentration was set at 25 or 75 mg L^{-1}. The Taguchi method tests selected combinations of factor levels in an orthogonal design, which produces good statistical results with reduced experimental work and cost.

P_B results for both microalgae were similar (Table 1 and Fig. 1), with values increasing from experiment 1 to experiment 4. P_L, on the other hand, did not vary greatly between experiments 1, 2, and 4, but was much higher in experiment 3. Sodium bicarbonate was used in experiment 2 and sodium acetate in experiment 3. Sodium nitrate concentrations were the same in both experiments. From these results, it can be seen that both carbon sources improved P_B but only sodium acetate positively affected P_L.

Statistical analysis of P_L results was carried out using Statistica v. 8.0 (Statsoft Inc., USA). Analysis of variance (ANOVA) for *N. gaditana* and *D. salina* P_L are shown in Tables 2 and 3, respectively. Fig. 2 shows the main effects plots and the Pareto charts, for both microalgae species.

ANOVA revealed that all variables (sodium nitrate, sodium acetate, and sodium bicarbonate concentrations) affected (P < 5%) the P_L of both microalgae (Tables 2 and 3). The main effects plots (Fig. 2) complement the ANOVA table; they show how variables can be adjusted to improve the results.

According to Fig. 2, P_L was highest with sodium acetate and sodium nitrate at the high level (2 g L^{-1} and 75 mg L^{-1}, respectively). The same was not observed with sodium bicarbonate. Sodium

Table 1. Taguchi L_4 orthogonal array design and experimental results

Exp.	Ac (g L^{-1})	Bic (g L^{-1})	Nit (mg L^{-1})	*Nannochloropsis gaditana*		*Dunaliella salina*	
				P_B (mg L^{-1} day^{-1})	P_L (mg L^{-1} day^{-1})	P_B (mg L^{-1} day^{-1})	P_L (mg L^{-1} day^{-1})
1	0	0	25	28.52 ± 0.06	1.43 ± 0.06	29.76 ± 5.72	1.20 ± 0.08
2	0	2	75	68.58 ± 1.25	2.24 ± 0.16	63.63 ± 0.58	1.57 ± 0.15
3	2	0	75	88.30 ± 5.13	10.25 ± 1.02	90.18 ± 5.12	12.12 ± 0.28
4	2	2	25	111.82 ± 0.07	3.60 ± 1.06	103.85 ± 4.07	3.31 ± 0.92

Ac, sodium acetate concentration; Bic, sodium bicarbonate concentration; Nit, sodium nitrate concentration; P_B, biomass productivity; P_L, lipid productivity

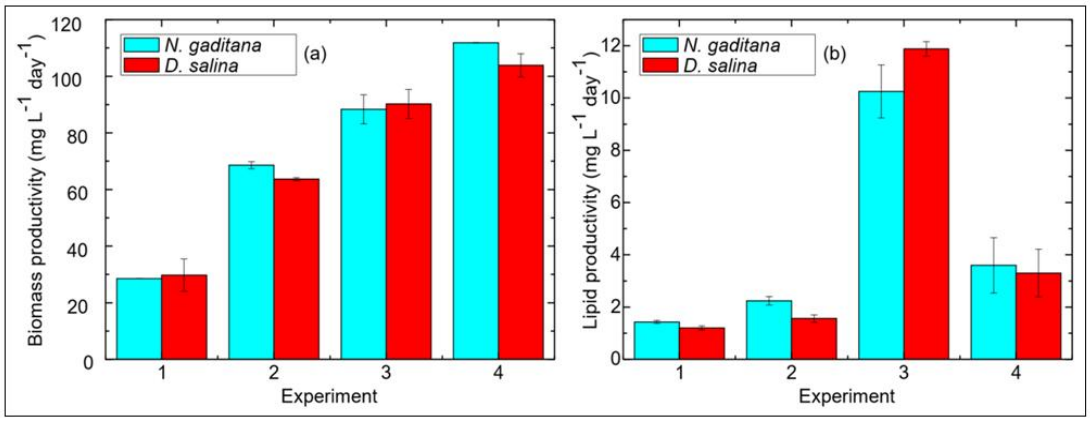

Fig. 1. Experimental results of (a) biomass productivity and (b) lipid productivity for *Nannochloropsis gaditana* and *Dunaliella salina*

Table 2. ANOVA of Nannochloropsis *gaditana*

Factor	SS	df	MS	*F*-value	*P*-value(%)
Sodium acetate concentration	51.71	1	51.71	47.39	.23
Sodium bicarbonate concentration	17.05	1	17.05	15.62	1.68
Sodium nitrate concentration	27.75	1	27.75	25.43	.73
Error	4.36	4	1.09		

SS, sum of squares; df, degrees of freedom; MS, mean squares

Table 3. ANOVA for the lipid productivity of *Dunaliella salina*

Factor	SS	df	MS	*F*-value	*P*-value (%)
Sodium acetate concentration	80.14	1	80.14	169.95	.02
Sodium bicarbonate concentration	35.70	1	35.70	75.71	.10
Sodium nitrate concentration	42.14	1	42.14	89.36	.07
Error	1.89	4	0.47		

SS, sum of squares; df, degrees of freedom; MS, mean squares

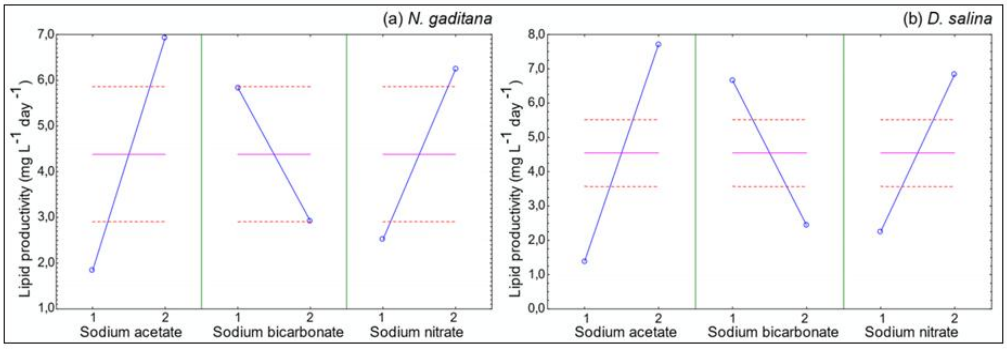

Fig. 2. Maieffects plots for lipid productivity of (a) *Nannochloropsis gaditana* and (b) *Dunaliella salina*

bicarbonate can improve P_B; however, sodium acetate is a better carbon source for P_L. Najafabadi et al. [3] reached a similar conclusion when studying *Chlorella vulgaris*. Fig. 3 shows the plots of model predictions for microalgal P_L, in which sodium bicarbonate concentration is fixed and sodium acetate and sodium nitrate concentrations vary. Fig. 3 shows that the higher the concentrations of sodium nitrate and sodium acetate, the higher the P_L. Therefore, within the studied range, the best productivity would be achieved with 2 g L^{-1} sodium acetate and 75 mg L^{-1} sodium nitrate (experiment 3). Under these experimental conditions, *N. gaditana* and *D. salina* achieved P_L values of 10.25 and 12.12 mg L^{-1} day^{-1}, respectively.

P_L values were similar to those obtained by Matos et al. [13]. The authors cultivated *N. gaditana* in a 1:3 (v/v) mixture of f/2 medium and desalination concentrate under a photoperiod of 16 h light and 8 h darkness and obtained a maximum P_L of 15.9 mg L^{-1} day^{-1} [13]. Mitra et al. [12] reported a maximum P_L of 14.63 mg L^{-1} day^{-1}.

The FAME profile of oils extracted from microalgae cultivated under the best conditions for P_L was determined using GC. Major FAME components are shown in Table 4.

N. gaditana oil was composed mainly of palmitic acid (C16:0), oleic acid (C18:1n9c), and stearic acid (C18:0). The major FAMEs of *D. salina* oil were palmitic acid, oleic acid, stearic acid, lauric acid (C12:0), myristic acid (C14:0), and linoleic acid (C18:2n6c).

D. salina oil had low amounts of C16:0 and gamma-linolenic acid (C18:3) but a high amount of C18:1. El Arroussi et al. [24] analyzed the FAME profiles of 57 microalgal strains from the Moroccan coast, including two *D. salina* strains. In their work, C18:3 levels in *D. salina* oils were 41.56 and 64.61%, much higher than the observed in the present work (2.27%). Likewise, the amount of C16:0 reported by the authors in *D. salina* oils (26.20 and 20.84%) was higher than that of the present study (10.37%). The amount of C18:1, however, was higher in the present study (27.48%) than in the study of El Arroussi et al. [24] (0%, not detected). The microalgal cultivation medium used in the referred work was similar to that of the present study but differed in the addition of sodium acetate. Thus, differences between FAME profiles might be related to the use of this carbon source.

Cho et al. [25] studied the effect of oxidative stress on biomass and lipid production by *D. salina* exposed to phenols. The major FAME components of the oil produced by control microalgae (without phenol exposure) were C16:0 (19.63%), C18:1 (1.60%), and C18:3 (45.58%). This FAME profile is more similar to

that found by El Arroussi et al. [24] than to that of the present work. Again, the cultivation medium used by Cho et al. [25] was similar to the report in this work, differing mainly in the use of sodium acetate as carbon source. These results support the conclusion that sodium acetate has a positive effect on the FAME profile of *D. salina* oil.

The FAME profile of *N. gaditana* oil revealed high amounts of C18:1 and C16:0. El Arroussi et al. [24] evaluated two *N. gaditana* strains cultivated in f/2 medium. Their results showed that *N. gaditana* oils contained 16.01 and 27.81% of C16:0 and 0 and 19.48% of C18:1. The amounts of C16:0 and C18:1 were lower than those obtained in our study (32.81 and 24.71%, respectively). The amounts of C20:5 were not reported by El Arroussi et al. [24].

Ferreira et al. [26] cultivated *N. gaditana* in a semi-continuous regime. A high dilution rate (40% daily renewal rate) provided a nutrient-sufficient condition, whereas a low dilution rate (10% daily renewal rate) induced a nutrient-depleted condition. The amount of C16:0 in the oil of *N. gaditana* cultivated under nutrient-sufficient and -depleted conditions (24.22% and 34.32%, respectively) was similar to that obtained in our work (22.18%). C20:5 content was relatively high under high nutrient availability (27.62%) and lower under nutrient limitation (9.79%). C18:1 (4.83–12.27%) and C18:2 (0.93–2.80%) contents were lower than those obtained in the present study (17.07–23.14%). The authors did not use sodium acetate as carbon source and used a higher concentration of nitrate (4 mM KNO_3) than it did in this work (0.88 mM NO_3^- instead of 75 mg L^{-1} $NaNO_3$).

Matos et al. [13] cultivated N. gaditana in a 1:3 (v/v) mixture of f/2 medium and desalination concentrate under different photoperiods and trophic conditions (autotrophic, mixotrophic, or heterotrophic). The carbon source was glucose (2 g L−1). The maximum C20:5 level was 5.6%, obtained under heterotrophic conditions. Glucose (mixotrophic condition) could improve the C20:5 content, in some of the presented conditions. C18:2 was highest in microalgal oil produced under heterotrophic cultivation, 26.6%, which is higher than the obtained in the present study, 17.07%. The amount of C18:1, however, was reduced by the presence of glucose. The highest C18:1 concentration (12.3%) was obtained under a heterotrophic condition with continuous illumination and was lower than the result of this study (23.24%). The results of the present work show that, contrary to glucose, sodium acetate did not improve the content of polyunsaturated fatty acids. Both studies show that the carbon source affects the FAME profile of microalgal oil.
Khadim et al. [27] studied the effect of varying KNO_3 and sodium bicarbonate concentrations on the productivity of *D. salina* cultivated in a flat-panel photobioreactor. The maximum P_B was 17.85 mg L^{-1} day^{-1}, which is lower than that obtained in experiment 3 of the present study, 90.18 mg L^{-1} day^{-1} (75 mg L^{-1} sodium nitrate, 2 g L^{-1} sodium acetate, and without sodium bicarbonate). Experiment 3 afforded a lipid yield of 13.44%, similar to the maximum lipid yield obtained by Khadim et al. [27], 16.36%.

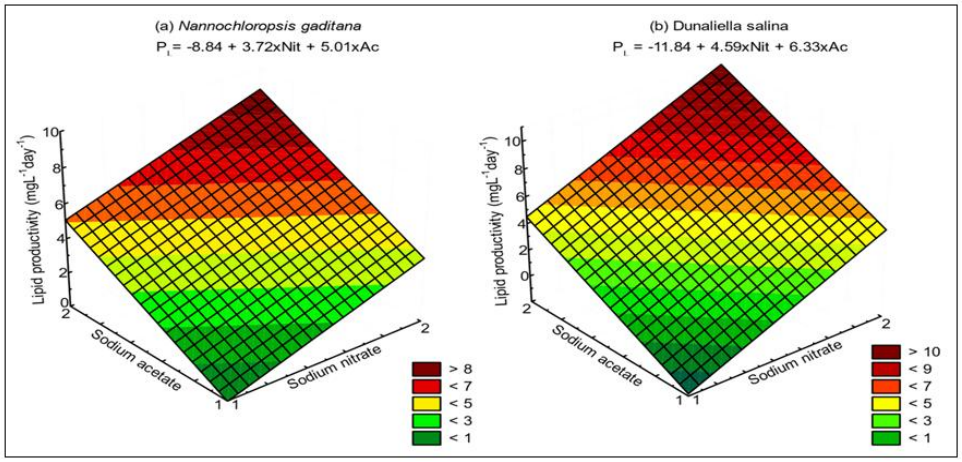

Fig. 3. Plot of model predictions for the lipid productivity (P_L) of (a) *Nannochloropsis gaditana* and (b) *Dunaliella* salina, in which sodium bicarbonate concentration was fixed and sodium acetate (Ac) and sodium nitrate (Nit) concentrations were allowed to vary freely

Table 4. Major FAMEs in oils extracted from *Nannochloropsis gaditana* and *Dunaliella salina* cultivated under the best conditions

FAME	*N. gaditana*	*D. salina*
Saturated fatty acids (SFAs) (%)		
Capric acid (C10:0)	0.12 ± 0.01	0.93 ± 0.02
Lauric acid (C12:0)	0.24 ± 0.02	20.4 ± 0.41
Myristic acid (C14:0)	0.84 ± 0.02	7.11 ± 0.14
Pentadecanoic acid (C15:0)	0.11 ± 0.01	n.d.
Palmitic acid (16:0)	32.81 ± 0.66	10.37 ± 0.21
Margaric acid (C17:0)	0.14 ± 0.03	0.32 ± 0.01
Stearic acid (C18:0)	10.15 ± 0.20	3.15 ± 0.06
Arachidic acid (C20:0)	3.21 ± 0.06	0.32 ± 0.00
Heneicosanoic acid (C21:0)	2.03 ± 0.04	0.47 ± 0.01
Behenic acid (C22:0)	0.2 ± 0.01	n.d.
Tricosanoic acid (C23:0)	0.36 ± 0.01	n.d.
Monounsaturated fatty acids (MUFAs) (%)		
Myristoleic acid (C14:1)	0.54 ± 0.01	1.34 ± 0.03
Palmitoleic acid (C16:1)	0.39 ± 0.01	2.34 ± 0.05
cis-10-Heptadecenoic acid (C17:1)	0.11 ± 0.02	0.97 ± 0.02
Oleic acid (C18:1n9c)	24.71 ± 0.49	27.48 ± 0.55
Erucic acid (C22:1n9)	0.59 ± 0.01	n.d.
Nervonic acid (C24:1n9)	0.41 ± 0.01	n.d.
Polyunsaturated fatty acids (PUFAs) (%)		
Linoleic acid (C18:2n6c)	0.93 ± 0.02	14.65 ± 0.29
Gama-linolenic acid (C18:3n6)	0.8 ± 0.016	2.27 ± 0.04
cis-11,14-Eicosadienoic acid (C20:2)	0.39 ± 0.08	0.19 ± 0.00
Arachidonic acid (C20:4n6)	2.06 ± 0.04	n.d.
cis-13,16-Docosadienoic acid (C22:2)	0.57 ± 0.01	n.d.
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	0.77 ± 0.01	n.d.

n.d. = not detected

Properties of *N. gaditana* and *D. salina* biodiesels were estimated on the basis of their FAME profiles (Table 5). In Table 5, we also report the biodiesel properties of other microalgal oils, obtained from literature data, for comparison purposes.

Table 5. Comparison of estimated properties for *Nannochloropsis gaditana* and *Dunaliella salina* oil biodiesel with literature data

Microalgae	DU	SV	IV g I_2 $100g^{-1}$ oil	CN	LCSF	CP (°C)	OS (h)	HHV	Ref.
Nannochloropsis gaditana	32.6	158.6	33.3	73.2	11.8	12.2	70.7	30.2	this study
Dunaliella salina	63.6	201.0	59.8	59.9	2.9	0.4	9.5	34.6	this study
Trichormus sp.	112.0	189.0	103.9	51.8	2.6	0.8	6.3	31.1	[28]
Nannochloropsis oculata			70.0	57.7		9.6		39.8	[29]
Chlorella sorokiniana			49.3–62.2	58.4–59.5		11.0–13.4		39.4–39.7	[30]
Scenedemus sp.			60.9–66.3	58.0–58.5		10.3–11.3		39.6–39.8	[30]
Nannochloropsis oceanica	57.4	200	94.7	52.3	6.7				[31]

DU, degree of unsaturation; SV, saponification value; IV, iodine value; CN, cetane number; LCSF, long chain saturated factor; CP, cloud point; OS, oxidation stability; HHV, higher heating value

Cetane number (CN) is a property associated with ignition delay time. A high CN value indicates good ignition properties and combustion quality [29,30]. The ASTM D6751 standard specification for biodiesel sets a minimum CV of 51, whereas EN 14214 establishes a lower limit of 47. The CN value predicted for *D. salina* biodiesel was similar to those of the literature, whereas the CN value for *N. gaditana* biodiesel was slightly higher than those presented in the literature. Both CN values were in accordance with the specifications.

The iodine value (IV) represents the amount of I_2 that can react with 100 g of oil. I_2 reacts with the double bonds of FAME; thus, the higher the IV, the more unsaturated the biodiesel [29,30]. *N. gaditana* and *D. salina* biodiesels (IV of 33.36 and 59.80, respectively) were within the upper limit of the EN 14214 standard for IV, 120. A high IV is associated with low oxidation stability. IV of *N. gaditana* biodiesel was markedly lower than that of other microalgal biodiesels, indicating a high saturated fatty acid content. Consequently, *N. gaditana* biodiesel had higher oxidation stability (OS) and long-chain saturated factor (LCSF) values than *D. salina* biodiesel and *Trichormus* sp. biodiesel [28]. The IV of *D. salina* biodiesel was similar to those of *Nannochloropsis oculata* [29], *Chlorella sorokiniana* [30], and *Scenedemus* sp. [30] biodiesels.

Cloud point (CP) and cold filter plugging point (CFPP) are important temperature parameters. CP is the temperature at which the fuel begins to form wax crystals and takes on a cloudy appearance. Because wax crystals can block pipes and internal systems, the lower the CP, the better the biodiesel [29,30]. *D. salina* biodiesel had the best predicted CP value (0.46°C), which was lower than that of the literature. This result indicates that biodiesel produced from *D. salina* oil obtained in this study could be used at low temperatures without precipitation of solid material.

CFPP is the lowest temperature at which the biodiesel exhibits adequate flow performance [29,30]. Operating a fuel below its CFPP can cause filter or pipeline blockage. Thus, fuels with low CFPP values are preferred. *D. salina* biodiesel showed a better CFPP value (−7.27°C) than *N. oculata* biodiesel (4.7°C) [31] and a similar value to *Trichormus* sp. biodiesel (−8.3°C) [28]. The standards for CFPP vary from country to country. A low CFPP means that the fuel can operate in colder environments.

4. CONCLUSION

Considering the obtained results, both studied carbon sources can increase the biomass productivity, for both *N. gaditana* and *D. salina microalgae*. The sodium acetate, however, was the only able to enhance the lipid productivity, thus being a better carbon source for oil production purposes. The FAME profiles of the obtained oils were considerably different between the two studied microalgae, which resulted in different biodiesel properties. *D. salina* oil showed a hither range of main FAMEs (C16:0), stearic (C18:1), oleic (C18:1), linoleic (C18:2), lauric (C12:0) and myristic (C14:0) acids. The main FAME for *N. gaditana* were C16:0, C18:0 and C18:1. *N. gaditana* obtained oil, showed a slightly higher cetane number (73.20), in relation to *D. salina* oil (59.59). Both results in accordance with the standard specifications. The biodiesel estimated for *D. salina*, however, due to its higher amounts of unsaturated fatty acids, showed better thermodynamic characteristics, with lower cloud point (0.46°C) and lower cold filter plugging point (-7.27°C).

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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