*Research Article*

# Preliminary evaluation of the green microalga *Dunaliella salina* as a potential feedstock for biodiesel: effect of molasses on growth and lipid profile

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**ABSTRACT.** This research aims to evaluate the effect of molasses as a source of organic carbon on the growth and lipid profile of *Dunaliella salina* cultivated under different light regimes to determine its strategy and potential use to produce biodiesel. Two sources of nutrients (F/2 medium, F/2; molasses, M) and two light conditions (12 h light: 12 h darkness, 12L:12D) conformed four treatments (F/2 12L:12D, F/2 D, M 12L:12D, and MD) that were investigated with three replicates each. The concentration of microalgae produced by the groups under light conditions was more than double that of those grown in darkness. The highest biomass produced ($46.91 \pm 6.47$ g $L^{-1}$) was obtained in the F/2 medium with 12L:12D treatment, while the lowest ($18.99 \pm 0.78$ g $L^{-1}$) was in the M 12L:12D group. Regardless of the culture conditions, the lipids were mainly composed of the C16:0 and C18 fatty acids. The use of molasses as a culture medium produced the highest amount of C18:1, regardless of the light regime. Molasses promoted the highest production of fatty acids methyl esters. Depending on the culture medium, *D. salina* cells showed different percentages of saturated (SFA), monounsaturated (MFA), and polyunsaturated (PUFA) fatty acids. The highest amount of saturated fatty acids was obtained in F/2 12L:12D. The highest amount of polyunsaturated fatty acids was presented in the mixotrophic culture (molasses and light). The cultures grown with molasses were rich in monounsaturated fatty acids. An inexpensive industrial by-product, Cane molasses could be a low-cost feedstock for biodiesel in cultivating *D. salina*.

**Keywords:** *Dunaliella salina*; molasses; feedstock; fatty acids profile; biofuel, biomass production

## INTRODUCTION

The current global economic development faces a severe crisis due to the energy supply since the reserves of fossil fuels tend to run out due to the growing demand (Martins et al. 2019). On the other hand, this energy source is causing negative impacts on human health, social development, economy, and environment (Khan et al. 2016), which are exacerbated even more in undeveloped countries (Nawas & Alvi 2018, Pereira 2018). For decades, the challenge to face this environ-mental and energy crisis has focused on searching for alternative energy sources that are sustainable and friendly to the environment (Handayani et al. 2019). One of the most promising alternative energy options is biodiesel (Gaurav et al. 2016, Londoño-Pulgarin et al. 2020), which has environmentally favorable properties since it mitigates the atmospheric $CO_2$ increase (Naik et al. 2010), is biodegradable, does not contain aromatic compounds, and is non-toxic (Hakeem et al. 2014). Commonly, biodiesel is mainly obtained from grain and seed oil (Fracchia et al. 2016, Wisniewski &

Kistowski 2020). However, the viability of this type of energy oil is questioned due to the space necessary to produce the seeds and all the agricultural inputs necessary for their production, including large volumes of water (Pikula et al. 2020).

The properties of biodiesel depend on the quality of the lipids; thus, the polyunsaturated fatty acids are less desirable and could adversely affect their properties since they decrease the cetane number and the oxidation stability of the biodiesel (Knothe 2009, Pinzi et al. 2009). Besides, triglycerides rich in saturated and monounsaturated fatty acids in the range of C16-C20, especially palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1), are ideal for producing biodiesel (Arias-Peñaranda et al. 2013, He et al. 2015). However, linoleic acid (C18:2) and linolenic acid (C18:3) provides flow properties for climates cold in very specific areas (He et al. 2015).

The production of biodiesel from microalgae is under intense investigation due to its promise of sustainable biofuels (Hallenbeck et al. 2016). They are the only sources of biodiesel that have the potential to displace diesel; for instance, the biodiesel production of some oil sources is in the range of 152 to 4747 kg of biodiesel produced per year, while microalgae (*Pavlova salina*, *Ankistrodesmus* sp., *Chlorella*, *Nannochloris* sp., *Neochloris oleoabundans* and *Botryococcus braunii*) are in the range of 51,921 to 121,104 kg of biodiesel produced per year (Mata et al. 2010). Many environmental factors influence microalgae lipids and fatty acids' growth, content, and composition (Gim et al. 2016). Therefore, some strategies are applied to improve the growth of microalgae and biochemical composition; these include the optimization of medium content (Mata et al. 2010, Engin et al. 2017), the conditions of the physical parameters (Nogueira et al. 2015), and the type of metabolism depending on the carbon source (Perez-Garcia et al. 2010, 2011, George et al. 2014). It is recognized that the high cost of carbon source for biodiesel production represents almost 50% of the cost of the medium in microalgae cultivation (Cheng et al. 2009); this way, alternative low-cost carbon sources as industrial by-product sugars have been reported as feedstock for biodiesel from microalgae cultivation (Hawed & Klöck 2014, Piasecka et al. 2017).

*Dunaliella salina* is a faster growth and easy cultivation green microalga and can accumulate polyunsaturated fatty acids required to produce biodiesel (Morais Jr. et al. 2020). This chlorophyte species can adapt its metabolism to a wide range of salinity and variations in temperature and intensity of light (Bonnefond et al. 2016). At the global trans-criptomic level, the response of *D. salina* to hypersaline stress is systematic work, possibly involving enhancement of photosynthesis and carbon fixation (He et al. 2020). The manipulation of culture conditions in *D. salina* can lead to fluctuations in quality and concentration of lipids (Fakhry & El Maghraby 2013) whether varying the concentrations of $CO_2$ (Abd El Baky et al. 2014), using different organic media (Gim et al. 2016) and changing photoperiod (Nzayisenga et al. 2020). So far, the use of molasses as a potential feedstock for growing *D. salina* has not been reported.

In this preliminary study, the use of industrially low-cost cane molasses as a carbon source for the laboratory-scale lipid profile of *D. salina* under different light conditions was evaluated to determine its strategy production as a biofuel source.

## MATERIALS AND METHODS

### Algal strain and culture conditions

The microalgae *Dunaliella salina* was obtained from Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Baja California, Mexico. The *Dunaliella* strain was kept in Petri dishes with 1.5% bacteriological agar (BD®), in test tubes (10 mL) and Erlenmeyer flasks (150 and 250 mL) containing F/2 medium (Guillard & Ryther 1962; SIGMA® G0154-500), which was sterilized using an autoclave at 121°C for 15 min. The culture was kept in seawater (salinity of 35 g $L^{-1}$) at 24°C with a photoperiod (12 h light:12 h darkness, 12L:12D). The test tubes were shaken twice a day to keep the microalgae in suspension, while the flasks were constantly aerated using glass tubes and an aquarium air pump. Once the microalgae reached the exponential phase, they were used as seed cells for heterotrophic batch cultivation, increasing the culture volume. This way, before starting the experiment, the microalga strain was split by increasing the volume every 10 days, starting with an inoculum of 50 mL until reaching a volume of 16 L needed for starting the bioassay (Medina et al. 2012).

### Treatment of molasses

The cane molasses was obtained from a local vendor (Ingenio Los Mochis, Sinaloa, México). Its proximal content and fatty acid profile are shown in Table 1.

The molasses was treated according to the methodology of Roukas (1998) and Liu et al. (2008) as follows: it was diluted in distilled water (10%, p/v), adjusting to pH 3 with $H_2SO_4$ during 24 h; after that, it was centrifuged at 5000 g for 15 min. The pH of the clarified liquid was adjusted at 8 using NaOH 10N; fina-

**Table 1.** Proximal content and fatty acid profile (%, dry weight) of cane molasses.

| Constituent | Dry weight basis (%) |
|---|---|
| Humidity | 18.55 |
| Crude protein | 2.31 |
| Crude fiber | 0.00 |
| Crude fat | 0.14 |
| Carbohydrates | 66.79 |
| Ash | 12.21 |
| **Macronutrients** | **%** |
| Na | 0.18 |
| K | 3.81 |
| Ca | 0.83 |
| Mg | 0.27 |
| P | 0.19 |
| **Micronutrients** | **mg kg$^{-1}$** |
| Fe | 214.23 |
| Mn | 26.74 |
| Zn | 7.13 |
| Cu | 10.35 |
| **Fatty acids** | **% FA** |
| C16:0 | 17.83 |
| C18:1 | 28.96 |
| C18:2 | 48.77 |
| C18:3 | 3.51 |
| C20:0 | 0.93 |

lly, the supernatant was autoclaved at 121°C for 15 min. Ten milliliters of treated molasses per liter of seawater (equivalent to 1 g L$^{-1}$ of cane molasses).

### Experimental design

Experimental units of 19 L inoculated with 4 L of *D. salina*, and 12 L of seawater containing culture medium (F/2 or molasses) were used to adjust an initial density of 400,000 cell mL$^{-1}$. The effect of molasses (M) on growth, biomass production, proximal composition, lipid distribution, and fatty acid profile of *D. salina* were compared with the F/2 medium, under two lighting regimes: 12L:12D and darkness (D); the four treatments (F/2 medium with 12L:12D, F/2 D, M with 12L:12D, and MD) were evaluated with three replicates. The light intensity for the 12L:12D treatments was adjusted to 2600-3200 lux with cold light lamps. The water temperature, salinity, and pH of the water (24 ± 2°C, 35 g L$^{-1}$, and 8.2, respectively), were kept within the acceptable ranges for the cultivation of microalgae (Vonshak 1993). Microalgae counts were performed daily using a hemocytometer (Camacho-Fernández et al. 2018).

### Harvest of *Dunaliella salina*

The microalgal biomass was harvested according to the methodology proposed by Besson & Guiraud (2013).

First, 10N NaOH was added to the experimental units to increase the pH to 10, it was allowed to settle, and the algal precipitate was obtained by extracting the largest amount of supernatant. The biomass was placed in trays and dried using an oven at 60°C for 72 h. Finally, the dry biomass of each replica was pulverized using a ball mill (Quiagn®), weighed, and stored frozen (-4°C) for later analysis.

### Biomass analysis

The amount of proteins, crude fiber, carbohydrates, ash, and moisture of microalgal biomass were determined with the standard methodology of AOAC (1998).

### Percentage in lipids

The evaluation of lipid concentration was using method 920.39 of AOAC (1998), for which fat extraction equipment was adapted (Extraction Unit E-816 brand BUCHI®). 2 g of sample were weighed on Whatman #41 filter paper (P1) to carry out the extraction and placed in thimbles inside the extraction equipment, where 150 mL of hexane was added to each thimble. The extraction temperature was kept at 100°C for 5 h. After that, the samples were placed at 70°C for 24 h to finally be weighed (P2), according to the following equation:

$$\% \text{ Fat} = \frac{(P2-P1)}{M} \times 100$$

where: P1: initial weight of the glass, P2: final weight of the glass, M: g of the sample.

### Fatty acids composition

Lipid acid profile was determined according to the method of Folch 1956 (AOAC 1998). Firstly, a sample of 2 g was mixed with 10 mL methanol and 20 mL chloroform and then homogenized using an Ultraturrax (IKA T18 digital), previous to vacuum filtered. After that, a solution of 0.88% potassium chloride in methanol:water (5:5 v/v) was added to the extract in order to separate the fatty acids in a Büchi rotary evaporator (Labortechnik AG, Switzerland), according to the Folch method (AOAC 1998). The samples were methylated by adding 5 mL solution of 0.5 N NaOH and 10 mL of boron-methanol trifluoride (BF$_3$), and 5 mL of heptane to the lipid content that remained in the ball flask. The methylation product was mixed with 0.5g of NaCl and 0.5g of Na$_2$SO$_4$ for purification; then, it was passed through a fiberglass-packed Pasteur pipette. The solution was recovered in a 2 mL amber vial, keeping it under a nitrogen atmosphere (AOAC 1998, method 969.33). Finally, a sample of 1 µL of the final extract was injected into an Agilent model 7820 gas chromatograph (USA), which was equipped with a flame ionization detector and a 30 m × 0.32 mm × 0.25

μm capillary column (Omega wax 320, Supelco). The temperature of the injector and detector was programmed at 260°C for 40 min using helium 3 mL min$^{-1}$ as a carrier gas. For the separation and quantification of the fatty acids, a mixture of 37 fatty acids of C4-C24 methyl esters (Supelco) was used as an external standard. The standardization method quantified the results using the area under the curve of the peaks of the identified fatty acids.

## Statistical analysis

All graphs and statistical analyses were conducted using Statistica 7 Statistical Software. Normality and homoscedasticity tests were not applied. One-way analysis of variance (ANOVA) tests was performed to determine significant differences among the treatments; then, differences among means were tested using Tukey's test ($P < 0.05$). The values are expressed as mean ± standard error.

## RESULTS

### Growth of *Dunaliella salina*

The growth of the microalgae showed significant differences between the treatments ($F_{(3,244)} = 16.654$, $P < 0.05$). The groups with light showed greater growth than those without light: F/2 medium with 12L:12D maintained an exponential growth until day 13 of culture (14.53E5 ± 0.45E5 cell mL$^{-1}$), while M 12L:12D grew until day 10 (9.91E5 ± 1.94E5 cell mL$^{-1}$) to decay at the end of the culture (7.20E5 ± 0.45E5 cell mL$^{-1}$). The groups without light did not show the typical phases of algal growth (onset, exponential, and plateau). The algal concentration decreased in the first days of culture (Fig. 1). On day 8 of culture, F/2 registered a final concentration (1.90E5 ± 0.36E5 cell mL$^{-1}$) of *D. salina*, which was lower than the initial one (5.46E5 ± 2.39E5 cell mL$^{-1}$).

The highest biomass produced (2.93 ± 0.40 g L$^{-1}$) was obtained in the F/2 12L:12D treatment, while the lowest quantity (1.19 ± 0.0.5 g L$^{-1}$) was recorded in the F/2D group (Fig. 2).

The organic matter biomass showed significant differences between the treatments ($F_{(7,16)} = 10.713$, $P < 0.05$), with an interval from 20.53 ± 0.32% in the MD group, to 18.09 ± 0.62% obtained for F/2D (Table 2).

The highest amount of lipids extracted from the organic matter of the microalgal biomass was found in the treatments using light. The M 12L:12L group registered the highest lipid percentage (3.62 ± 0.89%), being similar to F/2 12L:12D (3.17 ± 0.41%) but approximately three times higher than the treatments

without light (Table 3). The fiber was not detected in any of the treatments.

The fatty acid profile of *D. salina* cultured with molasses and the F/2 medium under light and dark conditions are summarized in Table 4. Regardless of the culture conditions, the lipids were mainly composed of the C16:0 and C18 (C18:0, C18:1, C18:2, C18:3). C18:0 was only found in the treatment F/2 12L:12D.

Using molasses as a culture medium produced the highest amount of C18:1, regardless of the illumination (38.93 ± 0.50 and 46.05 ± 0.57% for M 12L:12D and MD, respectively). Specifically, molasses promoted the highest production of C18:2 (22.85 ± 0.82%) and C16:0 (31.22 ± 1.66%) under light and dark conditions. Depending on the culture medium, *D. salina* cells showed different percentages of saturated (SFA), monounsaturated (MFA), and polyunsaturated (PUFA) fatty acids. The highest SFA content (39.10 ± 1.59%) was obtained in F/2 12L:12D. The cultures grown with molasses were rich in MFA (M 12L:12D = 38.93 ± 0.50%; MD = 46.05%) regardless of the light regime. The highest amount of PUFA (34.84 ± 1.56%) was presented in the mixotrophic culture (molasses and light).

## DISCUSSION

When culturing microalgae, the appearance and duration of the growth phases are a function of the amount of available nutrients, which, in static cultures as in the present trial, decrease as they are consumed (Bumbak et al. 2011). The nutritive substrates used exerted different effects on the growth dynamics of *Dunaliella salina*. The medium F/2 12L:12D promoted the growth of the microalgae, which remained in an exponential phase until the end of the culture (day 13), with its maximum concentration reached (14.53 ± 0.45E5 cell mL$^{-1}$) up to three times higher than those treatments using molasses as a carbon source, regardless of the light regime. This microalgal density was lower than that reported by Keerthi et al. (2015) for the same species with Walne's medium and with an optimized mineral medium (18.60 ± 0.10E5 and 20.10 ± 0.2E5 cell mL$^{-1}$, respectively), but similar to that documented by Colusse et al. (2020) with the F/2 medium (16.20 ± 1.06E5 cell mL$^{-1}$) and higher than that found using the Johnson medium (1.21 ± 0.30E5 cell mL$^{-1}$). The discrepancies between the previous results can be partially explained by differences in some cultivation factors such as the strain used, the initial density and the sowing phase, the material and volume of the experimental containers, and the cultivation conditions, among others.
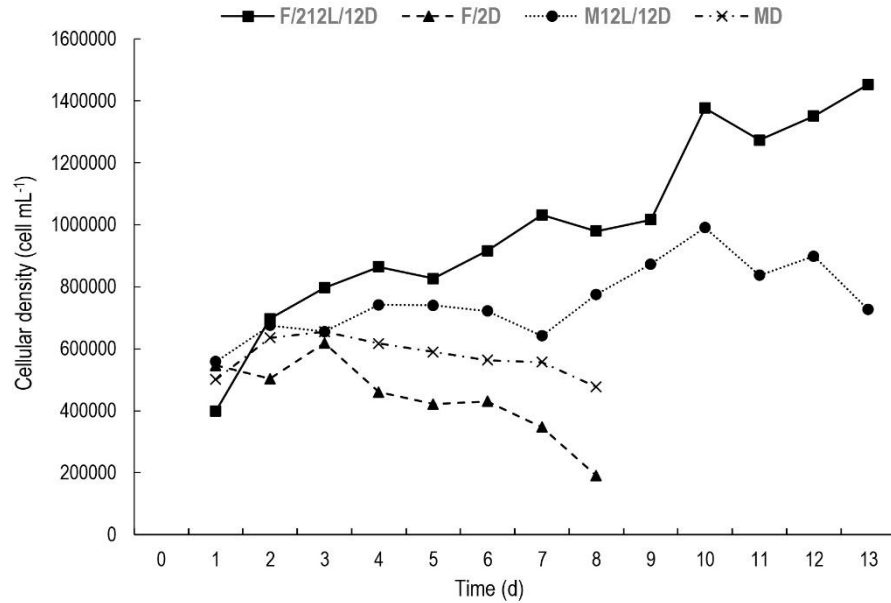
**Figure 1.** Dynamics of the growth (cell mL$^{-1}$) of *Dunaliella salina* cultivated with two culture mediums under different light regimes. F/2 12L:12D, F/2 medium under light conditions; M 12L:12D: molasses under light conditions; F/2D F/2 medium under dark conditions; MD: molasses under darkness conditions.
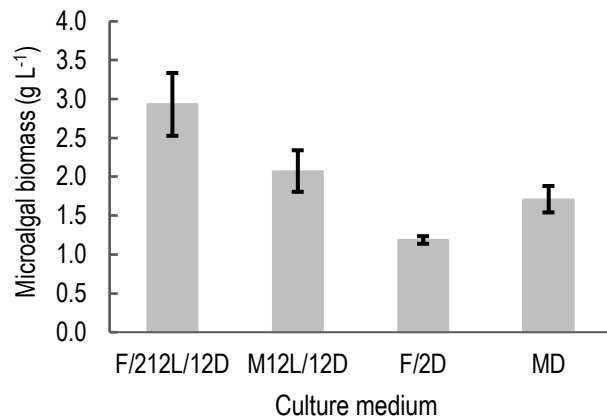


**Figure 2.** Biomass production (g L$^{-1}$) of *Dunaliella salina* cultivated with two culture mediums under different light regimes. F/2 12L/12D: F/2 medium under light conditions; M 12L:12D: molasses under light conditions; F/2D: F/2 medium under dark conditions; MD: molasses under dark conditions.

**Table 2**. Organic matter (%) in *Dunaliella salina* biomass cultivated with two culture mediums under different light regimes. F/2 12L:12D, F/2 medium under light conditions; M 12L:12D: molasses under light conditions; F/2D: F/2 medium under dark conditions; MD: molasses under dark conditions. In the column, the different letter means significant differences ($P \le 0.05$).

| Culture medium | Organic matter (%) | Ash (%) |
|---|---|---|
| F/2 12L:12D | 18.44 ± 0.32[ab] | 81.56 ± 0.32 |
| F/2D | 18.09 ± 0.62[b] | 81.91 ± 0.62 |
| M 12L:12D | 19.35 ± 0.42[ab] | 80.65 ± 0.42 |
| MD | 20.53 ± 0.32[a] | 79.47 ± 0.32 |

Specifically, a nutrient substrate deficient in the quality or quantity of its constituents prevents the optimal development of the crop. For example, Borowitzka et al. (1984) mention that nitrates represent the limiting nutrient for the growth of *D. salina*. Sodium nitrate (NaNO$_3$) is the main source of nitrogen in the F/2 medium (Kang et al. 2011), but it was not determined in molasses. On the other hand, the concentration of molasses used as a carbon source in microalgae cultivation varies the biomass production.

Piasecka et al. (2017) concluded that a concentration of less than 20 g L$^{-1}$ of molasses seems to be appropriate to obtain the maximum biomass of the *Parachlorella kessleri* microalgae, coinciding with Yeesang & Cheirslip (2014), who harvested the highest amount of biomass of *Botryococcus braunii* including 15 g L$^{-1}$ molasses. Although the amount of molasses used in this study (1 g mL$^{-1}$) is within the range proposed by the authors mentioned above, it may have been insufficient to reach the maximum biomass yield of *D. salina*. Increasing the amount of molasses in cultivating this microalga requires future research to optimize its biomass yield.

The culture medium also interferes with the metabolism of microalgae, affecting the production of molecules such as pigments (Wu et al. 2016, Camacho-Rodríguez et al. 2020), lipids (Abd El Baky et al. 2014),

**Table 3.** Proximate composition (%, mean ± standard error) of *Dunaliella salina* cultivated with different culture mediums and light conditions. *F/2 12L:12D: F/2 medium under light conditions; M 12L:12D: molasses under light conditions; F/2D: F/2 medium under dark conditions; MD: molasses under dark conditions. By column, the different letter means significant differences ($P \leq 0.05$).

| Treatments | Crude fat | Crude protein | Carbohydrates | Humidity |
|---|---|---|---|---|
| F/2 12L:12D* | $3.17 \pm 0.41^b$ | $31.62 \pm 2.87^a$ | $59.76 \pm 3.26^b$ | $5.44 \pm 0.09^b$ |
| M 12L:12D | $3.62 \pm 0.89^b$ | $33.33 \pm 1.68^a$ | $57.85 \pm 1.27^b$ | $5.20 \pm 0.11^{ab}$ |
| F/2D | $1.31 \pm 0.20^a$ | $50.27 \pm 1.36^b$ | $42.92 \pm 1.42^a$ | $5.51 \pm 0.18^b$ |
| MD | $1.14 \pm 0.06^a$ | $47.13 \pm 4.75^b$ | $46.83 \pm 4.78^a$ | $4.89 \pm 0.09^a$ |

**Table 4.** Fatty acid methyl ester (FAME) profile (%) of the *Dunaliella salina* microalga cultivated with different culture media and light conditions. *C16:0: palmitic acid, C18: stearic acid, C18:1: oleic acid, C18:2: linoleic acid, C18:3: linolenic acid, SFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, ND: no detected. By line, the different letter means significant differences ($P \leq 0.05$).

| FAME (%) | F/2 12L:12D | M 12L:12D | F/2D | MD |
|---|---|---|---|---|
| C16:0* | $33.55 \pm 2.18$ | $26.24 \pm 1.07$ | $27.24 \pm 1.43$ | $31.22 \pm 1.66$ |
| C18:0 | $5.55 \pm 0.62$ | ND | ND | ND |
| C18:1 | $33.39 \pm 2.41^a$ | $38.93 \pm 0.50^b$ | $42.60 \pm 3.08^c$ | $46.05 \pm 0.57^c$ |
| C18:2 | $7.24 \pm 0.64^a$ | $22.85 \pm 0.82^c$ | $11.17 \pm 1.15^b$ | $10.67 \pm 0.84^b$ |
| C18:3 | $20.27 \pm 3.52^{ab}$ | $27.08 \pm 1.84^c$ | $18.99 \pm 3.92^{ab}$ | $12.06 \pm 3.11^a$ |
| SFA | $39.10 \pm 1.59^c$ | $26.24 \pm 1.07^a$ | $27.24 \pm 1.43^a$ | $31.22 \pm 1.66^b$ |
| MUFA | $33.39 \pm 2.41^a$ | $38.93 \pm 0.50^b$ | $42.60 \pm 3.08^c$ | $46.05 \pm 0.57^c$ |
| PUFA | $27.51 \pm 3.93^{ab}$ | $34.84 \pm 1.56^c$ | $30.16 \pm 2.30^{bc}$ | $22.73 \pm 2.17^a$ |
| Degree of unsaturation MUFA+2(PUFA) | $88.40 \pm 9.52a$ | $108.6 \pm 4.55^b$ | $102.93 \pm 3.94^{ab}$ | $91.509 \pm 6.62^{ab}$ |

and acids fatty (Castillo-Casadiego et al. 2016). The lipid content in *D. salina* was affected by light rather than by the nutritive substrate used. The autotrophic cultures yielded higher lipid levels by sugars produced by $CO_2$ and light, coinciding with Smetana et al. (2017). Under heterotrophic conditions, Dubey et al. (2015) and Zheng et al. (2015) documented that microalga can yield higher lipid concentration through shorter metabolic pathways since organic carbon is directly converted into lipids. In this study, MD promoted a higher biomass and microalgae growth than F/2D, suggesting that, under dark conditions, the inclusion of molasses as an organic carbon source exerted a beneficial effect on *D. salina* lipid production. The lipid concentrations in the molasses treatments of the present work were three times lower than the groups that received illumination for 12 h, which coincides with the lipid accumulation trend of *Scenedesmus abundans* (Rai & Gupta 2017) with relation to biomass production in the presence of light. However, the adjustment of the photoperiod (Piligaev et al. 2015) represents a line of future research to optimize the biomass and lipid production of *D. salina* for its use as biodiesel.

The biodiesel quality is determined by the saturated to unsaturated fatty acids ratio (Radakovits et al. 2010).

The C16:1 and C18:1 fatty acids are most favorable for biodiesel production (Durett et al. 2008, Nascimento et al. 2012). Saturated fatty acids are resistant to degradation and, therefore, increase biodiesel's longevity and increase biodiesel's resistance to oxidation under hot climatic conditions, while unsaturated fatty acids enhance cold flow characteristics (Talebi et al. 2013). In the present study, the treatments that showed high concentrations of saturated fatty acids were F/2 12L:12D and MD. Liang et al. (2020) showed that high $CO_2$ concentrations (1000 ppm) reduced C16:0 and total saturated fatty acids, suggesting that molasses also reduced the total saturated fatty acids production.

Nowadays, microalgal biomass is a potential and sustainable renewable energy source for biodiesel production. Besides, molasses represents a low-cost agricultural by-product for microalgae production that would help in reducing the cost of biomass production (Bellou et al. 2014). As a Chlorophyceae, *D. salina* mostly presented C16:0 and C18:0 fatty acids; monounsaturated fatty acids (C18:1, cis-9) were found in all treatments. Liu et al. (2013) reported the highest content of C18:1 in *Chlorella zofingiensis* (35.2% of total fatty acids) grown in the dark under heterotrophic and mixotrophic conditions using molasses as carbon source; in our study, the MD group obtained 46.05% of

C18:1, reinforcing the potential of molasses as an alternative carbon source for cultivating microalgae for the production of biodiesel. On the other hand, the M 12L:12D group contained the higher concentrations of C18:2 and C18:3, suggesting that molasses encouraged the production of PUFA's. Similar observations were given by Perez-Garcia et al. (2011) and Piasecka et al. (2017), who mentioned that the molasses promoted polyunsaturated fatty acids.

There was no trend in SFA, MUFA, and PUFA found in the experimental treatments. The highest levels for SFA, MUFA, and PUFA were obtained in the F/2 12L:12D (39.10 ± 1.59%), MD (46.05 ± 0.57%), and M 12L:12D (34.84 ± 1.56%) groups, respectively. It has been noted that SFA, MUFA, and PUFA vary according to the microalgae strain and species. Comparing with our results and working with different *D. salina* strains, Suárez et al. (2007) reported lower mean values of SFA (24.09%) and MUFA (7.19%), but higher levels of PUFA (67.37%), meanwhile, Fakhry & El Maghraby (2013) obtained 34.76, 32.18, and 33.06% of SFA, MUFA, and PUFA, respectively. Differences in results may be due to several factors such as general culture conditions, source of nutrients, and the algal strain itself. Abd El Baky et al. (2014) studied the effect of different $CO_2$ levels on the fatty acid profile of *D. salina,* obtaining 82-85% of SFA, 1.8 to 3.8% of MUFA, and 8.35 to 13.84% of PUFA. These authors noted that the percentages of lipids vary among the same species of *D. salina* depending on the culture conditions.

At the industrial level, one disadvantage of biodiesel from the M12L:12D treatment is its content of high PUFA levels, which has negative effects by decreasing its oxidation stability and reducing the cetane number (Pinzi et al. 2009), as well as being susceptible to the appearance of undesirable odors and flavors because the formation of aldehydes and alcohols caused by peroxides and hydroperoxides. However, this group may exhibit low fluency temperature due to its low content of saturated fatty acids (Bahadur et al. 2013).

## CONCLUSIONS

The preferable biodiesel fuel consists of long-chain fatty acids (C16 and C18) produced by *D. salina* in this preliminary study with the M 12L:12D and MD treatments. Molasses as a culture medium affected *D. salina* growth, biomass, biochemical content, and fatty acid profile. The addition of molasses to the culture medium significantly changed the yield and composition of fatty acids methyl esters. Since microalgae containing lower contents of PUFA's are recommended for biodiesel, the MD group should be studied as a potential feedstock for biofuel by optimizing the culture conditions of *D. salina*. Cane molasses, a low-cost industrial by-product, could be a low-cost feedstock for biodiesel in cultivating *D. salina*.

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