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EFFECTS OF TEMPERATURE AND NITROGEN LIMITATION ON GROWTH KINETICS, PROXIMATE COMPOSITION AND FATTY ACID PROFILE OF *Nannochloropsis* sp.

EFECTOS DE LA TEMPERATURA Y LIMITACIÓN DE NITRÓGENO EN LA CINÉTICA DE CRECIMIENTO, LA COMPOSICIÓN PROXIMAL Y EL PERFIL DE ÁCIDOS GRASOS DE *Nannochloropsis* sp.

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Abstract

Microalgae have received significant interest as a potential feedstock for the production of biofuels, food, and feed. The effects of temperature and nitrogen limitation on the growth, proximate composition, and fatty acid profile of *Nannochloropsis* sp. were studied using solvent-oil extraction aided by ultrasonic process as a promoter of microalgae cell disruption. Experiments included a control algal culture grown in F medium (F) at 25°C and four cultures grown in F with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, 35°C, and 40°C. The results showed that temperature had significant effects on the cell density and growth rate of *Nannochloropsis* sp. The highest values were obtained when microalgae cultured in F at 25°C, whereas the lowest were obtained in F/2 at 35°C. The protein, carbohydrate, and lipid contents were the highest in F at 25°C and had an increasing tendency in F/2 from 25°C to 30°C; however, the lowest values were obtained in F/2 at 35°C. Both the temperature and nitrogen limitation highly affected the fatty acid profile of *Nannochloropsis* sp. The proportion of polyunsaturated compounds decreased with increasing temperature from 25°C to 35°C, whereas it increased with nitrogen limitation. These results generate alternatives in *Nannochloropsis* sp culture conditions that allow have qualitatively best products in relation to their potential use in food and biofuels.

Keywords: fatty acids, biofuels, *Nannochloropsis* sp., nitrogen limitation, ultrasonic cell disruption.

Resumen

Las microalgas han recibido gran atención en su utilización como materia prima potencial para la producción de biocombustibles, alimentos y otros productos. En este trabajo se estudió el efecto de la temperatura y la limitación de nitrógeno sobre el crecimiento, la composición proximal y el perfil de ácidos grasos en *Nannochloropsis* sp. Para extraer el aceite, se procedió con la utilización de solventes, asistido con ultrasonido. Los experimentos incluyeron un cultivo control en medio F (F) a 25°C y cuatro cultivos F con la mitad de la concentración original de nitrato de sodio (F/2), a 25°C, 30°C, 35°C, y 40°C.Se obtuvo el mayor contenido de proteínas, carbohidratos y lípidos en medio F a 25°C, observándose en F/2 una tendencia creciente en el intervalo de 25°C a 30°C; los valores más bajos fueron obtenidos en F/2 a 35°C. La temperatura y la limitación de nitrógeno influyeron de manera significativa en el perfil de ácidos grasos; la proporción de compuestos poliinsaturados disminuyó con el aumento de temperatura de 25°C a 35°C, mientras que estos se incrementaron con la limitación de nitrógeno. Estos resultados permiten visualizar alternativas en las condiciones de cultivo de *Nannochloropsis* sp, que eventualmente, pudieran conducir hacia la obtención de productos con elevado potencial energético y alimenticio.

Palabras clave: ácidos grasos, biocombustibles, *Nannochloropsis* sp, limitación de nitrógeno, ultrasonido.

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1 Introduction

Microalgae are unicellular photosynthetic organisms with variable size and shape that mainly live in aquatic environments and survive both in marine and fresh waters. They develop various ecological and physiological strategies and contribute 48.5% of primary productivity in the oceans, since they use light energy and carbon dioxide to produce biomass (Field *et al*., 1998; Peña-Salamanca *et al*., 2005; Converti *et al*., 2009; Moazami *et al*., 2012).

The intensive cultivation of marine microalgae has several applications, such as the production of biofuels, food with high nutritional value, aquaculture feed, and pharmaceutical and nutricosmetic products (Converti *et al*., 2009; Liu *et al*., 2013; Robles-Heredia *et al*., 2016). Due to the high photosynthetic efficiency and biomass production rates of marine microalgae, only small tracts of land are required for culture, and as a result, there is no competition with food production (Soto-León *et al*., 2014). Microalgae have colonized a variety of environments, ranging from tropical to arctic waters, since they have some resistance to drought, salinity, or low light conditions. Their versatile metabolism allows them to adapt to hostile environments, such as of low nitrogen, a necessary element for protein synthesis in the cell. The metabolism of microalgae is mainly directed to the synthesis of carbohydrates and lipids (Anzueto, 2008); therefore, their growth requires a sufficient supply of carbon and solar radiation or light (Yoo *et al*., 2010). Under optimal growth conditions, microalgae synthesize membrane lipids and fatty acids as esters of glycerol, while under stressful conditions, many microalgae adapt their metabolism to the formation and accumulation of lipids in the form of triacylglycerol, which is deposited as lipid bodies in the cytosol that constitute energy reserves and carbon sources (Hu *et al*., 2008). Various factors can induce changes in the growth, metabolic response, and physiological activity of different microalgae species. Under similar culture conditions, microalgae of the same genus or even the same species may vary in weight and proximate composition (Nieves *et al*., 2009; Alsull *et al*., 2012; Zhiyong *et al*., 2013; Olofsson *et al*., 2012).

Previous studies have shown that culture temperature, as well as the composition of culture medium, have significant effects on the growth rate (Montagnes *et al*., 2003), cell size (Atkinson *et al*., 2003) and proximate composition (Thompson *et al*., 1992), and the fatty acid profile (Converti *et al*., 2009; Renaud *et al*., 1999; Taoka *et al*., 2009) of microalgae. Additionally, the lipid content of microalgae can vary between species, while the chemical composition of oil depends on various factors, including microalgae strain, substrate composition, and environmental conditions (Gong and Jiang, 2011). Many microalgae species increase the production of unsaturated fatty acids with decreasing culture temperature (Wu *et al*., 2013).

Microalgae have received considerable interest, because they are considered a natural source of functional compounds such as polyunsaturated fatty acids of the ω-3 (eicosapentaenoic acid [C20:5] and docosahexaenoic acid [C22: 6]) and ω-6 families (arachidonic acid [C20: 4]), liposoluble antioxidants (carotenoids and tocopherols), and water-soluble antioxidants (polyphenols). Additionally, they produce and store lipids as fatty acids, phospholipids, and glycolipids (Cha *et al*., 2012; Arredondo-Vega *et al*., 2013) that can be used for the production of biodiesel by transesterification reactions catalyzed by acids or bases (Muthukumar *et al*., 2012).

The general process for obtaining oil from microalgae consists of three basic stages: cultivation, harvesting, and oil extraction. When the amount of biomass reaches the maximum value in the bioreactor, the separation and/or concentration are carried out using sedimentation, flocculation, centrifugation, and/or filtration, followed by cell lysis and oil extraction. Oil from microalgae biomass can be obtained by Soxhlet extraction with n-hexane (Halim *et al*., 2011; Ranjan *et al*., 2010), supercritical fluid extraction with carbon dioxide (Andrich *et al*., 2005) or methanol (Patil *et al*., 2011), and the method of Bligh and Dyer with a solvent mixture of chloroform/methanol (Bligh and Dyer, 1959). However, there are methods that instead of solvents, they use ball mills and pressure (Richmond, 2004), enzymes (Sander and Murthy, 2009), microwave-assisted pyrolysis (Du *et al*., 2011), pulsed electric field and hydrothermal liquefaction (Brown *et al*., 2010) or ultrasound-assisted solvent extraction. In the ultrasound-assisted extraction, sound waves are propagated through the medium causing cavitation, generating energy for disruption in the cell membranes or cell walls, facilitating both solvent penetration into the cell and the release of components into the continuous phase (Moreno-Castro *et al*., 2015).

Nannochloropsis sp. is a microalgae of very small size, around 4 μm in diameter, which hinders its separation from the culture medium (Barros *et al*., 2015). The specific conditions of the culture

medium affect both the cellular productivity and the biochemical composition of the harvested microalgae, being the limitation of nitrogen and light radiation, two of the most important influlence factors (Angles *et al*., 2017; Fabregas *et al*., 2004). The oil content in *Nannochloropsis* sp. can oscillate at 40-50% dry basis. The composition of the oil includes the presence of palmitic acid, palmitoleic acid, oleic acid, myristic acid, stearic acid and eicosapentaenoic acid (EPA); Grouping them by their degree of unsaturation, the proportion corresponds to the following order: saturated fatty acids (SFA) > monounsaturated fatty acids (MUFA) > polyunsaturated fatty acids (PUFA) (Angles *et al*., 2017).

The objectives of this study were to evaluate the effects of temperature and nitrogen limitation on: (a) the growth of *Nannochloropsis* sp., (b) the proximate composition of microalgae biomass, and (c) the fatty acid profile of oil extracted by the Bligh and Dyer method, aided with ultrasonic irradiation.

2 Materials and methods

2.1 Strain

The strain of *Nannochloropsis* sp. that was evaluated in this study was selected for its high content in fatty acids as reported by Chisti (2007) and obtained from the Department of Aquaculture, Center for Scientific Research and Higher Education of Ensenada (CICESE).

2.2 Cultivation and growth kinetics

Seawater from the Mazatlan Bay, Mexico was filtered using activated carbon water filters of 10, 5, and 1 microns and disinfected using 5% v/v sodium hypochlorite at a rate of 1 ml L^{-1}. Then, the residual chlorine content in seawater was removed by adding 56 mg of sodium thiosulfate per ml of sodium hypochlorite that was added (Hemerick, 1973) using air bubbling for 20 min. Throughout the experimental process, the salt concentration of seawater was maintained at 35 g L^{-1}, which is the natural salinity in the Mazatlan Bay. The microalgae inoculum was developed by consecutive transfers and used when the cell density reached a concentration of 3×10^5 cells ml^{-1}. F medium (F) nutrients (Guillard and Ryther, 1962) with some modifications as described by Voltolina *et al*., (1989) and F nutrients with half the original concentration of sodium nitrate (75 g L^{-1}; F/2) were added at a rate of 1 ml L^{-1} of seawater for each treatment. Culture media were distributed in 20 plastic containers of 19 L, and microalgae cultures were maintained using a static batch culture technique. The experiments included a control microalgae culture grown in F at 25°C and four cultures grown in F/2 at 25°C, 30°C, 35°C, and 40°C. The treatments with four replicates each were arranged in a completely randomized design. The containers were maintained at the desired temperature using 50-W titanium heaters with an accuracy of ±0.1°C. Continuous aeration was supplied to cultures by a 2.5-hp blower power, previously filtered with a cartridge of 1 micron; the light intensity was 6,000-6,500 lux (120-130 mol m^{-2} s^{-1}). Cell concentration was determined by counting using a compound light microscope (Olympus, Tokyo, Japan) equipped with a Neubauer chamber of 0.1 mm^3. The cell division (μ) per d and the cumulative rate of cell division ($\Sigma\mu$) were determined as described by Nieves (Nieves *et al*., 1998; Nieves, 2000).

2.3 Proximate composition

The proximate composition of microalgae was estimated using conventional analytical techniques. Samples were filtered through 25 mm Whatman GF/C glass-fiber filters in quadruplicate for each type of analysis (proteins, carbohydrates, and lipids). Samples were stored at -79°C until processing. Proteins were extracted by continuous heating for 15 min and 0.1 N sodium hydroxide and determined by the method of Lowry *et al*., (1951); carbohydrates were extracted with sulfuric acid and determined as described by DuBois *et al*., (1956); and lipids were extracted as described by Bligh and Dyer (1959) and determined as described by Pande *et al*., (1963).

2.4 Microalgae harvesting, oil extraction and purification process

Microalgae harvesting was carried out by coagulation-flocculation using 1 ml L^{-1} aluminum chloride hydroxide, followed by centrifugation until obtaining a concentrated paste of microalgae. (Barros *et al*., 2015; Papazi *et al*., 2010).

Oil extraction was performed as described by Bligh and Dyer (1959) with some modifications. 50 ml of a mixture of chloroform: methanol (2:1) was used for 10 g of wet microalgae suspension that was subjected to ultrasonic irradiation using a high efficiency ultrasonic processor (Hielscher Ultrasonics, Teltow, Germany) with an output power of 200 W and mechanical vibrations generated

by electronic excitation of 24 kHz. A sonotrode (Hielscher Ultrasonics) was used to transmit the sound power, varying the amplitude, which is directly related to the density and acoustic power. The ratio of chloroform: methanol: water was adjusted at 2:1:0.8 prior to biomass separation from the liquid phase by centrifugation, while the obtained liquid phase was subjected to a final adjustment of the chloroform: methanol: water ratio (2:2:1.8) and transferred to a separator funnel for recovering the non-aqueous phase after 12 h (Soto-León *et al*., 2014). Then, the non-aqueous phase was transferred to an activated charcoal column and eluted with chloroform to remove chlorophyll. Excess solvent was removed using a rotary evaporator (Yamato Scientific, Tokyo, Japan) under a vacuum at 40°C.

2.5 *Fatty acid profile*

The fatty acid profile of microalgae oil was analyzed using the sono-transesterification reaction. This reaction was developed using a methanol: oil molar ratio of 6:1 and 0.85 wt% potassium hydroxide as a catalyst in an ultrasonic processor (Hielscher Ultrasonics, Teltow, Germany) for 60 s as described by Soto-León *et al*., (2014). Glycerol was removed by rotary evaporation using a vacuum, and the oil phase was filtered using 0.22 mm Whatman GF/C glass-fiber filters. The quantitative analysis of fatty acid methyl ester composition was performed using a gas chromatograph-mass spectrometer equipped with an automatic liquid sampler (Agilent Technologies, Santa Clara, CA, USA). The capillary column was coated with Omega Wax 250, and helium was used as a gas carrier at a flow rate of 1 ml min^{-1} with 1 μl injection volume. Samples were analyzed with the column held initially at 50°C, and then the temperature was increased to 270°C at a 5°C min^{-1} increment and held at this temperature for 10 min. A 37-component reference standard (Supelco, Bellefonte, PA, USA) was used for discriminating the gas chromatograph-mass spectrometer signals (Hammann *et al*., 2013).

2.6 *Statistical analysis*

The effect of temperature on growth kinetics and proximal analysis registered in each temperature level was performed using one factor ANOVA, coming to perform the Tukey test at 95% confidence interval level to determine the difference between treatments. Statistical analysis was done using the Statistica 8.0 software.

The rates of cell division and growth rate in each treatment were analyzed by analysis of covariance; where it was significant it proceeded to make multiple comparisons to elucidate the best treatment and relate it with proximal composition and the amount of fatty acids.

3 Results and discussion

3.1 *Growth kinetics of Nannochloropsis sp.*

No data were obtained from microalgae grown in F/2 at 40°C, since the culture collapsed between the first 48-72 h. As shown in Fig. 1, the cell density of *Nannochloropsis* sp. significantly increased after 3 d of culture, ranging between 3.178×10^6 cells ml^{-1} and 4.117×10^6 cells ml^{-1}, with no significant differences between treatments at d 4 of culture ($p < 0.05$). At d 5 of culture, cell density increased significantly slower at 35°C than that at 25°C or 30°C. Generally, an increase in temperature in nitrogen limited cultures means a decrease in biomass production, as has been demonstrated in studies with different microalgal species such as *Nannochloropsis* sp. (Vadiveloo *et al*., 2015) and *Nannochloropsis oculata* (Converti *et al*., 2009; Camacho-Rodríguez *et al*., 2015; Mitra *et al*., 2015).

The μ showed a sustained growth until d 5 of the culture, when the exponential growth phase was concluded (Fig. 2). The lowest average μ per d was obtained in F/2 at 35°C (0.165 μ d^{-1}), while the highest at 30°C with an average of 1.362 μ d^{-1} (Table 1). The cell density, μ, and $\Sigma\mu$ of *Nannochloropsis* sp. were not significantly different between the control and the cultures grown in F/2 at 25°C or 30°C; however, these parameters were significantly lower ($p < 0.05$) in the culture grown in F/2 at 35°C compared with all other treatments and the control (Table 1). This is consistent with the work of Converti *et al*., who found a decrease of μ with increasing temperature in cultures of *N. oculata* (Converti *et al*., 2009).

Cell density, μ per day, and $\Sigma\mu$ were also evaluated at day 13. As presented in Table 2, the highest cell density was obtained in F/2 at 25°C, while the lowest was in F/2 at 35°C. The $\Sigma\mu$ showed a tendency to decrease with increasing temperature. Both the cell density and $\Sigma\mu$ of *Nannochloropsis* sp. were not significantly different between the control and the cultures grown in F/2 at 25°C and 30°C; however, these two parameters were significantly lower ($p <$

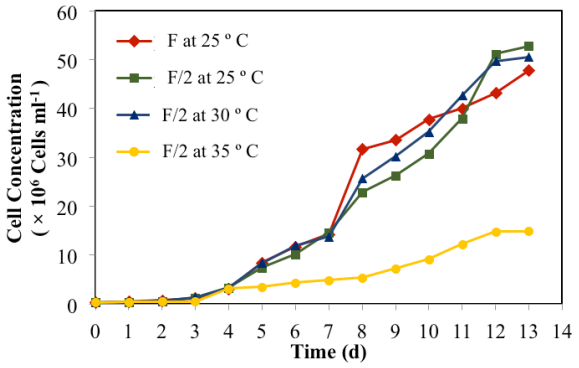

Fig.1. Cell density ($\times 10^6$ cells ml^{-1}) of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C.

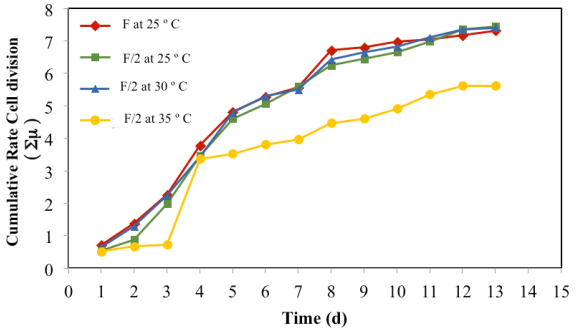

Fig. 2. Cumulative rate of cell division ($\Sigma\mu$) of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C.

0.05) in the culture grown in F/2 at 35°C compared with all other treatments and the control (Table 2). Additionally, no significant differences were identified in μ between the control and all treatments ($p < 0.05$).

3.2 *Proximate composition of Nannochloropsis sp.*

As presented in Table 3, no significant differences were identified in the protein content of *Nannochloropsis* sp. cultured in F at 25°C and that cultured in F/2 at 25 or 30°C ($p < 0.05$); however, the protein content obtained in F/2 at 35°C (32.022 μg ml^{-1}) was significantly lower ($p < 0.05$) compared with that obtained in F at 25°C (72.672 μg ml^{-1}). The maximum carbohydrate content was obtained in F/2 at 30°C (53.097 μg ml^{-1}), which was significantly higher ($p < 0.05$) than that obtained in F/2 at 35°C (15.998 μg ml^{-1}). In F/2, both the protein and carbohydrate contents tended to increase with increasing temperature from 25 to 30°C and then decreased significantly ($p < 0.05$) at 35°C (Table 3). The lipid content of *Nannochloropsis* sp. cultured in F was significantly higher (57.467 μg ml^{-1}; $p < 0.05$) than that in F/2 at different temperatures. In F/2, the lowest lipid content was obtained at 35°C (21.663 μg ml^{-1}) that was not significantly different than that at 25°C (26.311 μg ml^{-1}), while the highest was obtained at 30°C (40.594 μg mL^{-1}) that was significantly higher ($p < 0.05$) than those at 25°C and 35°C. These results are in line with those reported by other authors that have observed that increasing temperature and nitrogen limitation result in increased production of lipids and carbohydrates with a tendency to decrease in protein content (Converti *et al.*, 2009; Bondioli *et al.*, 2012).

3.3 *Fatty acid profile of Nannochloropsis sp.*

In this work, it was confirmed that ultrasound-assisted solvent extraction is an efficient method that justifies its heavy use for obtaining various components of interest from microalgae (Parniakov *et al.*, 2015; Chemat *et al.*, 2016).

Table 1. Mean values and standard error of cell density, cell division (μ) per day, and the cumulative rate of cell division ($\Sigma\mu$) of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C at d 5 of culture (exponential growth phase).

	F at 25°C	**F/2 at 25°C**	**F/2 at 30°C**	**F/2 at 35°C**
$\times 10^6$ cells ml^{-1}	$8.460^b \pm 0.832$	$7.410^b \pm 0.533$	$8.395^b \pm 0.857$	$3.481^a \pm 0.133$
μ d^{-1}	$1.032^b \pm 0.046$	$1.144^b \pm 0.120$	$1.362^b \pm 0.189$	$0.165^a \pm 0.140$
$\Sigma\mu$	$4.804^b \pm 0.139$	$4.615^b \pm 0.103$	$4.781^b \pm 0.163$	$3.533^a \pm 0.054$

Same letters indicate no significant differences at $p < 0.05$.

Table 2. Mean values and standard error of cell density, cell division (μ) per day, and the cumulative rate of cell division ($\Sigma\mu$) of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C at d 13 of culture.

	F at 25°C	**F/2 at 25°C**	**F/2 at 30°C**	**F/2 at 35°C**
$\times 10^6$ cells ml^{-1}	$47.813^{b}\pm2.907$	$52.734^{b}\pm0.269$	$50.625^{b}\pm1.652$	$14.906^{a}\pm0.624$
μ d^{-1}	$0.145^{a}\pm0.130$	$0.101^{a}\pm0.227$	$0.064^{a}\pm0.173$	$0.003^{a}\pm0.058$
$\Sigma\mu$	$7.311^{b}\pm0.087$	$7.458^{b}\pm0.007$	$7.396^{b}\pm0.048$	$5.631^{a}\pm0.063$

Same letters indicate no significant differences at $p < 0.05$.

Table 3. Mean values and standard error of proximate composition (protein, carbohydrate, and lipid contents) of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C.

	F at 25°C	**F/2 at 25°C**	**F/2 at 30°C**	**F/2 at 35°C**
P*	$72.672^{b}\pm6.770$	$41.367^{ab}\pm4.279$	$47.384^{ab}\pm0.704$	$32.022^{a}\pm1.896$
C*	$41.010^{ab}\pm5.505$	$22.630^{ab}\pm1.438$	$53.097^{b}\pm5.670$	$15.998^{a}\pm1.010$
L	$57.467^{c}\pm4.146$	$26.311^{a}\pm0.400$	$40.594^{b}\pm 1.814$	$21.663^{a}\pm1.505$

P, proteins; C, carbohydrates; L, lipids.

Same letters indicate no significant differences at $p < 0.05$.

* Non-parametric analysis of variance.

Table 4. Fatty acid profile of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C.

	F at 25°C	**F/2 at 25°C**	**F/2 at 30°C**	**F/2 at 35°C**
Component				
Capric acid (C10:0)	0.066	-	-	0.0976
Lauric acid (C12:0)	0.9808	1.0078	-	1.0859
Myristic acid (C14:0)	0.6189	0.7305	1.0367	1.4374
Myristoleic acid (C14:1)	-	-	-	0.0602
Palmitic acid (C16:0)	27.7825	20.8383	25.1089	27.3496
Palmitoleic acid (C16:1)	2.0774	3.8964	1.5647	3.1769
Heptadecanoic acid (C17:0)	0.0587	-	-	0.0388
Cis-10-heptadecenoic acid (17:1)	0.334	-	-	2.0335
Stearic acid (C18:0)	0.9982	-	0.7066	-
Elaidic acid (C18:1n9t)	0.2396	-	-	-
Oleic acid (C18:1n9c)	10.36	11.7002	14.8627	11.8473
Linoleic acid (C18:2n6c)	24.7024	25.1187	28.6097	25.2042
Araquidic acid (C20:0)	0.0581	-	0.0577	-
?-linolenic acid (C18:3n6)	1.212	1.864	1.9068	1.6523
Cis-11-eicosenoic acid (20:1)	0.1673	-	0.149	0.1505
Linolenic acid (C18:3n3)	23.1741	26.9439	18.5812	18.8958
Cis-11,14-eicosadieonic acid (C20:2)	-	0.4165	0.4904	0.3504
Behenic acid (C22:0)	0.0536	0.3523	-	-
Cis-8,11,14-eicosatrienoic acid (C20:3n6)	0.3187	0.3528	0.3152	0.2632
Erucic acid (C22:1n9)	1.2121	0.4502	0.9449	1.1081
Cis-11,14,17-eicosatrienoic acid (C20:3n3)	0.3028	0.6549	0.3752	0.7041
Tricosanoic acid (C23:0)	3.7569	4.1348	4.1001	3.7362
Cis-13,16-docosadienoic acid (C22:2)	-	0.056	-	-
Lignoceric acid (C24:0)	0.5562	0.2746	0.3079	0.1759
Cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3)	0.9696	1.208	0.8821	0.6322

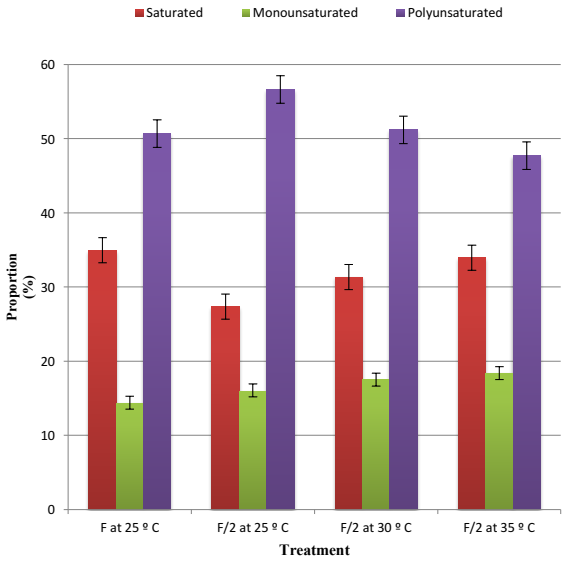

Fig. 3. Proportion of saturated, monounsaturated, and polyunsaturated fatty acids of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C.

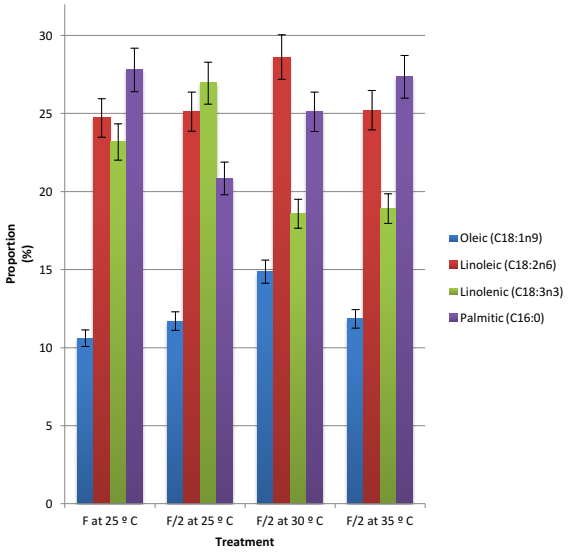

Fig. 4. Proportion of oleic (C18:1n9), linoleic (C18:2n6), linolenic (C18:3n3), and palmitic (C16:0) in the fatty acid profile of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C.

The fatty acid profile of *Nannochloropsis* sp is presented in Table 4; nitrogen limitation at 25°C increased the level of unsaturated compounds by 10.49% (50.67% in F to 56.61% in F/2) and the level of monounsaturated compounds, mainly of oleic acid, by 10.28% (14.39% in F to 16.04% in F/2). However, the level of saturated compounds decreased by 21.75% (34.93% in F to 27.33% in F/2).

Saturated and monounsaturated compounds increased with increasing temperature in F/2, showing that an increase in temperature counteracts the effect of nitrogen limitation (Fig. 3). Both the temperature and nitrogen limitation promoted the production of monounsaturated compounds in *Nannochloropsis* sp., which are considered important for biofuel production. The proportions of saturated (25-35%), monounsaturated (13-18%), and polyunsaturated (48-50%) compounds in the fatty acid profile of *Nannochloropsis* sp. were in agreement with those reported by Cabrera *et al*. for *Nannochloropsis* oculata; however, the specific fatty acid profile differed from previous studies (Converti *et al*., 2009).

The concentration of polyunsaturated fatty acids decreased with increasing temperature, indicating that elongation and desaturation are interrupted when the culture temperature is relatively high (Guschina and Harwood, 2006; Nelson and Cox, 2006). Moreover, at relatively low temperatures, the Δ12 desaturase enzyme is expressed at its maximum capacity, allowing microalgae to increase the synthesis of polyunsaturated fatty acids. It seems that this increase ensures the fluidity of cell membranes and leads to the development of a phospholipid bilayer with greater diffusion and displacement. Therefore, our results are consistent to those reported where there have been significant PUFA´s levels (Mitra *et al*., 2015), particularly EPA (Camacho-Rodríguez *et al*., 2015) with temperature increment.

Fig. 4 shows the proportion of four components (oleic, linoleic, linolenic, and palmitic acids) in the fatty acid profile of *Nannochloropsis* sp., which are considered important for biofuel and food production (Baumgardt *et al*., 2016). The proportion of palmitic acid, which was the most abundant compound in F (27.8%), was decreased in F/2 (20.83%) due to nitrogen limitation, while that of linolenic acid increased from 23.17 in F to 26.94% in F/2 and became the most abundant compound. The proportions of oleic and linoleic acids slightly increased from 10.6 and 24.7% in F to 11.7 and 25.11% in F/2, respectively (Fig. 4). Therefore, nitrogen limitation led to major changes in metabolic pathways and particularly those of lipid biosynthesis.

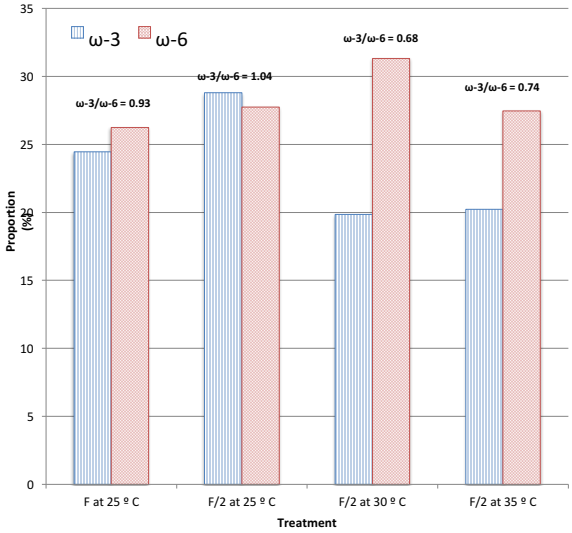

Fig. 5. Proportion of ω-3 and ω-6 groups in the fatty acid profile of *Nannochloropsis* sp. cultured in F medium (F) at 25°C (control) and F medium with half the original concentration of sodium nitrate (F/2) at 25°C, 30°C, and 35°C.

The fatty acid profile of *Nannochloropsis* sp. was also studied based on the presence of ω-3 (C18: 3n-3, C20: 3n-3, and C20: 5n-3) and ω-6 (C18: 2n-6, C18: 3n-6, and C20: 3n-6) groups (Fig. 5). The results showed that the limitation of nitrogen enhanced the biosynthesis of ω-3 groups at 30°C (32.5%) and of ω-6 groups at 25°C (28.1%) compared with the control (26.7% and 24.8%, respectively).

Hu and Gao (2003) suggested the use of *Nannochloropsis* sp. for food production due to its high content in eicosapentaenoic acid (C20: 5n3), the main polyunsaturated fatty acid of the ω-3 family. However, this study showed that the proportion of this component in the fatty acid profile was very low (0.6-1.12%). The results also revealed that palmitic acid was the major component of saturated fatty acids and that linolenic acid (18.5-26.94%) was the main component in the ω-3 family, results that were in agreement with those reported by Hu and Gao (2006), but in disagreement with those by Cabrera *et al*. (2005). Any variation in the fatty acid profile of microalgae can be attributed to metabolic differences between species and environment conditions (Hu *et al*., 2008). Therefore, temperature and nitrogen reduction significant effects on the ω-3/ω-6 ratio of *Nannochloropsis* sp. (Fig. 5). The ω-3/ω-6 ratio was the highest (0.93±0.01) in F at 25°C and the lowest in F/2 at 30°C (0.68±0.02). The ω-3/ω-6 ratio is very important, since it is considered important in human nutrition for the prevention of cardiovascular diseases. According to the Food and Agriculture Organization of the United Nations (FAO), the optimal ratio ω-3/ω-6 is 1:1 or 1:2. In this work, the ω-3/ω-6 ratio in F/2 at 25°C (1.04±0.02) was the best.

Conclusions

Nannochloropsis sp has great potential as a feedstock for the production of biofuels and food. Under conditions of nitrogen limitation, cell density, growth and fatty acid profiles are highly affected by the temperature of the culture. It has been observed that cumulative cell division, cell density decrease according increase temperature, showing the best result for both F and F/2 media at 25ºC with no significant differences. But, it was observed for another hand, an increased accumulation of lipid at 30°C with a high content of proteins, carbohydrates and polyunsaturated acids. Finally, due to the significant effect on the ω-3/ω-6 ratio *Nannochloropsis* sp has a significant potential to be use in food when growing at 25ºC using F/2 media.

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References

Alsull, M., Wan Omar, M. (2012). Responses of *Tetraselmis* sp. and *Nannochloropsis* sp. isolated from Penang National Park Coastal Waters, Malaysia, to the combined influences of salinity, light and nitrogen limitation. *International Conference on Chemical, Ecology and Environmental Sciences (ICEES'2012)*. Bangkok. pp. 142-145.

Andrich, G., Nesti, U., Venturi, F., Zinnai, A., Fiorentini, R. (2005). Supercritical fluid extraction of bioactive lipids from the microalga *Nannochloropsis*?sp. *European Journal of Lipid Science and Technology 107*, 381-386.

Angles, E., Jaouen, P., Pruvost, J., Marchal, L. (2017). Wet lipid extraction from the microalga *Nannochloropsis* sp.: Disruption, physiological effects and solvent screening. *Algal Research 21*, 27-34.

Anzueto, M. A. (2008). *Evaluación del cultivo continuo de tres especies microalgales*. Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Baja California, México.

Arredondo-Vega, B. O., Vázquez-Pérez, R. N., Hernández-Uribe, F. (2013). Compuestos de alto valor agregado a partir de microalgas y cianobacterias. *Ciencia, Tecnología e Innovación para el Desarrollo de México 5*.

Atkinson, D., Ciotti, B. J., Montagnes, D. J. S. (2003). Protists decrease in size linearly with temperature: ca. 2.5% $°C^{-1}$. *Proceedings of the Royal Society of London B: Biological Sciences 270*, 2605-2611.

Barros, A. I., Gonçalves, A. L., Simões, M., Pires, J. C. M. (2015). Harvesting techniques applied to microalgae: A review. *Renewable and Sustainable Energy Reviews 41*, 1489-1500.

Baumgardt, F. J. L., Zandoná-Filho, A., Brandalize, M. V., da Costa, D. C., Antoniosi-Filho, N. R., Abreu, P. C. O. V., Corazza, M. L., Pereira-Ramos, L. (2016). Lipid content and fatty acid profile of *Nannochloropsis oculata* before and after extraction with conventional solvents and/orcompressed fluids. *The Journal of Supercritical Fluids 25*, 89-95.

Bligh, E. G., Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology 37*, 911-917.

Bondioli, P., Della-Bella, L., Rivolta, G., Chini-Zittelli, G., Bassi, N., Rodolfi, L., Casini, D., Prussi, M., Chiaramonti, D., Tredici, M. R. (2012). Oil production by the marine microalgae *Nannochloropsis* sp. F& M-M24 and Tetraselmis suecica F& amp; M-M33. *Bioresource Technology 114*, 567-572.

Brown, T. M., Duan, P., Savage, P. E. (2010). Hydrothermal liquefaction and gasification of *Nannochloropsis* sp. *Energy & Fuels 24*, 3639-3646.

Cabrera, T., Bae, J. H., Bai, S. C., Hur, S. B. (2005). Comparison of the nutritucional value of *Chlorella ellipsoidea* and *Nannochloropsis oculata* for rotifers and *Artemia Nauplii. Journal of Fisheries Science and Technology 8*, 201-206.

Camacho-Rodríguez, J., Cerón-García, M. C., Fernández-Sevilla, J. M., Molina-Grima, E. (2015). The influence of culture conditions on biomass and high value product generation by *Nannochloropsis gaditana* in aquaculture. *Algal Research 11*, 63-73.

Cha T. S., Fong-Lee, N. G., Aziz, A., Loh, S. H. (2012). Effect of nitrate on oil content and fatty acid composition of *Nannochloropsis* sp. at early stationary growth phase. *Journal of Sustainability Science and Management 7*, 30-36.

Chemat, F., Rombaut, N., Sicaere, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., Abert-Vian, M. (2016). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. *Ultrasonics Sonochemistry 34*, 540-560.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances 25*, 294-306.

Converti, A., Casazza, A. A., Ortiz, E. Y., Perego, P., Del Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing: Process Intensification 48*, 1146-1151.

Du, Z., Li, Y., Wang, X., Wan, Y., Chen, Q., Wang, C., Ruan R. (2011). Microwave-assisted pyrolysis of microalgae for biofuel production. *Bioresource Technology 102*, 4890-4896.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry 28*, 350-356.

Fábregas, J., Maseda, A., Domínguez, A., Otero, A. (2004). The cell composition of *Nannochloropsis* sp. changes under different irradiances in semicontinuous culture. *World Journal of Microbiology & Biotechnology 20*, 31-35.

Field C. B., Behrenfeld M.J., Randerson J.T., Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science 281*, 237-240.

Gong, Y., Jiang, M. (2011). Biodiesel production with microalgae as feedstock: from strains to biodiesel. *Biotechnology Letters 33*, 1269-1284.

Guillard, R. R. L., Ryther, J. H. (1962). Studies of marine planktonic diatoms: *Cyclotella nana* hustedt and *Detonula confervacea* (cleve) gran. *Canadian Journal of Microbiology 8*, 229-239.

Guschina, I. A., Harwood, J. L. (2006). Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research 45*, 160-186.

Halim, R., Gladman, B., Danquah, M. K., Webley, P. A. (2011). Oil extraction from microalgae for biodiesel production. *Bioresource Technology 102*, 178-185.

Hammann, S., Tillmann, U., Schröder, M., Vetter, W. (2013). Profiling the fatty acids from a strain of the microalgae *Alexandrium tamarense* by means of high-speed counter-current chromatographyand gas chromatography coupled with mass spectrometry. *Journal of Chromatography A 1312*, 93-103.

Hemerick, G. (1973). Mass culture. En: *Handbook of Phyological Methods*, pp. 255-273. Cambridge University Press, London, England.

Hu, H., Gao, K. (2003). Optimization of growth and fatty acid composition of a unicellular marine picoplankton, *Nannochloropsis* sp., with enriched carbon sources. *Biotechnology Letters 25*, 421-425.

Hu, H., Gao, K. (2006). Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. *Biotechnology Letters 28*, 987-992.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal 54*, 621-639.

Liu, Z., Liu, C., Hou, Y., Chen, S., Xiao, D., Zhang, J., Chen, F. (2013). Isolation and characterization of a marine microalga for biofuel production with astaxanthin as a co-product. *Energies 6*, 2759-2772.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry 193*, 265-275.

Mitra, M., Patidar, S. K., Mishra, S. (2015). Integrated process of two stage cultivation of *Nannochloropsis* sp. for nutraceutically valuable eicosapentaenoic acid along with biodiesel. *Bioresource Technology 193*, 363-369.

Moazami, N., Ashori, A., Ranjbar, R., Tangestani, M., Eghtesadi, R., Nejad, A. S. (2012). Large-scale biodiesel production using microalgae biomass of *Nannochloropsis*. *Biomass and Bioenergy 39*, 449-453.

Montagnes, D. J. S., Kimmance, S. A., Atkinson, D. (2003). Using Q10: Can growth rates increase linearly with temperature? *Aquatic Microbial Ecology 32*, 307-313.

Moreno-Castro, L. E., Quintero-Ramos, A., Ruiz-Gutiérrez, M. G., Sánchez-Madrigal, M. A., Meléndez-Pizarro, C. O., Pérez-Reyes, I., Lardizábal-Gutiérrez, D. (2015). Nixtamalization assisted with ultrasound: effect on mass transfer and physicochemical properties of nixtamal, masa and tortilla. *Revista Mexicana de Ingeniería Química 14*, 265-279.

Muthukumar, A., Elayaraja, S., Ajithkumar, T. T., Kumaresan, S., Balasubramanian, T. (2012). Biodiesel production from marine microalgae *Chlorella marina* and *Nannochloropsis salina. Journal of Petroleum Technology and Alternative Fuels 3*, 58-62.

Nelson, D. L., Cox, M. M. (2006). *Lehninger Principios de Bioquímica*. Ediciones Omega, Barcelona, España.

Nieves, M. (2000). *Efecto de productos de naturaleza zeolítica sobre el crecimiento y la calidad dietética de microalgas para la acuicultura*. Universidad de Colima, Colima, Mexico.

Nieves, M., López, D. J., Medina, M. A., Piña, P., Leal, S., López-Elías, J. A. (2009). *Revista de Investigaciones Marinas 30*, 123-133.

Nieves, M., Voltolina, D., Barrera, A. (1998). A new parameter for comparison of microalgae growth. *Rivista Italiana di Acquacoltura 33*, 177-184.

Olofsson, M., Lamela, T., Nilsson, E., Bergé, J. P., del Pino, V., Uronen, P., Legrand, C. (2012). Seasonal variation of lipids and fatty acids of the microalgae *Nannochloropsis oculata* grown in outdoor large-scale photobioreactors. *Energies 5*, 1577-1592.

Pande, S. V., Khan, R. P., Venkitasubramanian, T. A. (1963). Microdetermination of lipids and serum total fatty acids. *Analytical Biochemistry 6*, 415-423.

Papazi, A., Makridis, P., Divanach, P. (2010). Harvesting *Chlorella minutissima* using cell coagulants. *Journal of Applied Phycology 22*, 349-355.

Parniakov, O., Apicella, E., Koubaa, M., Barba, F. J., Grimi, N., Lebovka, N., Pataro, G., Ferrari, G., Vorobiev, E. (2015). Ultrasound-assisted green solvent extraction of high-added value compounds from microalgae *Nannochloropsis* sp. *Bioresource Technology 198*, 262-267.

Patil, P. D., Gude, V. G., Mannarswamy, A., Deng, S., Cooke, P., Munson-McGee S., Rhodes I., Lammers P., Nirmalakhandan N. (2011). Optimization of direct conversion of w*et al*gae to biodiesel under supercritical methanol conditions. *Bioresource Technology 102*, 118-122.

Peña-Salamanca, E. J., Palacios-Peñaranda, M. L., Ospina-Álvarez, N. (2005). *Algas como indicadoras de contaminación*. Universidad del Valle Cali, Colombia.

Ranjan, A., Patil, C., Moholkar, V. S. (2010). Mechanistic assessment of microalgal lipid extraction. *Industrial & Engineering Chemistry Research 49*, 2979-2985.

Renaud, S. M., Thinh, L.-V., Parry, D. L. (1999). The gross chemical composition and fatty acid composition of 18 species of tropical australian microalgae for possible use in mariculture. *Aquaculture 170*, 147-159.

Richmond, A. (2004) *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd.

Robles-Heredia, J. C., Sacramento-Rivero, J. C., Ruiz-Marín, A., Baz-Rodríguez, S., Canedo-Lopez, Y., Narváez-García, A. (2016). Evaluation of cell growth, nitrogen removal and lipid production by *Chlorella vulgaris* to different conditions of aireation in two types of annular photobioreactors. *Revista Mexicana de Ingeniería Química 15*, 361-377.

Sander, K. B., Murthy, G. S. (2009). Paper Number: 1035636. Jun 21-24. Reno, Nevada: *American Society of Agricultural and Biological Engineers annual international meeting*.

Soto-León, S., Zazueta-Patrón, I. E., Piña-Valdez, P., Nieves-Soto, M., Reyes-Moreno, C., Contreras-Andrade, I. (2014). Extracción de lípidos de Tetraselmis Suecica: Proceso asistido por ultrasonido y solventes. *Revista Mexicana de Ingeniería Química 13*, 723-737.

Taoka, Y., Nagano, N., Okita, Y., Izumida, H., Sugimoto, S., Hayashi, M. (2009). Influences of culture temperature on the growth, lipid content and fatty acid composition of *Aurantiochytrium* sp. strain mh0186. *Marine Biotechnology 11*, 368-374.

Thompson, P. A., Guo, M.-X., Harrison, P. J., Whyte, J. N. C. (1992). Effects of variation in temperature on the fatty acid composition of eight species of marine phytoplankton. *Journal of Phycology 28*, 488-497.

Vadiveloo, A., Moheimani, N. R., Cosgrove, J. J., Bahri, P. A., Parlevliet, D. (2015). Effect of different light spectra on the growth and productivity of acclimated *Nannochloropsis* sp. (Eustigmatophyceae). *Algal Research 8*, 121-127.

Voltolina, D., Bückle-Ramírez, L. F., Morales-Guerrero, E. L. (1989). *Manual de metodologías y alternativas para el cultivo de microalgas*. Centro de Investigación Científica y de educación Superior de Ensenada (CICESE), Baja California, Mexico.

Wu, Y.-H, Yu, Y., Hu, H.-Y. (2013). Potential biomass yield per phosphorus and lipid accumulation property of seven microalgal species. *Bioresource Technology 130*, 599-602.

Yoo, C., Jun, S.-Y., Lee, J.-Y., Ahn, C.-Y., Oh, H.-M. (2010). Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresource Technology 101*, S71-S74.