Volumen 33, Nº 1. Páginas 93-101
IDESIA (Chile) Enero-Febrero, 2015

Lipid content and fatty acid profiles in ten species of microalgae

Contenido de lípidos y perfil de ácidos grasos en diez especies de microalgas

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ABSTRACT

The present study aimed to evaluate the lipid content and fatty acid profiles of 9 marine species (*Nannochloropsis oculata, Thalassiosira pseudonana, Phaeodactylum tricornutum, Isochrysis galbana, Tetraselmis suecica, Tetraselmis chuii, Chaetoceros muelleri, Thalassiosira fluviatilis* and *Isochrysis* sp.) and 1 freshwater microalga species (*Chlorella vulgaris*) that were cultivated in Erlenmeyer flasks with 800 mL of culture medium under stationary autotrophic conditions while exposed to a continuous photon flux density of approximately 175 µmol m^{-2} s^{-1}, a temperature of 25±2 ºC and constant airflow. *N. oculata* and *T. pseudonana* exhibited the highest lipid content, although they had a high proportion of monounsaturated fatty acids relative to polyunsaturated fatty acids. *C. vulgaris* and *Isochrysis* sp. exhibited high levels of polyunsaturated fatty acids, whereas *P. tricornutum*, *N. oculata* and *T. suecica* exhibited the highest eicosapentaenoic acid levels, and *I. galbana* and *C. muelleri* had the highest docosahexaenoic fatty acid levels. The freshwater species *C. vulgaris* had the highest levels of linoleic and linolenic acids, followed by the marine species *T. chuii*.

Key words: lipids, fatty acid profile, microalgae.

RESUMEN

*El presente estudio pretende como objetivo evaluar el perfil de ácidos grasos y contenido de lípidos de nueve microalgas marinas (*Nannochloropsis oculata, Thalassiosira pseudonana, Phaeodactylum tricornutum, Isochrysis galbana, Tetraselmis suecica, Tetraselmis chuii, Chaetoceros muelleri, Thalassiosira fluviatilis *e* Isochrysis *sp.) y una de agua dulce (*Chlorella vulgaris*) y la cultura autotrófica estacionaria, usando frascos que contienen 800 ml de medio de cultivo, proporcionando constante la densidad del flujo de fotones de iluminación en los cultivos de alrededor de 175 mol* m^{-2} s^{-1}*, temperatura 25 ± 2 °C y aireación. La especie* N. oculata *y* T. pseudonana *mostraron los niveles más altos de lípidos, pero con una alta proporción de ácidos grasos monoinsaturados en relación con poliinsaturados. La especie* C. vulgaris *e* Isochrysis *sp. mostraron altos niveles de ácidos grasos poliinsaturados;* P. tricornutum, N. oculata *y* T. suecica *revelaron los niveles más altos de ácido eicosapentaenoico y* C. muelleri *e* I. galbana *alcanzaron los más altos niveles del ácido graso docosahexaenoico.* C. vulgaris *reveló un mayor contenido de ácidos linoleico y linolénico, seguido de las especies marinas* T. chuii.

Palabras clave: *lipídicos, perfil de ácidos grasos, microalga.*

Introduction

Microalgae are important constituents of ecosystems that range from marine and freshwater to desert and from hot to cold, and thus possess varied compositions of several chemical components such as proteins, lipids, fatty acids, carbohydrates and pigments. The concentrations of each component depend on the nature of the organism, the environmental or culture conditions and the physiological state of the culture itself. Differences in the chemical composition of microalgae cultures, including lipid content and fatty acid profiles, can be found not only between different species but also within the same species; these differences depend on the composition of the culture medium,

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Fecha de Recepción: 6 Agosto, 2013.
Fecha de Aceptación: 27 Noviembre, 2014.

airflow, carbon content, luminosity, photoperiod, culture temperature and pH, among others (Miao & Wu, 2004).

Some cyanobacteria produce large amounts of polyunsaturated fatty acids (PUFAs; 20-60%). Saturated and monounsaturated fatty acids (SFAs and MUFAs, respectively) are among the most prominent stored lipid components in eukaryotic algae and generally reach values greater than 80% of the total lipid fraction (Richmond, 2004). Some marine microalgae species contain high levels of long-chain polyunsaturated fatty acids (LC-PUFAs) in their lipid fractions, such as eicosapentaenoic (EPA - $20:5_{n-3}$) and docosahexaenoic acids (DHA – $22:6_{n-3}$) (Medina *et al.,* 1998).

Studies have shown that the ingestion of algae-derived ω-3 fatty acids has beneficial effects on visual and neural development and on the prevention of diseases such as heart conditions, hypertension, cancer, diabetes, cystic fibrosis, asthma, arthritis, depression and schizophrenia, a finding that is also reflected by the large number of microalgae-based products that have biological activities in humans (Derner *et al.,* 2006). Ethanol or supercritical fluid lipid extraction has increased in commercial importance with regard to the production of lipid-based cosmetic formulations such as creams or lotions, because these provide both nutritional and protective effects to the skin. Other microalgae-derived lipids, including glycolipids and phospholipids, should not be overlooked in future skin care developments.

Many microalgae species are used in food production because they produce several substances, including vitamins, mineral salts, pigments, lipids and fatty acids. The main applications of microalga-derived fatty acids include the enrichment of fish food (*e.g.,* in aquiculture), possible uses in biodiesel production, an essential fatty acid source in the human diet as both a food additive and a human health supplement, biological carbon sequestration and sewage treatment. The amounts of commercially interesting compounds that can be obtained from microalgae are unpredictable, thus making studies of species and chemical composition of microalgae indispensable, in addition to a strong interest in clean, sustainable and organic technologies to generate products for human use (Derner *et al.,* 2006).

Much is known about the uses of certain fatty acids, especially long-chained ones. Still, few studies on the lipid contents and fatty acid profiles of microalgae have been performed in Brazil. Thus the present study aims to identify differences between microalgae species with respect to the above characteristics.

Materials and Methods

The experiments were performed in a cell culture room at the Department of Food Science (Departamento de Ciência dos Alimentos – CAL), Federal University of Santa Catarina (Universidade Federal de Santa Catarina – UFSC) in Florianópolis, Santa Catarina State (SC), Brazil. The studied microalgae included the freshwater species *Chlorella vulgaris* and the marine species *Nannochloropsis oculata, Thalassiosira pseudonana, Phaeodactylum tricornutum, Isochrysis galbana, Tetraselmis suecica*, *Tetraselmis chuii, Chaetoceros muelleri, Thalassiosira fluviatilis* and *Isochrysis* sp. The experimental design comprised randomised blocks of time with 3 repetitions.

The culture room was maintained at a temperature of 25±2 ºC with continuous artificial culture radiation (75 µmol m^{-2} s^{-1} on the first day of culture and increasing to 150 µmol m^{-2} s^{-1} from the second day until harvest). Modified WC medium was used to culture *C. vulgaris* (Guillard & Lorenzen, 1972), and f/2 Guillard's medium was used to culture the marine species (Guillard, 1975).

The experimental phase included the inoculation of a predetermined volume of algal culture, depending on the desired initial density, into 800 mL of culture medium. For each repetition, this 800 mL volume was added to 1000 mL Erlenmeyer flasks, autoclaved for 30 min at 121 °C and stored for 48 h at 25 °C. The freshwater culture medium was corrected to pH 6.5 with 10% HCl; the saltwater medium remained at approximately pH 8.0, thus no correction was necessary. Culture airflow was provided with a mini compressor (Boyu Acq-003 50/L).

To harvest the cultures, the culture in each flask was centrifuged twice for 20 min at 4000 rpm and 10 °C. The samples were frozen, lyophilised, centrifuged, dried in an incubator at 50 °C and ground, followed by lipid extraction. Fatty acid methyl esters were obtained by lipid esterification as previously described by Metcalfe & Schimitz (1966), and the fatty acid profiles were determined by gas chromatography (Varian 3400CX, equipped with a flame ionisation detector; Varian Medical Systems, Inc., Palo Alto, CA, USA).

The lipid content and fatty acid profile data obtained were submitted to a variance analysis and Tukey's test to identify differences between the mean values of each treatment. The ESTAT software package was used for this purpose.

Results and Discussion

The total lipid content per dry mass (TLDM) levels were found to be higher in *N. oculata* than in *T. suecica*, *T. fluviatilis*, *C. muelleri*, *T. chuii* and *C. vulgaris* (Table 1). Servel *et al.* (1994) reported higher levels in *I. galbana* (25.6%) and *C. calcitrans* (11.8%) and lower levels in *Nannochloropsis* sp. (5.6%). Mata *et al.* (2010) reported TLDM values of 22.7-29.7% in *N. oculata*, 18-57% in *P. tricornutum,* 20.6% in *T. pseudonana,* 7% to 40% in *Isochrysis galbana*, 8.5-23% in *Tetraselmis suecica*, 5-58% in *Chlorella vulgaris*, from 7.1-33% in *Isochrysis* sp. and 33.6% for *C. muelleri*, thus corroborating the data obtained in the present study, in which only *C. muelleri* exhibited a lower value (Table 1).

According to Michael and Borowitzka (1988), the ideal TLDMs for the different classes of algae cited are as follows: *Cyanophyceae* 2-23%, *Chrysophyceae* 12-72%, *Prymnesiophyceae* 5-48%, *Cryptophyceae* 3-17%, *Xanthophyceae* 6-16%, *Bacillariophyceae* 1-39%, *Chlorophyceae* 1-70% and *Euglenophyceae* 1-17%. In the present study, the highest TLDM among the *Bacillariophyceae* was found in *T. pseudonana* (24.67%), among *Prymnesiophyceae* in *Isochrysis* sp. (21.08%) and among *Chlorophyceae* in *C. vulgaris* (16.08%), which agrees with the wide range of values cited above (Table 1).

Renaud *et al.* (1999) studied 18 microalgae species and found TLDM values of 17% in *Chaetoceros* sp. with a mean percentage of 15.4% in the order *Bacillariophyceae*, 13.2% in *Tetraselmis* sp. with a mean percentage of 12.3% in *Prasinophyceae*, 23.4% in *Isochrysis* sp. and 17.5% in *Prymnesiophyceae*. In the present study, a similar value was obtained for *Isochrysis* sp. with a mean percentage of 20.3%, and a lower value for *C. muelleri*, despite a very similar overall average percentage in the order *Bacillariophyceae* (15.6%), as well as a lower value for the order *Prymnesiophyceae* (*T. suecica* and *T. Chuii*) with a mean percentage of 9.6% (Table 1). Renaud *et al.* (2002) obtained TLDMs of 20.7% and 16.8% for *Isochrysis* sp. and *Chaetoceros* sp., respectively, which are similar and higher, respectively, than the values obtained for the same genera in the present study (Table 1). Servel *et al.* (1994) reported a lower TLDM in *T. Suecica*, and Castro Araújo & Garcia (2005) found higher content in *Chaetoceros* sp.

Table 1. Total lipid content as percentage of dry mass (TLDM), saturated fatty acid content as percentage of total lipids (SFAC/TL) and total fatty acids (SFAC/TFA) and monounsaturated and polyunsaturated fatty acid content as percentages of total fatty acids (MUFAC/TFA and PUFAC/TFA, respectively) and of total lipids (MUFAC/TL and PUFAC/TL, respectively) in ten microalgae species.

Species	TLDM	SFAC/ TL	SFAC/ TFA	MUFAC/ TFA	MUFAC/TL	PUFAC/ TFA	PUFAC/ TL
				(%)			
T. pseudonana	24.7 ab	9.0 ab	36.7 cd	44.7 a	11.0 a	12.9 cd	3.2 bc
N. oculata	27.2 a	12.7 a	46.9 abc	44.5 a	12.1 a	8.8 d	2.4 bcd
C. muelleri	10.2 cd	3.0 c	29.4 d	36.3 ab	3.7 bcd	15.0 bcd	1.5 cd
T. suecica	8.4 d	3.5 c	41.9 bc	30.8 b	2.6 cd	26.0 abc	2.2 bcd
I. galbana	19.5 abc	4.6 a	58.0 a	28.8 b	5.6 b	12.5 cd	2.5 bcd
Isochrysis sp.	21.1 ab	8.8 ab	41.7 bc	24.4 b	5.2 bc	27.3 ab	5.8 a
T. chuii	10.7 cd	5.4 bc	50.0 ab	24.2 b	2.6 cd	25.6 abc	2.7 bcd
C. vulgaris	16.1 bcd	6.5 bc	40.2 bcd	23.5 b	3.8 bc	31.9 a	5.1 a
T. fluviatilis	9.3 d	5.3 bc	57.3 a	23.2 b	2.2 cd	15.0 bcd	1.4 d
P. tricornutum	18.1 abcd	8.5 ab	46.8 abc	4.2 c	0.8 d	18.3 abcd	3.3 b
Mean	16.52	7.4	44.9	28.46	4.95	19.3	3.0
CV (%)	20.30	22.41	9.16	15.78	20.45	25.1	19.71

* Mean values not followed by the same letter differ from each other according to Tukey's test, with an error probability of 5%.

Higher TLDM values for the genus *Isochrysis* were reported by Valenzuela-Espinoza *et al.* (2002) and Servel *et al.* (1994). Some studies also reported higher TLDM values in the genus *Chaetoceros* than those obtained in the present study (Saavedra & Voltolina, 1994; Servel *et al.,* 1994), whereas others reported similar values (López-Elías *et al.,* 2003; López-Elías *et al.,* 2005). Rebolloso-Fuentes *et al.* (2001) reported TLDM values that were similar to those obtained in the present study for *P. tricornutum* (Table 1).

The species *C. vulgaris* was found to have TLDM values compatible with some studies (Richmond, 2004) and lower than those reported by other research groups (Medina *et al.,* 1998). According to Richmond (2004), the variations in TLDM values are due to differences in culture conditions, cultivation type, nutrient concentration, growth rate, life cycle phase, environmental conditions and the state of the cells in the culture.

The saturated fatty acid content of the total lipids (SFAC/TL) and the total fatty acids (SFAC/TFA) were calculated and are expressed as % (p/p). *N. oculata* exhibited the highest SFAC/TL, although this value was not significantly different from those of the species *T. pseudonana*, *Isochrysis* sp., *I. galbana* and *P. tricornutum*. *I. galbana* exhibited the highest SFAC/TFA, although this value did not differ significantly from those of the species *T. fluviatilis*, *P. tricornutum*, *N. oculata* and *T. chuii* (Table 1). The species *I. galbana* and *T. fluviatilis* contain more than 50% SFA, including both TL and TFA. In other words, they produce lower levels of unsaturated fatty acids (UFA), which are the most desirable for human and animal nutrition. López-Elías *et al.* (2003) found a 60% SFAC/TL level in *Chaetoceros* sp., a higher value than that observed in the present study (Table 1).

The SFAC/TFA and SFAC/TL values listed in Table 1 show that *I. galbana* exhibited the highest SFAC/TFA, although it did not differ significantly from *T. fluviatilis*, *T. chuii*, *N. oculata* or *P. tricornutum*. *N. oculata* exhibited higher SFAC/TL, but again did not differ significantly from *I. galbana*, *T. pseudonana*, *Isochrysis* sp. or *P. tricornutum*. According to Qi *et al.* (2002) *I. galbana* is rich in PUFAs, however this finding did not agree with the present study, because the highest content observed was that of SFAs, with an average of 58% of the total fatty acids (Table 1). Nevejan *et al.* (2003) found a lower SFA content in *I. galbana* (33.7% of total fatty acids).

Regarding the MUFA content found in the total lipids (MUFAC/TL) and the total fatty acids (MUFAC/TFA), the species *N. oculata* exhibited a higher MUFAC/TL that did not differ significantly from that of *T. pseudonana*, and *T. pseudonana* exhibited the highest MUFAC/TFA, although this was not significantly different from those of the species *N. oculata* and *C. muelleri* (Table 1). López-Elías *et al.* (2003) determined a mean MUFAC/TL of 5.23% for *Chaetoceros* sp., which is lower than the value obtained in the present study for the same genus (Table 1). Renaud *et al.* (1999) reported MUFAC/TFA values of 23.3%, 14.7% and 11.3% in *Chaetoceros* sp., *Tetraselmis* sp. and *Isochrysis* sp., respectively; these are lower values than those obtained in the present study for the same genera (Table 1), whereas Renaud *et al.* (2002) reported similar values for *Chaetoceros* sp. (39.4%) and *Isochrysis* sp. (20.5%).

The MUFAC/TFA of *I. galbana*, *T. suecica*, *Nannochloropsis* sp. and *C. calcitrans* were determined by Servel *et al.* (1994) to be 14.8%, 17.1%, 24.0% and 29.1%, respectively, which are lower than, respectively, those obtained in the present study for the same genera (Table 1). Qi *et al.* (2002) and Nevejan *et al.* (2003) obtained MUFAC/TFAs of 20.7% and 23.2%, respectively, for *I. galbana*, which are very similar to the values obtained in the present study for that species (Table 1). Evaluating the MUFAC/TFA in *N. oculata*, *P. tricornutum* and *T. pseudonana*, Tonon *et al.* (2002) obtained values of 47.26%, 34.61% and 31.45%, respectively, which are similar to, lower than and higher than, respectively, those obtained for the same species in the present study (Table 1).

Regarding the PUFA content found in total fatty acids (PUFAC/TFA) and total lipids (PUFAC/TL), the species *C. vulgaris* exhibited a higher PUFAC/TFA that was not significantly different from those of the species *Isochrysis* sp., *T. suecica*, *T. chuii* and *P. Tricornutum*. *Isochrysis* sp. exhibited a higher PUFAC/TL that did not differ significantly from that of *C. vulgaris*. The PUFAC/TFA determined for *C. vulgaris* was similar to that reported by Moraes & Vieira Costa (2008). *N. oculata* exhibited a lower PUFAC/TFA; however, because it accumulated higher TLDM in our study, the PUFAC/TL value did not match the earlier reports (Table 1).

Renaud *et al.* (1999) obtained PUFAC/TFA values of 47.2%, 56.35% and 56.4% in *Chaetoceros* sp., *Tetraselmis* sp. and *Isochrysis* sp., respectively, which were higher than the values obtained in the present study for the same genera (Table 1). Further, Renaud *et al.* (2002) reported PUFAC/TFAs of 19.5% and 37.4% in *Chaetoceros* sp. and *Isochrysis* sp., respectively, and López-Elías *et al.* (2003) reported a PUFAC/TFA value of 34.77% in *Chaetoceros* sp.; these values are also higher than those obtained in the present study (Table 1). Saavedra & Voltolina (1994) found MUFAC/TFA, SFAC/TFA and PUFAC/TFA values of 47.83%, 43.84% and 0.93%, respectively, in *Chaetoceros* sp., a trend that was also observed in the present study, although lower values for MUFAC/TFA (36.3%) and SFAC/TFA (29.35%) and higher values for PUFAC/TFA (15.01%) were also observed (Table 1).

Servel *et al.* (1994) obtained PUFAC/TFA values of 61.5%, 58.6%, 53.3% and 32.5% for *I. galbana*, *T. suecica*, *Nannochloropsis* sp. and *C. calcitrans*, respectively; these comprised 17.0%, 20.9%, 2.8% and 8.7% of the total lipids. The values reported by the authors are higher than those listed in Table 1, in which the only similar value was that obtained for the PUFAC/TL of *N. oculata*. According to Qi *et al.* (2002), *I. galbana* is rich in PUFAs with a total value of 48.9%, which is higher than the average obtained for this species in the present study (Table 1). Nevejan *et al.* (2003) also observed high PUFAC/TFA values for *I. galbana* (41.7%). Tonon *et al.* (2002) obtained PUFAC/TFA values of 12.13%, 33.08% and 21.20% for *N. oculata*, *P. tricornutum* and *T. pseudonana*, respectively; however, the values obtained in the present study were lower for the same species (Table 1). The highest fatty acid content was the SFA 18:0 stearic acid value obtained for *T. fluviatilis*, 37.95%, which was followed by the MUFA 16:1 of 16.12% (Table 2).

C. muelleri is a prominent food source for animals of high commercial value, especially crustaceans and bivalve molluscs. The importance of this species is due to its fatty acid profile, which comprises 5-20% $20:5_{(n-3)}$, 0.2-1% $22:6_{(n-3)}$ and less than 0.2% $22:4_{(n-6)}$ (Brown *et al.,* 1997). This profile is very different from that found in the present study for the same species, which showed higher content of the fatty acids 16:1 (36.33%), 18:0 (14.38%), 14:0 (11.87%) and the PUFA 20:3 (9.69%) (Table 2). Servel *et al.* (1994) observed a high 16:1 palmitoleic acid content in this genus, however that study also found high levels of the PUFA $20:5_{n-3}$/EPA (34%). Saavedra & Voltolina (1994) found a higher percentage of the fatty acid 16:1 (47.83%), followed by 16:0 (21.82%) and 14:0 (18.29%) in *Chaetoceros* sp. The highest percentage found for this species in the present study was that of the fatty acid 16:1 (36.33%), this was followed by 18:0 (14.38%), 14:0 (11.87%) and a considerable percentage of the PUFA 20:3 (9.69%).

Rousch *et al.* (2003) compared *P. tricornutum* and *C. muelleri* and observed a higher number of fatty acids in the *C. muelleri* profile, in which 14:0, 16:0, 16:1, 16:2, 16:3, 18:0, $18:1_{(n-9)}$, $18:2_{(n-6)}$ and $20:5_{(n-3)}$ were the main fatty acids detected. López-Elías *et al.* (2003) obtained the following average fatty acid percentages for *Chaetoceros* sp. (in decreasing order): 14:0 (31.57%), 16:0 (20.46%), 18:3 (16.58%), 20:5 (10.22%), 18:0 (7.98%), 18:1 (5.23%), 20:4 (4.14%), 18:2 (1.73%), 22:6 (1.56%) and 18:4 (0.53%). This profile differs strongly from the one found in the present study, especially because no 16:1/palmitoleic acid was detected (Table 2). Renaud *et al.* (1999) obtained a higher SFAC value for 14:0 (18.8%), MUFAC for $18:1_{n-7}$ (20.2%) and PUFAC for $20:5_{n-3}$ (16.7%) in *Chaetoceros* sp. Similarly, Renaud *et al.* (2002) observed a higher SFAC value for 14:0 (23.6%), MUFAC for $16:1_{n-7}$ (36.5%) and PUFAC for $20:5_{n-3}$ (8.0%) in cultivated *Chaetoceros* sp. There were similarities with respect to MUFAC; specifically, in addition to a SFAC value of 14.28% for 16:0, 18:0 was also predominant with a value of 14.25%. The predominant PUFAC was 20:3, with 10.0% of the total fatty acids.

In the present study the mean percentages of SFAs (46.88%) and MUFAs (44.49%) found in *N. oculata* were similar, however, the PUFA percentage (8.76%) was much lower (Table 1). 16:0 palmitic acid was the most abundant SFA found in *N. oculata*, comprising 33.17% of the total fatty acids. The MUFA 16:1 palmitoleic acid was the second most abundant, comprising 30.96% of the total fatty acid content, followed by 18:1 with 13.53%. The long chain PUFA (LC-PUFA) present in the highest amounts was $20:5_{n-3}$/EPA (eicosapentanoic), which represented 5.36% of the total fatty acid content (Table 2). Similar results were reported by Servel *et al.* (1994) for *Nannochloropsis* sp., however the EPA content in that study was much higher (30.1%) than the value obtained in the present study.

Table 2. Fatty acid profiles (% of total fatty acids) obtained from ten microalgae species.
CV= *Chlorella vulgaris;* ISO= *Isochrysis* sp.; TP= *Thalassiosira pseudonana*; PTRI= *Phaeodactylum tricornutum*; TFL= *Thalassiosira fluviatilis*; CMU= *Chaetoceros muelleri*; NANO= *Nannochloropsis oculata*; TS= *Tetraselmis suecica*; TCHU= *Tetraselmis chuii*; ISOGA= *Isochrysis galbana*.

NCFA	CV	ISO	TP	PTRI	TFL	CMU	NANO	TS	TCHU	ISOGA
08:0	–	–	–	0.63	–	0.11	–	–	–	0.88
10:0	–	0.97								
12:0	–	–	–	–	–	–	0.31	–	–	–
14:0	1.72	15.47	0.60	5.95	13.35	11.87	8.14	0.56	1.51	21.65
14:1	1.15	0.92	13.75	–	–	–	–	0.13	0.97	3.98
15:0	–	–	0.51	1.38						
16:0	21.17	12.61	2.69	26.96	3.35	0.96	33.17	27.32	23.43	20.84
16:1	8.03	3.59	24.21	–	16.12	–	30.96	5.56	5.25	3.693
17:0	1.29	–	27.33	2.73	–	–	–	1.02	0.473	0.99
18:0	15.47	1.59	3.11	6.09	37.95	14.38	1.07	12.68	2.68	4.72
18:1	–	18.29	4.51	4.19	7.07	–	13.53	25.11	17.55	21.12
18:1c	13.46									
18:1t	0.89	0.94	1.80							
18:2	7.44	3.54	3.38	3.57	1.22					
18:2t	–	–	–	–	–	0.243	–	–	0.20	0.193
18:2c	–	–	–	–	–	–	–	2.23	6.19	0.873
18:3d	22.17	18.36	3.99	1.71	6.69	2.84	2.43	14.57	17.67	5.54
18:3	–	–	–	–	–	–	–	3.88	–	1.20
20:0	0.21	9.46	1.46	–	–	–	3.23	–	18.32	10.91
20:3	0.57	–	3.33	–	7.12	9.69	–	–	1.51	0.87
20:4	–	–	–	–	–	–	–	0.12		
20:5	0.43	3.05	2.15	13.03	–	0.12	5.36	5.24		–
22:0	–	0.16	–	–	0.76	0.157	0.95	–	3.61	–
22:1	–	–	0.397	–	–	–	–	–	0.43	
22:2	0.44	2.18								
22:6	0.86	0.18	–	–	–	2.12	0.973	–	–	3.92
24:0	–	1.48	1.01	3.11	1.87	1.88	–	–	–	1.38
24:1	–	0.67								
Total	97.32	95.10	93.98	69.34	98.18	100.14	100.13	100.00	100.00	99.80

NCFA= Number of carbons on fatty acid chains.

According to Schneider *et al.* (1995), the most abundant fatty acids in the genus *Nannochloropsis* are 16:0, $16:1_{n-7}$ and $20:5_{n-3}$, a profile that was confirmed in the present study (Table 2). Tonon *et al.* (2002) observed large variations in the fatty acid profile, depending on the extraction method and the growth phase during which the analysis was made, although there was a predominance of the fatty acids 16:1, 16:0, 18:1 and 20:5 in *N. oculata*, a tendency that was confirmed for this species in the present study (Table 2).

SFAs (41.91%) were predominant in *T. suecica*, followed by MUFAs (30.79%) and PUFAs (26.03%). *T. suecica* contained high levels of the SFAs 16:0 (27.32%) and 18:0 (12.68%), followed by the MUFA 18:1 (25.11%) and the PUFAs 18:3δ (14.57%) and 20:5 (5.24%) (Table 2). Similar results were also reported by Servel *et al.* (1994), although the authors found a high level of the fatty acid $18:4_{n-3}$ (21.6%). SFAs were predominant in *T. chuii* (50.02%), followed by PUFAs (25.58%) and MUFAs (24.19%); the latter two were found at similar levels in the present study (Table 1). The highest values for *T. chuii* were for the SFA 16:0 palmitic acid (23.43%) and 20:0 eicosanoic acid (18.32%), followed by the PUFA 18:3/δ-linolenic acid (17.67%) and the MUFA 18:1/ oleic acid (17.55%) (Table 2).

The highest mean fatty acid percentage in *I. galbana* was of SFAs (58.04%), followed by MUFAs (28.80%) and at lower levels PUFAs (12.57%),

as shown in Tables 1 and 2. *I. galbana* contained higher levels of the SFA 14:0 tetradecanoic acid (21.65%) and 16:0 palmitic acid (20.84%), followed by the MUFA 18:1 oleic acid (21.12%), the SFA 20:0 eicosanoic acid (10.91%) and the PUFA 18:3δ-linolenic acid (5.44%), as shown in Table 2. Similar results were reported by Servel *et al.* (1994) for the same species, although the authors reported a high percentage of the PUFA $18:4_{n-3}$, which was not observed in the present study. Poisson & Ergan (2001) reported high levels of the fatty acids 18:1, 14:0, 16:0 and 22:3 for *I. galbana* in cultures grown in Jones or Provasoli 1/3 medium; regardless of whether the cultures were analysed at day 4, 8 or 14, higher levels of the fatty acid 18:1 were always observed. In the present study there were similarities in the values obtained for the fatty acids 14:0, 16:0 and 18:1. High levels of the fatty acids 14:0, 16:0 and 18:1 were also previously reported for *I. galbana* (Qi *et al.,* 2003).

Sánchez *et al.* (2000) reported high percentages of 14:0 tetradecanoic acid (27.8%), 16:0 (20.5%), 18:1 (10.4%), and $22:6_{(n-3)}$/DHA (9.7%) in *I. galbana.* In the present study values of 21.65%, 20.84%, 21.12 % and 3.9% were obtained for the same fatty acids, respectively, which are very similar to the above-cited results. According to Qi *et al.* (2002), *I. galbana* is rich in PUFAs such as DHA ($C22:6_{n-3}$, $\Delta^{4,7,10,13,16,19}$) which represents 12% of total fatty acids and is thus higher than the mean value obtained in the present study (Table 2). The above-mentioned authors also obtained a higher value for the fatty acid 18:3, a similar value for 14:0 and lower values for 16:0, 16:1 and 18:1.

The highest fatty acid concentration in *C. vulgaris* was that of the PUFA 18:3 (22.17%), followed by the SFAs 16:0 (21.17%), 18:0 (15.47%) and 18:1 (13.46%) (Table 2). Similarly, Mendes *et al.* (1995) reported the fatty acids 18:1, 16:0 and 18:3 to be the main lipid constituents, representing 41%, 22% and 9%, respectively, and thus exhibiting higher, similar and lower levels, respectively, than those found in the present study (Table 2). The highest percentage of fatty acids in *C. vulgaris* was of SFAs (40.2%), followed by PUFAs (31.9%) and a lower MUFA content (23-5%), as shown in Table 2.

According to Ramadhas *et al.* (2005), soybean oil has the following profile: 11.75% 16:0/palmitic acid, 3.15% 18:0/stearic acid, 23.27% 18:1/oleic acid, 55.53% 18:2/linoleic acid and 6.31% 18:3/ linolenic acid. As shown in Table 2, the fatty acid profile of *C. vulgaris* is much more complex and the percentages of linoleic and oleic acids are much lower, whereas the percentages of palmitic, stearic and linolenic acids are higher.

The highest percentage of fatty acids in *T. pseudonana* was that of MUFAs (44.67%), followed by SFAs (36.72%) and PUFAs (12.85%). Among SFAs, the highest percentage was that of 17:0/heptadecanoic or margaric acid, with 27.33% of the total fatty acid content. High levels of the MUFAs 14:1 (13.75%) and 16:1 (24.21%) were observed. The highest PUFA level was that of the fatty acid 18:3, although this was similar to the values obtained for 18:2 and 20:3 (Table 2). Tonon *et al.* (2002) observed the following profile for *T. pseudonana*: 7.28% 14:0, 22.64% 16:0, 26.15% 16:1, 1.94% 18:0, 5.30% 18:1, 16.66% 20:5 and 4.54% 22:6, with a predominance of the fatty acids 16:1, 16:0 and 20:5, a tendency that was maintained independently of the extraction method and the growth phase during which the analysis was made. However, this tendency was not confirmed for this species in the present study (Table 2).

There was a high percentage of non-determined fatty acids in *P. tricornutum*, comprising 30.66% of the total fatty acid content. However, the predominance of SFAs, mainly 16:0, followed by the PUFA 20:5 (Table 2), was observed, which is typical for Diatomaceae during cold periods. High levels of the fatty acids 20:5, 16:1, 16:0 and 14:0 were reported by Alonso *et al.* (2000) for *P. tricornutum*, and these differ from the profile obtained for the same species in the present study (Table 2).

In *Isochrysis* sp., the highest fatty acid content was that of the PUFA 18:3 (18.36%), followed by the MUFA 18:1 (18.29%) and the SFA 14:0 (15.47%). However, the highest fatty acid percentage in *Isochrysis* sp. was for SFAs (41.73%), followed by PUFAs (27.30%), whereas the lowest content was that of MUFAs (24.41%) (Table 1). Renaud *et al.* (1999) reported higher levels of the SFAs 14:0 (17.3%) and 16:0 (12.0%), the MUFA $18:1_{n-7}$ (6.9%) and the PUFAs $18:4_{n-3}$ (19.0%), $18:5_{n-3}$ (10.6%) and $22:6_{n-3}$ (9.9%) for *Isochrysis* sp. Cultivating *Isochrysis* sp. under different temperatures, Renaud *et al.* (2002) reported the highest levels for SFA 14:0 (25.9%), the MUFA $16:1_{n-7}$ (11.3%) and the PUFA $18:4_{n-3}$ (10.0%) at a temperature of 25 °C. In the present study, high levels of the SFAs 14:0, 16:0 and 20:0, the MUFA 18:1 and the PUFA 18:3 were observed for *Isochrysis* sp. (Table 2).

Conclusions

The species *N. oculata* exhibited the highest lipid content, with low levels of polyunsaturated fatty acids and high levels of monounsaturated fatty acids, which together comprised more than 50% of the total fatty acids.

The species *T. pseudonana* exhibited a high lipid content, high levels of monounsaturated fatty acids and low levels of polyunsaturated fatty acids.

The species *C. vulgaris* and *Isochrysis* sp. exhibited high levels of polyunsaturated fatty acids. With regard to LC-PUFAs (long-chain polyunsaturated fatty acids), the species *P. tricornutum*, *N. oculata* and *T. suecica* exhibited the highest levels of EPA (eicosapentaenoic acid), and the species *I. galbana* and *C. muelleri* exhibited the highest levels of DHA (docosahexaenoic acid).

The freshwater species *C. vulgaris* had the highest levels of linoleic and linolenic acids, followed by the marine species *T. chuii*.

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