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Comparative characterization of three *Tetraselmis chui* (Chlorophyta) strains as sources of nutraceuticals

Gleyci A. Oliveira Moser[1] · José Juan Barrera-Alba[2] · María J. Ortega[3] · Catharina Alves-de-Souza[4] · Ana Bartual[5,6]

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Abstract

Species of the genus *Tetraselmis* have traditionally been used as a valuable nutritional source in aquaculture for their high fatty acid content (5–10% dry weight). Their use in nutraceutical production for humans is growing worldwide. Among them, *Tetraselmis chui* is generally reported as rich in polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). We evaluated the potential of three *T. chui* strains for the production of these nutraceuticals: the model strain CCAP 8/6, which is broadly used in the aquaculture industry due to its high PUFA content, and two strains (TCBG-1 and TCBG-2) isolated from Guanabara Bay (Rio de Janeiro, Brazil). The two Brazilian strains grew faster than CCAP 8/6 with higher percentage of PUFAs (up to 70% of total FA at the exponential growth phase). They also produced unique fatty acids in significant quantities: TCBG-1 produced arachidonic acid (ARA) and EPA during the exponential phase (> 20% of total FA), while TCBG-2 produced these PUFAs in addition to DHA (> 18% of total FA) at the late exponential phase. A two-stage growth system using co-cultures of the two Brazilian strains is proposed as an optimal model for PUFA production, based on their simultaneous scaling cultivation in photobioreactors. Furthermore, both strains are suitable candidates for upscaling in open systems in tropical regions since they are adapted to the environmental conditions in Guanabara Bay, where they form massive blooms by outcompeting other microalgae.

Keywords Docosahexaenoic acid (DHA) · Eicosapentaenoic acid (EPA) · Fatty acids · Intraspecific variation · Microalgae · *Tetraselmis chui*

Introduction

Polyunsaturated fatty acids (PUFAs) and derivatives, such as fatty acid amides (FAAs), are organic molecules with numerous biotechnological applications (Patil et al. 2007; Wang et al. 2012). Notably, eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) are important omega-3 sources, while arachidonic acid (ARA, C20:4n6) is a vital omega-6 PUFA (Balic et al. 2020). EPA and DHA are applied in the treatment of diseases such as atherosclerosis, cancer, rheumatoid arthritis, psoriasis, "Alzheimer," and macular degeneration (Li et al. 2019), while ARA is essential for pre- and post-natal development (Crawford et al. 2000). Additionally, PUFAs, such as gamma

✉ José Juan Barrera-Alba
barrera.alba@unifesp.br

[1] Laboratório de Ecologia e Cultivo Do Fitoplâncton Marinho, Departamento de Oceanografia Biológica, Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, Rio de Janeiro, RJ CEP 20550-900, Brazil

[2] Departamento de Ciências do Mar, Instituto do Mar, Universidade Federal de São Paulo, Rua Carvalho de Mendonça, 144. Encruzilhada, Santos, SP CEP 11070-100, Brazil

[3] Departamento de Química Orgánica, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Avda. República Saharaui, S/N, 11510 Puerto Real, Cádiz, Spain

[4] Algal Resources Collection, MARBIONC, Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC NC28409, USA

[5] Instituto Universitario de Investigaciones Marinas (INMAR), Universidad de Cádiz, Avda. República Saharaui, S/N, 11510 Puerto Real, Cádiz, Spain

[6] Departamento Biologia, Facultad de Ciencias del Mar Y Ambientales, Universidad de Cádiz, Puerto Real, República Saharaui, s/n, 11510 AvdaCádiz, Spain

linoleic acid (GLA), are potential agents in cancer treatment (Wang et al. 2012).

In the last decades, omega-3-PUFA have been categorized as nutraceuticals, that is, compounds that have benefits for human health and aid prevention and/or treatment of disease and/or health disorder(s) (De Felice 1995; Kalra 2003; Da Costa 2017). Due to its high levels of EPA and DHA, wild oily fish is considered a health food and fishoil is in high demand for use in aquaculture feeds as a source of these PUFAs (Miller et al. 2008; Chauton et al. 2015). The global nutraceutical market size has been value at US$ 417.66 billion in 2020 (Liong 2015; MS&S Report 2021) . Based in the rising geriatric population, increasing healthcare costs, and changing lifestyle and food innovation, it is expected to grow at compound annual growth rate (CAGR) of 8% from 2020 to 2028 (Da Costa 2017). Commercial production of healthy oils from wild fish stocks has led to economic, ethical, and environmental concerns (Tocher et al. 2019) and due to increasing competition in the global market, fish oil is being substituted by vegetable alternatives such as microalgae (Steinrücken et al. 2017; Koyande et al. 2019).

Microalgae are considered an essential and sustainable alternative to fish oil as a source of PUFAs, EPA, and DHA (Tocher et al. 2019). According to Patil et al. (2007), microalgae may have higher lipid stability compared to traditional PUFA sources due to their rich antioxidant carotenoids and vitamins and bioencapsulated lipids by their cell walls. Additionally, microalgae have critical advantages for commercial production over transgenic plants or fungi: they are easy and fast to cultivate, have high productivity, and a natural capacity for adaptation in diverse and even adverse environmental conditions (Adarme-Vega et al. 2014).

Species of the genus *Tetraselmis* (Chlorophyta, Chlorodendrophyceae) have traditionally been used as animal feed in aquaculture for their high valuable lipids content (Rahman et al. 2017). *Tetraselmis* spp. are unicellular flagellates, with a flattened elliptical morphology with 4 equal flagella. In nature, they can survive from marine/euryhaline to freshwater habitats. Their protein contents vary from 35 to 40% dry weight (DW), including all the essential amino acids, total lipids range from 5 to 13.98% DW (Rahman et al. 2017; Qazi et al. 2021), and carbohydrates from 30 to 35% DW (Muller-Feuga 2000). Strains of the species *Tetraselmis chui* are generally rich in PUFAs, EPA, and DHA, as well as fat-soluble carotenoids (Sarpal et al. 2019). Carotenoids and phenolic compounds have also been shown to be important contributors to antioxidant activity in microalgal biomass (Goiris et al. 2012) and *T. chui* is a promising potential source of antioxidants (Banskota et al. 2019). In Europe, various companies are producing *Tetraselmis* biomass for aquaculture (Araujo et al. 2021). Moreover, the Spanish company "Fitoplancton Marino S.L." was granted authorization in 2014 to market dried *T. chui* as human food supplements in the European Union (under trade name TetraSOD) endorsed by the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN 2017; Mantecón et al. 2019). This company markets dried *T. chui* in different food categories, such as sauces, special salts, and condiments (AECOSAN 2017). This fact opens the market for the study of the production of *Tetraselmis* strains to be integrated into human diets and encourages the screening of strains acclimated to different environmental/geographical conditions to scale up its cultivation from photobioreactors (PBRs) to open systems. We also prospected the production of polyunsaturated aldehydes (PUA) from the different strains of *Tetraselmis*. PUA are produced by lypoxidation of PUFA when membrane cell is damaged and membrane integrity is compromised. These oxilipins were firstly described in diatoms (Miralto et al. 1999) and several biological functions have been proposed for them as grazer defense (Ianora et al. 2004), cell to cell signaling (Vardi et al. 2006), or antibacterial activity (Ribalet et al. 2008). In the last decade, an applied interest of these molecules has been revealed as anticancer compounds for future development (Sansone et al. 2014; Martínez-Andrade et al. 2018).

In this study, our objective was to evaluate the potential of three strains of the unicellular chlorophyte *T. chui* as PUFA source for nutraceutical applications and to enquire the production of other lipid derivatives as PUA. We include two Brazilian strains freshly isolated from Guanabara Bay (Rio de Janeiro, Brazil) as potential candidates for large-scale cultivation in that latitude. We propose the Brazilian strains of *T. chui* as plausible candidates for future production as nutraceuticals and human food in a similar manner as it has been developed in Europe.

Materials and methods

Microalgal culturing and molecular identification of the Brazilian strains

The model *Tetrselmis chui* strain CCAP 8/6 (hereafter TC1) was obtained from the culture collection of Marine Culture Service of University of Cádiz (Spain). The two Brazilian *T. chui* strains TCBG-1 and TCBG-2 (hereafter TC2 and TC3) were freshly isolated from a bloom in Guanabara Bay (Rio de Janeiro, Brazil; 22°56.58′S; 43°08.42′W). The three strains were maintained semi-continuously in 2-L stock cultures using filtered (0.2 µm) natural seawater enriched with F/2 medium (Guillard and Ryther 1962). These stock cultures were maintained at 20 °C under a continuous light regime and constant irradiance of 75 µmol quanta m^{-2} s^{-1} (Maddux and Jones 1967; Sigaud and Aidar 1993; Go et al. 2012).

The genetic characterization of the two recently isolated Brazilian strains was based on the marker SSU rDNA. For each strain, 50 mL of culture was centrifuged, and the DNA extracted following the CTAB protocol (Lebret et al. 2012). The detailed method descriptions for DNA extraction, PCA, sequencing, and phylogenetic analysis are presented in the supplementary materials (Supplementary material 1).

Experimental design

Experimental cultures were obtained from the scaling-up of stock cultures in air agitated, 12-L tubular photobioreactors (PBRs) (Fig. 1) using 0.2-µm filtered natural seawater enriched with F/2 medium (Supplementary Table 6) at equivalent light and temperature conditions as the stock's cultures explained above. For each strain, the scale-up procedure started with stock cultures of 100 mL in Erlenmeyer flasks transferred in triplicate to 500-mL bottles and then in triplicate 1-L flasks, each subsequently used to inoculate one PBR at initial cell density of 10^4 cells mL^{-1} (Fig. 1). The PBRs were maintained at pH 8 through variable CO_2 injections in the aeration system (a pressure of 0.1 bar) that automatically add CO_2 to the PBRs by setting a pH set point of 8.2. The triplicate PBRs for each strain were followed until late exponential growth for 12–13 days. Samples for the determination of growth rates and pigment composition were obtained every day, while PUFAs and polyunsaturated aldehydes (PUAs) were determined twice, during exponential (day 5th or 6th) and late exponential (day 10th or 11th) growth phases.

Microalgal growth

Microalgal growth was assessed based both on in vivo fluorescence and cell counts. Samples (3 mL) were collected in triplicates from each PBR for the analysis of in vivo fluorescence using a Turner Design TD 700 fluorometer immediately after sampling. These samples were then fixed with formaldehyde (1%) and cells were automatically counted through image flow cytometry (IFC) analysis (Amnis ImageStream XMkII, Luminex Corporation). Additionally, cell morphometric characteristics of the strains (i.e., diameter and length) were also measured daily in 800–2000 individuals per strain. Cell volume and area were estimated using formulas based on the spheroid geometric shape (Sun and Liu 2003) using the morphometric cell characteristics obtained by IFC.

Growth rates were calculated from cell counts according to an exponential model (Fogg and Thake 1987) . The specific growth rate (μ, day^{-1}), the slope of the growth rate

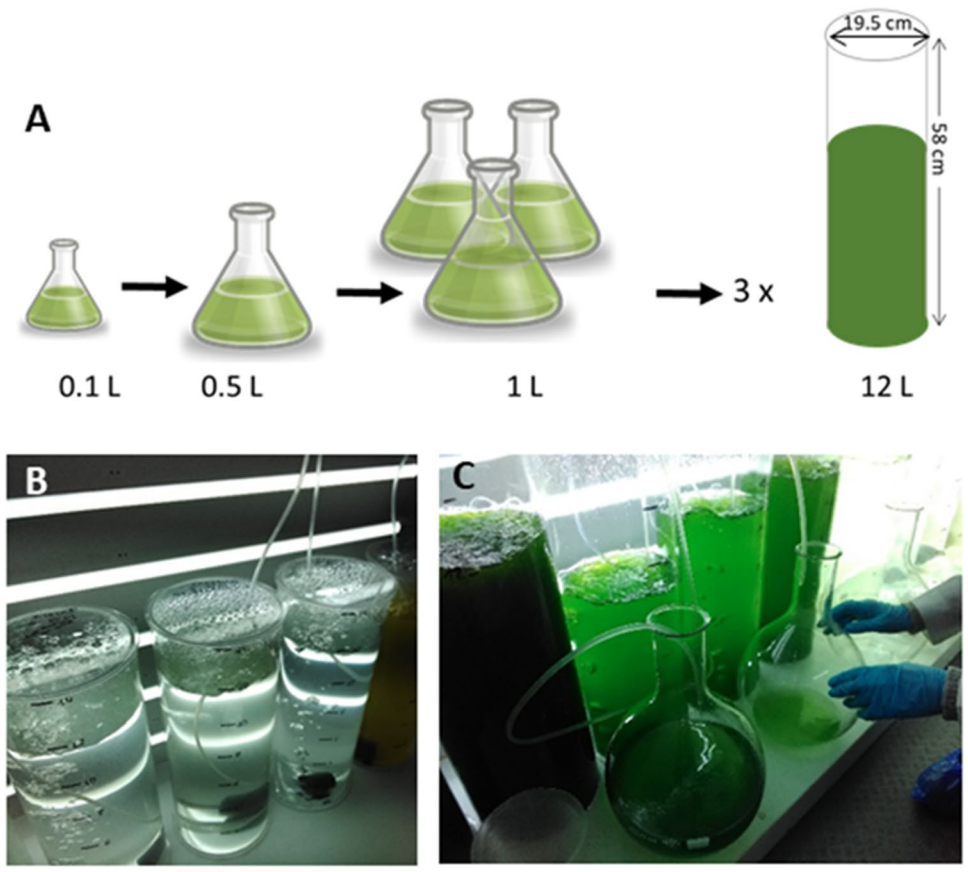

Fig. 1 (A) Diagram showing the scaling up procedure during the experiments; (B) Tubular photobioreactors (PBRs) used in the experimental set-up on day 0. (C) PBRs during sampling during exponential growth phase

curve in the exponential phase, was calculated according to the following:

$$\mu = \frac{\ln(\alpha_2/\alpha_2)}{(t_2 - t_1)}$$

where α_1 and α_2 are the cell counts at the beginning and the end of the exponential growth phase, at times 1 (t_1) and 2 (t_2), respectively.

Pigment composition

Samples for pigment composition (3–50 mL, depending on cell density) were concentrated by filtration through GF/F filters that were immediately frozen in liquid nitrogen and preserved at − 80° C until analysis. Chlorophyll-*a* (Chl.*a*), chlorophyll-*b* (Chl.*b*), phaeopigments, and carotenoids were extracted using 90% acetone for 24 h at 4 °C in the dark. Pigment concentrations were then determined by spectrophotometry using equations of Jeffrey and Humphrey (1975) for Chl.*a* and Chl.*b* for green algae and Parsons et al. (1984) for carotenoids as follows:

$$\begin{aligned} Chlorophyll\ a &= 11.93E_{664} - 1.93E_{647} \\ Chlorophyll\ b &= 20.36E_{647} - 5.50E_{664} \\ Carotenoids &= 7.6\left(E_{480} - 1.49E_{510}\right) \end{aligned}$$

where E_x stands for the extinction coefficient at "*x*" wavelength (corrected by the 750-nm reading).

Lipid extraction and analysis

Total lipids (TL) were extracted following the protocol described by Folch et al. (1957) modified by Iverson et al. (2001). Cells from 500 mL were collected by centrifugation (4000 rpm at 4 °C for 15 min), frozen in liquid N_2, lyophilized, and preserved at − 80 °C until extraction. Freeze-dried cells were treated six times with a solution of chloroform:methanol (2:1) and sonicated (ultrasound bath, 200 W-50 Hz) for 10 min. The combined extracts were filtered, evaporated under reduced pressure, and conserved at − 80 °C until the analysis of fatty acid methyl esters (FAMEs). Fatty acids for total lipid extract were transmethylated for analysis by GC–MS. A portion of lipids (15–20 mg) was treated with 1 mL of MeOH/HCl (10:1) and heated under reflux for 1 h. After cooling, the mixture was extracted with *n*-hexane (3 × 3 mL). The organic layers were combined, rinsed with brine, and dried over anhydrous $MgSO_4$ yielding a residue after evaporation that was purified in a small silica gel column.

Fatty acid methyl esters were analyzed using a high-resolution SYNAPT G2 instrument (Waters, USA) equipped with a quadrupole-time-of-flight (QTOF) analyzer using atmospheric pressure ionization (API) in positive ionization mode.

Polyunsaturated aldehyde extraction and analysis

For PUAs, 100-mL samples were collected by centrifugation (4000 rpm at 4 °C for 15 min). The obtained pellet was treated with 2 mL of 25 mM *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) in 100 mM of Tris–HCl at pH 7.2, and conserved at − 20 °C until extraction following the protocol of Morillo-García et al. (2014). For extraction, cell suspensions were rapidly thawed, and 500 µL of internal standard was added (5 nM benzaldehyde). Subsequently, mechanical disruption was induced by ultrasound (Bandelin Sonoplus, HD2070, 97%) on ice and kept for 1 h at room temperature to ensure complete derivatization of aldehydes. Extraction was performed into hexane:methanol:water (2:1:2) separation funnel (methanol for liquid chromatography, Licrosolv, 99.8%; hexane for liquid chromatography, Licrosolv, 98%). Analysis of extracts was carried out in an Agilent 7890A GC system (Agilent Technologies Inc., USA) coupled to a Waters Synapt high-resolution mass spectrometer G2 Q-TOF (Waters, USA) equipped with APGC ionization source. The identification of analytes was based on the comparison of retention times and accurate mass measurements to those of commercially available pure standards, 2*E*,4*E*-heptadienal (90%, Sigma-Aldrich, Germany), 2*E*,4*E*-octadienal (≥ 96%, Sigma-Aldrich), and 2*E*,4*E*-decadienal (85%, Sigma-Aldrich). Chromatograms were evaluated with the QuanLynx software (version 4.1, Waters, USA).

Statistical analysis

All statistical analyses were performed in R software (R Core Team 2019) using the basic "stats" package (detailed R routine presented in Supplementary material 2). The Kruskall-Wallis nonparametric test was used to compare the three tested strains during early and late exponential growth phases regarding % DW of total fatty acids (TFAs), saturated fatty acids (STAs), monounsaturated fatty acids (MUFAs), PUFAs, EPA, DHA, ALA, and ARA. Data for each one of these descriptors were organized into six groups: (1) TC1 — exponential, (2) TC2 — exponential, (3) TC3 — exponential, (4) TC1 — late exponential, (5) TC2 — late exponential, and (6) TC3 — late exponential. For each descriptor, the total significant difference ($p < 0.05$) between these six groups was first explored using the *kruskal.test* function [number of observations (n) = 18, degrees of freedom (df) = 5], followed by pairwise comparison using the Wilcoxon rank-sum test with the *pairwise.wilcox.test* function. Additionally, the same statistical tests were performed

to differentiate pigment content, morphometry ($n = 18$, df = 5), and growth rates. The latter only considered three groups related to the three *T. chui* strains: TC1, TC2, and TC3 ($n = 9$, df = 2).

Results

Strain identification, growth rates, and morphometry

The phylogenetic analysis of the SSU region of the DNAr gene placed the two Brazilian strains (TC2 and TC3) in the *T. chui* genetic clade together with strain TC1 and other *T. chui* sequences obtained from GenBank (Supplementary Fig. 6). The three *T. chui* strains showed growth rates greater than 0.7 day^{-1} (Table 1). The strain TC1 showed the highest growth rate ($p < 0.05$) compared with TC2 and TC3. This is consistent with higher cell size and cell volume of TC2 and TC3 (Table 1).

Pigment content

High pigment concentrations were obtained for the three *T. chui* strains (Table 2). Total chlorophyll (Chl.*a* + Chl.*b*) ranged from 30.22 mg g^{-1} for TC2 to 42.56 mg g^{-1} for TC1 during the exponential growth phase, decreasing significantly towards the end of the cultivation period. Regarding total carotenoids, levels in the three strains ranged from 15.93 to 17.17 mg g^{-1} (Table 2). TC1 and TC3 showed a significantly higher ($p < 0.05$) concentration of Chl.*a*, Chl.*b*, and carotenoids compared to TC2 during the exponential growth phase. During late exponential conditions, no significant differences in pigments content were observed.

Strain productivity and total fatty acids

The biomass productivity in terms of dried weight (DW) of TC2 and TC3 strains was significantly higher (> 0.2 g L^{-1}) (Kruskall-Walis test, $p < 0.05$) than that for TC1 (0.09 g L^{-1}) (Table 3). The TFA content during the exponential phase varied from 16 to 21% DW in the three strains (Table 3). This value increased during the late exponential phase, reaching 32.2% DW in TC3. The % DW of PUFAs during

Table 1 Growth rates (μ, $n = 3$) and cell morphological characteristics ($n > 800$ cells) of the three tested *Tetraselmis chui* strains (mean and standard deviation). *MLD*, maximum linear dimension; *S/V*, surface to volume ratio. *TC1*, *T. chui* CCAP 8/6; *TC2*, *T. chui* TCBG-1; *TC3*, *T. chui* TCBG-2

Strain		Growth	Morphometry							
			Exponential phase				Late exponential phase			
		μ (day^{-1})	MLD (μm)	Area (μm^2)	Volume (μm^3)	S/V	MLD (μm)	Area (μm^2)	Volume (μm^3)	S/V
TC1	Mean	1.06	12.9	346	598	0.6	12.6	307	491	0.6
	sd	0.01	1.8	77	201	0.1	1.6	56	134	0.1
TC2	Mean	0.87	14.1	415	784	0.5	14.6	444	867	0.5
	sd	0.01	1.7	89	260	0.1	1.8	89	269	0.1
TC3	Mean	0.80	14.0	437	855	0.5	15.5	509	1063	0.5
	sd	0.09	1.6	90	267	0.1	1.9	107	341	0.1

Table 2 Pigment concentrations of the three *Tetraselmis chui* strains. Values represent the mean ± SD ($n = 3$). Data are expressed as mg g^{-1} of DW. *Chl.a*, chlorophyll *a*; *Chl.b*, chlorophyll *b*; *Carot*, total carotenoids; *TC1*, *T. chui* CCAP 8/6; *TC2*, *T. chui* TCBG-1; *TC3*, *T. chui* TCBG-2. Statistical comparison for each pigment among both growth phases was performed by a Kruskal–Wallis test ($n = 18$, df = 5) followed pairwise comparisons through Wilcoxon rank-sum test ($p < 0.05$). *Significant differences with all other conditions. [a,b,c,d,e,f]Significant differences with other conditions and similarity with the others of the same group

Strain		Exponential phase			Late exponential phase		
		Chl.*a* (mg g^{-1})	Chl.*b* (mg g^{-1})	Carot (mg g^{-1})	Chl.*a* (mg g^{-1})	Chl.*b* (mg g^{-1})	Carot (mg g^{-1})
TC1	Mean	28.34[a]	14.22[b]	15.93[c]	12.13[d]	6.40[e]	7.71[f]
	SD	1.63	1.08	0.72	0.29	0.11	0.15
TC2	Mean	19.73*	10.49*	12.03*	10.02[d]	5.27[e]	9.22[f]
	SD	2.52	1.44	1.58	3.13	1.71	3.30
TC3	Mean	25.13[a]	14.66[b]	17.17[c]	9.91[d]	4.41[e]	7.37[f]
	SD	0.92	1.97	1.40	2.80	1.55	1.75

Table 3 Dry weight (DW) and fatty acid contents of the three tested *Tetraselmis chui* strains. *TFA*, total fatty acids; *Σ MUFA*, total monounsaturated fatty acids; *Σ PUFA*, total polyunsaturated fatty acids. Data are expressed as mean ± SD; $n = 3$. *TC1*, *T. chui* CCAP 8/6; *TC2*, *T. chui* TCBG-1; *TC3*, *T. chui* TCBG-2

Strain		Exponential phase					Late exponential phase				
		DW (g L^{-1})	TFA (mg L^{-1})	TFA (% DW)	Σ MUFA (% DW)	Σ PUFA (% DW)	DW (g L^{-1})	TFA (mg L^{-1})	TFA (% DW)	Σ MUFA (% DW)	Σ PUFA (% DW)
TC1	Mean	0.09	14.8	16.3	2.27	11.5	0.58	127.0	21.9	3.74	12.14
	sd	0.00	1.0	1.1	0.37	1.21	0.05	14.2	2.6	0.76	2.79
TC2	Mean	0.22	42.9	19.6	6.09	9.12	0.67	126.5	19.0	5.18	10.74
	sd	0.02	12.7	6.4	2.55	2.88	0.05	65.9	11.9	2.67	5.83
TC3	Mean	0.26	56.8	21.5	2.99	14.83	0.67	215.5	32.2	10.79	13.34
	sd	0.12	31.6	3.3	0.45	2.01	0.10	103.7	13.9	4.86	5.52

the exponential growth phase varied among strains ranging from a minimum of 9.12% in strain TC2 to 14.83% in TC3 (Table 3) and increased slightly during the late exponential phase in the three strains.

Most TFAs (~70%; Table 3) produced by strains TC1 and TC3 during exponential growth phase were PUFAs (70 ± 4.88% and 69.06 ± 1.79% of TFA, respectively) (Fig. 2; Table 3) while TC2 showed significantly higher MUFA content ($p < 0.05$; Fig. 3) when compared with TC1. In strain TC2, most TFAs (46.59%) were PUFAs. The PUFA proportion decreased during the late exponential phase for TC1 and TC3 (55% and 42%, respectively) while it increased for TC2 (56.39%). Although no quantitatively significant differences were observed for total % DW of PUFAs among strains (Fig. 3D; Table 3), their PUFA profiles varied (Fig. 2) with significant differences observed when compared to the different types of these fatty acids ($p < 0.05$; Fig. 3). The three strains produced interesting molecules for nutraceutical uses, such as omega-3 (e.g., EPA and DHA) and omega-6 fatty acids (e.g., ARA) (Fig. 2; Tables 4 and 5). The fatty acid profile during the exponential phase (expressed as % of TFA) of strain TC1 was mainly composed of 13.94% palmitic acid (C16:0), 16.16% hexadecatetraenoic acid (C16:4), and 25.42% α-linoleic acid (C18:3-*n*3; ALA) (Fig. 2; Table 4). During the late exponential phase, this profile changed to 10.07% α-linoleic acid (C18:3-n3; ALA), 15.56% stearidonic acid (C18:4 *n*-3; SDA), and 18.78% docosahexanoic acid (C22:6 *n*-3; DHA), indicating an increase in the proportion of long-chain fatty acids with culture age (Fig. 2; Table 5). The strain TC1 lacked some other PUFAs such as EPA.

TFA contents of TC2 exponential growing cells were composed of a similar palmitic acid (C16:0) composition to TC1 (12.49%) but with a higher content of EPA (28.9%) and a lower content of SDA (6.58%). During late exponential phase, TC2 reduced the EPA content to 8.06% and increased the ALA content to 21.29% (Table 5). Regarding strain TC3, TFA content during exponential phase was mainly composed of 27.86% docosahexanoic acid (C22:6 *n*-3; DHA), 14.32% stearidonic acid (C18:4 *n*-3; SDA), and 15.56% α-linoleic acid (C18:3 *n*-3; ALA) (Fig. 2; Table 4), maintaining a high percentage of EPA (25.15%) during late exponential growth phase and increasing the percentage of MUFAs to 33.26% of TFA (Table 4).

The percentage of DW of the different fatty acids varied significantly per strain and/or growth phase. The strain TC1 did not produce significant quantities of DHA during the exponential phase (Fig. 3; Table 4) but accumulated significant quantities of it with culture age reaching 4.1% of DW ($p < 0.05$; Fig. 3) during the late exponential growth phase (Table 5). Strain TC2 showed minimal DHA content during exponential growth phase (0.48% DW) (Fig. 3; Table 4), without any production during late exponential growth

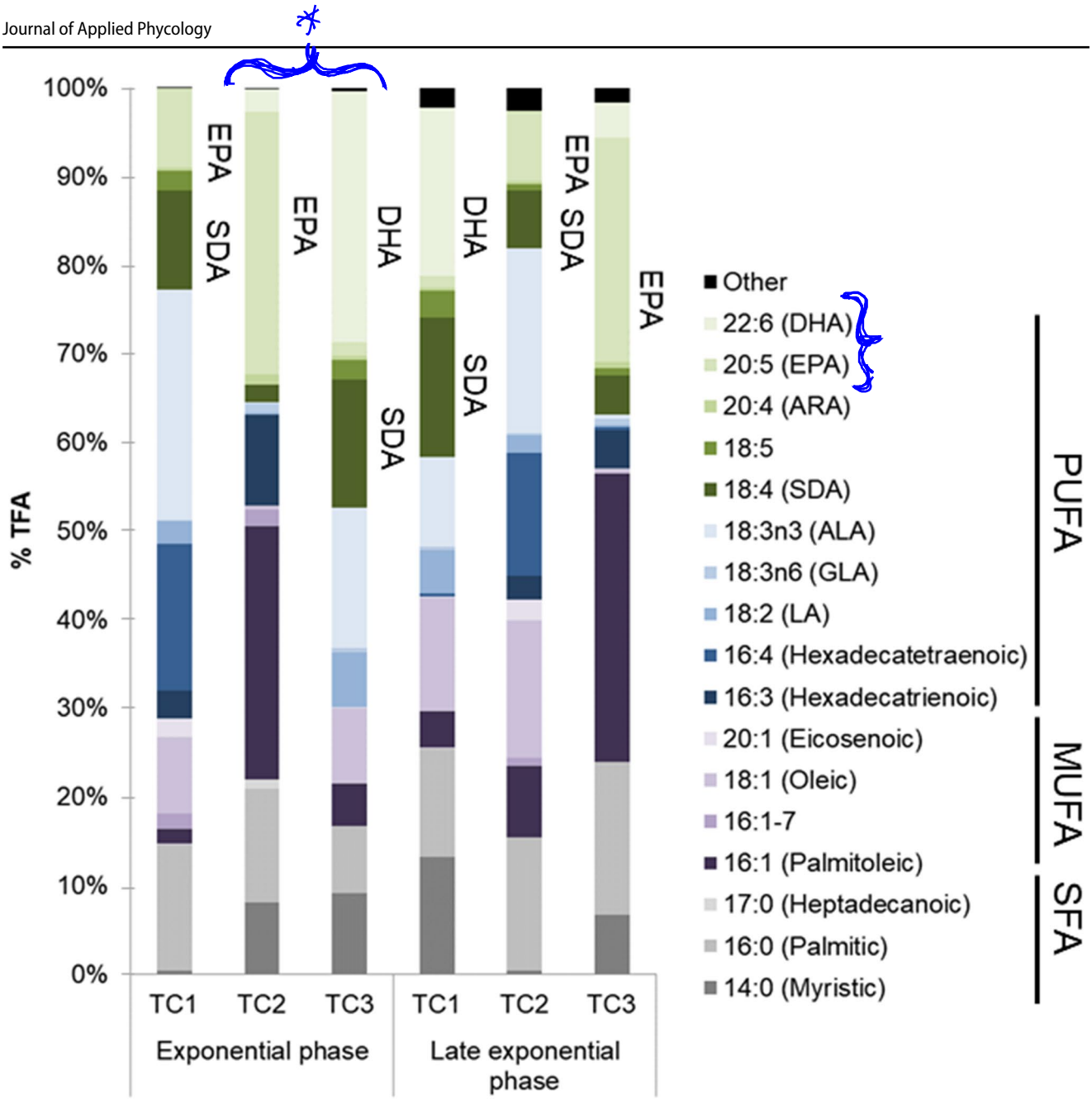

Fig. 2 Relative fatty acid composition of the three *Tetraselmis chui* strains during the exponential and late exponential growth phases. Data are average values ($n = 3$) of replicates. Other = fatty acids with amounts < 1% of total fatty acids (TFA) in all strains. TC1, *T. chui* CCAP 8/6; TC2, *T. chui* TCBG-1; TC3, *T. chui* TCBG-2

(Fig. 3; Table 5). Comparatively, the strain TC3 showed the highest DHA content at exponential growth phase (5.99% of DW), decreasing to 1.27% during the late exponential stage. Strains TC2 during exponential growth and TC3 during late exponential phase were composed of a significantly (Fig. 3; $p < 0.05$) higher content of EPA than the other two strains (> 5% of DW), which accounted for 25% of TFA content. Furthermore, TC3 during exponential phase and TC1 during late exponential phase contained significantly (Fig. 3; Table 4) high quantities of DHA (> 5% DW) compared to the strain TC2.

Polyunsaturated aldehydes

The three strains produced mainly two polyunsaturated aldehydes, 2*E*,4*E*/*Z*-Heptadienal (HD) and 2*E*,4*E*/*Z*,7-Decatrienal (DT) (Table 6). Strain TC2 also produced 2*E*,4*E*/*Z*-Octadienal (OD). With culture age, TC2 and TC3 reduced the production of HD during late exponential

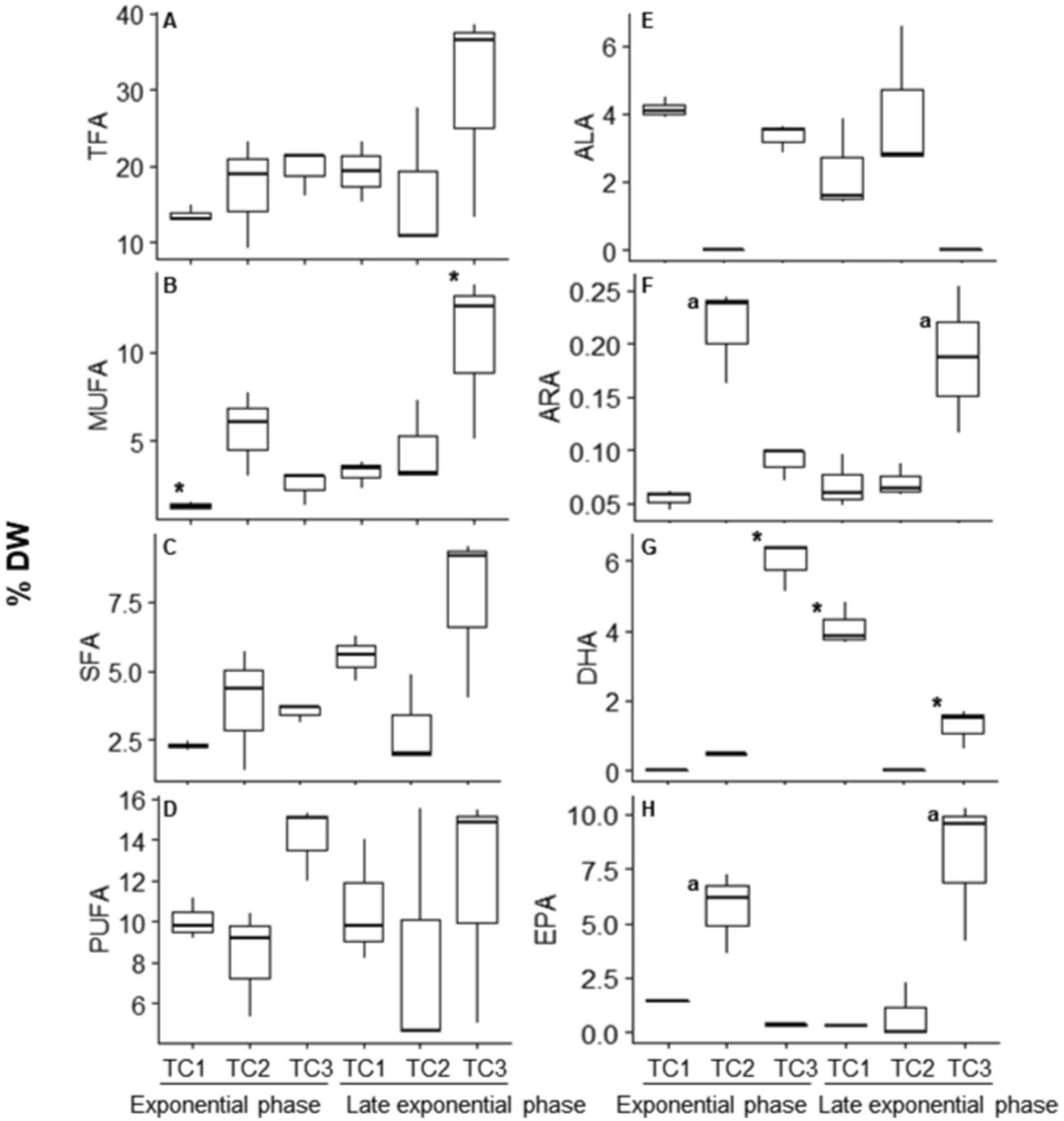

Fig. 3 Statistical comparison of total fatty acids (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), α-linoleic acid (ALA), and arachidonic acid (ARA) content as % dry weight (% DW) of the three tested *Tetraselmis chui* strains in the exponential and late exponential growth phases. Kruskal Wallis ($n = 18$, df = 5) followed by pairwise comparisons through Wilcoxon rank-sum test ($p < 0.05$). * indicates differences with all other conditions; [a] indicates differences with other conditions and similarity with the others of the same group

growth while no significant variation was observed for TC1. Polyunsaturated aldehydes levels ranged from 0.33 to 1.88 fmol/cell, which are comparable to that obtained for some diatom species in culture (Wichard et al. 2005).

Discussion

Our results have displayed the intraspecific variability of the fatty acid profile of *T. chui* strains as well as their nutritional value, especially in the freshly isolated Brazilian strains TC2

Table 4 Fatty acid profiling. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) as percentage of dry weight (% DW) and percentage of total fatty acids (% TFA) of the three strains of *Tetraselmis chui* (mean and standard deviation; $n=3$) at exponential phase (* indicates values < 0.01)

	% DW						% TFA					
Component	TC1		TC2		TC3		TC1		TC2		TC3	
(Acid methyl esters)	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
SFAs	**2.52**	**0.14**	**4.65**	**1.94**	**3.64**	**0.33**	**15.41**	**0.22**	**23.15**	**2.58**	**17.03**	**1.16**
C8:0 (caprylic)	*	*	—	—	*	*	0.04	0.06			*	*
C10:0 (capric)	*	*	*	*	*	*	0.01	0.01	0.03	0.03	*	*
C11:0 (undecanoic)	*	*	—	—	*	*	*	*			*	*
C12:0 (lauric)	*	*	0.01	*	*	*	0.03	0.01	0.07	0.01	0.03	*
C13:0 (tridecanoic)	*	*	*	*	*	*	*	*	0.01	0.00	0.01	*
C14:0 (myristic)	0.06	*	**1.54**	0.21	**1.93**	0.14	0.37	0.03	**7.84**	1.05	**8.95**	0.67
C15:0 (pentadecanoic)	0.01	*	0.15	0.01	0.06	*	0.06	0.01	0.76	0.04	0.26	0.01
C16:0 (palmitic)	**2.28**	0.01	**2.45**	0.30	**1.61**	0.10	**13.94**	0.05	**12.49**	1.55	**7.46**	0.47
C17:0 (heptadecanoic)	*	*	**0.20**	0.02	0.02	*	0.06	0.02	**1.01**	0.09	0.10	*
C18:0 (stearic)	0.13	0.02	0.14	0.01	0.01	*	0.82	0.13	0.69	0.05	0.04	*
C20:0 (arachidic)	0.01	*	0.01	*	0.01	*	0.07	0.01	0.05	0.01	0.05	0.01
C22:0 (behenic)	—	—	0.04	0.01	0.02	*	—	—	0.20	0.05	0.10	0.02
MUFAs	**2.27**	**0.37**	**6.09**	**2.55**	**3.00**	**0.45**	**13.94**	**2.30**	**30.24**	**3.48**	**13.93**	**0.21**
C14:1 (myristoleic)	*	*	0.02	*	0.06	0.01	0.04	0.01	0.09	0.01	0.29	0.03
C15:1 (cis-10-pentadecenoic)												
C16:1 (palmitoleic)	**0.27**	0.04	**5.46**	0.64	**0.99**	0.05	**1.64**	0.27	**27.82**	3.28	**4.60**	0.23
C16:1 –7	**0.29**	0.05	**0.36**	0.04	0.02	*	**1.75**	0.32	**1.84**	0.20	0.11	0.01
C17:1 (cis-10-heptadecenoic)	—	—	—	—	0.07	0.01	—	—	—	—	0.30	0.04
C18:1n9c (oleic)	**1.38**	0.21	0.10	0.02	**1.79**	0.07	**8.43**	1.31	0.49	0.12	**8.34**	0.34
C20:1n9 (cis-11-eicosenoic)	**0.34**	0.07	—	—	0.01	*	**2.06**	0.42			0.06	0.01
C22:1n9 (erucic)	*	*	—	—	0.05	0.01	0.02	0.00			0.23	0.03
PUFAs	**10.86**	**1.07**	**7.02**	**2.16**	**14.74**	**1.99**	**66.46**	**4.58**	**35.93**	**0.93**	**68.64**	**1.78**
C16:2	0.14	0.01	0.14	0.01	0.09	*	0.87	0.09	0.71	0.04	0.42	0.02
C16:3	**0.50**	0.04	**1.96**	0.10	—	—	**3.04**	0.23	**9.96**	0.51	—	—
C16:4	**2.64**	0.28	—	—	0.01	0.01	**16.16**	1.72	—	—	0.05	0.04
C18:2n6c (linoleic) — LA	**0.40**	0.05	0.05	*	**1.30**	0.07	**2.47**	0.31	0.24	0.02	**6.06**	0.33
C18:3n6 (γ-linolenic) — GLA	0.02	*	**0.20**	0.01	0.10	0.01	0.15	0.02	**1.01**	0.07	0.48	0.05
C18:3n3 (α-linolenic) — ALA	**4.15**	0.06	0.02	0.01	**3.35**	0.10	**25.42**	0.39	0.08	0.05	**15.56**	0.48
C18:4 — DAS	**1.79**	0.31	**0.38**	0.02	**3.08**	0.19	**10.93**	1.90	**1.94**	0.11	**14.32**	0.78
C18:5	**0.36**	0.11	—	—	**0.47**	0.05	**2.20**	0.70	—	—	**2.20**	0.22
C20:2 (cis-11,14-eicosadienoic)	—	—	—	—	—	—	—	—	—	—	—	—
C20:3n6 (cis-8,11,14-eicosatrienoic) — DGLA	—	—	0.04	*	0.02	0.02	—	—	0.19	0.02	0.11	0.10
C20:3n3 (cis-11,14,17-eicosatrienoic) — ERA	—	—	—	—	0.01	0.01	—	—	—	—	0.03	0.03
C20:4n6 (arachidonic) — ARA	0.06	0.01	**0.22**	0.04	0.09	*	0.33	0.06	**1.13**	0.18	0.42	0.02
C20:5n3 (cis-5,8,11,14,17-eicosapentaenoic) — EPA	**1.44**	0.09	**5.68**	0.05	**0.33**	0.03	**8.79**	0.53	**28.90**	0.25	**1.55**	0.14
C22:6n3 (cis-4,7,10,13,16,19-docosahexaenoic) — DHA	—	—	**0.48**	0.13	**5.99**	0.21	—	—	**2.43**	0.66	**27.86**	0.99

Highest values highlighted in bold

and TC3. The three tested strains grew optimally in the cultured conditions (> 0.7 day^{-1}) in the PBRs (Table 1), in the upper range of most species used for microalgal production (Supplementary Table 7). The two Brazilian strains showed higher growth rates at the experimental temperature (20 °C), corresponding to the lower range of water temperature observed at Guanabara Bay during the dry season (Paranhos 1993). Their growth could even be enhanced at higher temperatures, as described in the literature (Rukminasari et al. 2019).

Cell sizes of the three strains (Table 1) were in the typical ranges reported for other *T. chui strains.* Malibari et al.

Table 5 Fatty acid profiling. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) as percentage of dry weight (% DW) and percentage of total fatty acids (% TFA) of the three strains of *Tetraselmis chui* (mean and standard deviation; $n = 3$) at late exponential phase (* indicates values < 0.01)

	% DW						% TFA					
Component	TC1		TC2		TC3		TC1		TC2		TC3	
(Acid methyl esters)	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
SFAs	**5.79**	**0.84**	**3.09**	**1.70**	**7.97**	**3.26**	**26.50**	**3.07**	**16.20**	**0.43**	**25.00**	**0.86**
C8:0 (caprylic)	*	*	*	*			0.01	0.01	0.00	0.00		
C10:0 (capric)	*	*	*	*	0.01	0.01	0.01	0.00	0.01	0.00	0.02	0.02
C11:0 (undecanoic)	*	*	*	*	*	*	0.01	0.00	0.00	0.00	0.00	0.00
C12:0 (lauric)	0.01	*	*	*	0.02	*	0.06	0.02	0.03	0.00	0.06	0.00
C13:0 (tridecanoic)	*	*	*	*	*	*	0.02	0.00	0.00	0.00	0.01	0.00
C14:0 (myristic)	**2.87**	0.24	0.07	0.01	**2.13**	0.04	**13.12**	1.11	0.37	0.04	**6.61**	0.13
C15:0 (pentadecanoic)	0.06	0.01	0.01	*	0.29	0.03	0.27	0.04	0.05	0.00	0.90	0.08
C16:0 (palmitic)	**2.63**	0.45	**2.90**	0.09	**5.53**	0.31	**12.04**	2.04	**15.28**	0.48	**17.17**	0.95
C17:0 (heptadecanoic)	0.04	*					0.17	0.06				
C18:0 (stearic)	0.14	0.05	0.07	0.05	0.01	*	0.63	0.22	0.39	0.24	0.04	0.01
C20:0 (arachidic)	0.01	*	0.01	*	0.01	*	0.06	0.02	0.07	0.01	0.04	0.01
C22:0 (behenic)	0.02	0.02			0.05	0.01	0.10	0.08			0.16	0.04
MUFAs	**3.74**	**0.76**	**5.18**	**2.66**	**10.79**	**4.86**	**17.05**	**2.64**	**27.43**	**0.52**	**33.26**	**1.04**
C14:1 (myristoleic)	0.03	0.05	*	*	0.02	0.01	0.13	0.23	0.01	0.01	0.07	0.02
C15:1 (cis-10-pentadecenoic)					0.03	*					0.09	0.01
C16:1 (palmitoleic)	**0.87**	0.33	**1.57**	0.06	**10.43**	0.29	**3.99**	1.50	**8.26**	0.29	**32.41**	0.90
C16:1 –7			0.17	0.04					0.91	0.14		
C17:1 (cis-10-heptadecenoic)												
C18:1n9c (oleic)	**2.78**	0.63	**2.99**	0.05	0.21	0.04	**12.69**	3.07	**15.77**	0.26	0.65	0.13
C20:1n9 (cis-11-eicosenoic)	0.02	0.01	**0.47**	0.09	0.01	0.01	0.11	0.03	**2.46**	0.46	0.03	0.03
C22:1n9 (erucic)	0.03	*	*	*	*	*	0.13	0.02	0.01	0.01	0.01	0.01
PUFAs	**12.05**	**2.77**	**10.16**	**5.47**	**11.73**	**4.77**	**54.67**	**6.63**	**53.42**	**0.34**	**36.83**	**1.40**
C16:2	0.09	0.01	0.05	*	0.22	0.04	0.44	0.05	0.28	0.01	0.68	0.13
C16:3			**0.51**	0.04	**1.37**	0.07			**2.69**	0.20	**4.25**	0.20
C16:4	0.08	0.05	**2.67**	0.17	0.11	0.09	0.35	0.24	**14.06**	0.88	0.34	0.28
C18:2n6c (linoleic) — LA	**1.06**	0.19	**0.39**	0.01	0.08	0.03	**4.85**	0.87	**2.07**	0.03	0.26	0.09
C18:3n6 (γ-linolenic) — GLA	0.07	0.03	0.06	0.01	0.20	0.08	0.31	0.15	0.25	0.02	0.63	0.26
C18:3n3 (α-linolenic) — ALA	**2.20**	1.07	**4.05**	0.06	0.15	0.12	**10.07**	4.91	**21.29**	0.31	0.46	0.38
C18:4 -SDA	**3.40**	0.16	**1.25**	0.07	**1.43**	0.30	**15.56**	0.74	**6.58**	0.36	**4.44**	0.93
C18:5	**0.66**	0.21	0.13	0.02	0.27	0.13	**3.03**	0.94	0.70	0.08	0.84	0.41
C20:2 (cis-11.14-eicosadienoic)	0.01	0.02					0.05	0.09				
C20:3n6 (cis-8.11.14-eicosatrienoic) — DGLA	*	*			0.02	0.01	0.01	0.02			0.06	0.04
C20:3n3 (cis-11.14.17-eicosatrienoic) — ERA	*	*					0.01	0.01				
C20:4n6 (arachidonic) — ARA	0.07	0.02	0.08	0.02	0.22	0.14	0.31	0.08	0.41	0.11	0.69	0.43
C20:5n3 (cis-5.8.11.14.17-eicosapentaenoic) — EPA	**0.30**	0.05	**1.53**	0.11	**8.09**	0.20	**1.35**	0.22	**8.06**	0.58	**25.15**	0.63
C22:6n3 (cis-4.7.10.13.16.19-docosahexaenoic) — DHA	**4.11**	0.23			**1.27**	0.02	**18.78**	1.05			**3.95**	0.07

Highest values highlighted in bold

(2018) presented an extensive cell morphology of *Tetraselmis* sp. When compared with this study, TC1 showed cell volume similar to *Tetraselmis* sp. growing in f/2 medium, while TC2 and TC3 registered cell volume superior to this strain growing in SFW medium. Also, TC1, TC2, and TC3 had higher DW than that strain of *Tetraselmis* sp. (Malibari et al. 2018).

Very recently Pereira et al. (2018) showed that monoalgal cultures of *Tetraselmis* sp. (strain CTP4) can be successfully scaled up to industrial PBRs for 60 days without culture collapse or contamination by competing microalgae,

Table 6 Polyunsaturated aldehyde content of the three tested *Tetraselmis chui* strains (mean ± SD; $n = 3$). *HD*, 2*E*,4*E*/*Z*-heptadienal; *OD*, 2*E*,4*E*/*Z*-octadienal; *DT*, 2*E*,4*E*,7*E*/*Z*-decatrienal. Means (± sd) are expressed both as 100*fg cell^{-1} and as fg µg^{-1}

Strain	Units	Exponential phase			Late exponential phase		
		HD	OD	DT	HD	OD	DT
TC1	100*fg cell^{-1}	0.91 (±0.11)	0	0.88 (±0.05)	1.04 ±(0.11)	0	0.36 (±0.09)
	fg µg^{-1}	58.2 (±11.2)	0	56.0 (±7.7)	36.1 (±12.8)	0	12.6 (±2.6)
TC2	100*fg cell^{-1}	1.71 (±0.44)	0.29 (±0.25)	0.63 (±0.07)	0.46 (±0.18)	0.76 (±1.17)	0.09 (±0.15)
	fg µg^{-1}	83.8 (±9.6)	13.2 (±11.5)	31.2 (±2.3)	19.5 (±11.1)	28.6 (±41.5)	4.6 (±7.9)
TC3	100*fg cell^{-1}	1.27 (±0.51)	0	0.58 (±0.17)	0.31 (±0.12)	0	0.06 (±0.11)
	fg µg^{-1}	61.3 (±30.5)	0	28.1 (±12.6)	13.6 (±8.7)	0	2.2 (±3.8)

demonstrating the robustness of this species to large-scale production. The strains used in our study grew faster (Table 1 and Supplementary Table 7) and had a higher PUFA concentration (up to 70% of TFA for TC1 and TC3 during the exponential growth phase) and pigment content than *Tetraselmis* sp. CTP4 (Pereira et al. 2018). These strains seem to be good candidates for scaling up to high-volume PBRs for mass production. Considering that TC2 and TC3 strains were recently isolated from naturally occurring blooms at Guanabara Bay, this facilitates a good physiological preadaptation to these climate conditions for a plausible open system production at that latitude.

The results show the versatility of strain TC3 to produce different PUFA depending on culture age, with a high production of DHA during the exponential phase, changing to a high accumulation of EPA during the late exponential growth. Additionally, the omega-3:omega-6 ratio of the three strains was always over 1:1, ranging from 8.4 to 25.4 (Fig. 4), which validates the nutritional value of strains for food supplements (Simopoulos 2004). TC1 and TC3 showed an increase in the % DW of TFA with age, while remained at 19% DW for the strain TC2 at both growth phases (Table 3). The significant effect of growth phase in the EPA and DHA content among strains has been also reported for other microalgae (Boelen et al., 2017). Nutrient deprivation has different effects on lipid metabolism and TFA on other *Tetraselmis* strains. In fact, (Adarme-Vega et al. 2014) found that *Tetraselmis* sp. had the highest omega-3 FA at nutrient replete conditions, decreasing with nutrient stress but affecting differently to EPA proportion. These authors also demonstrated that when nutrients are depleted, there could be an autophagic process to provide intracellular nitrogen for limited de novo synthesis, and that the gene expression of genes for FA synthesis decreased under nutrient stress

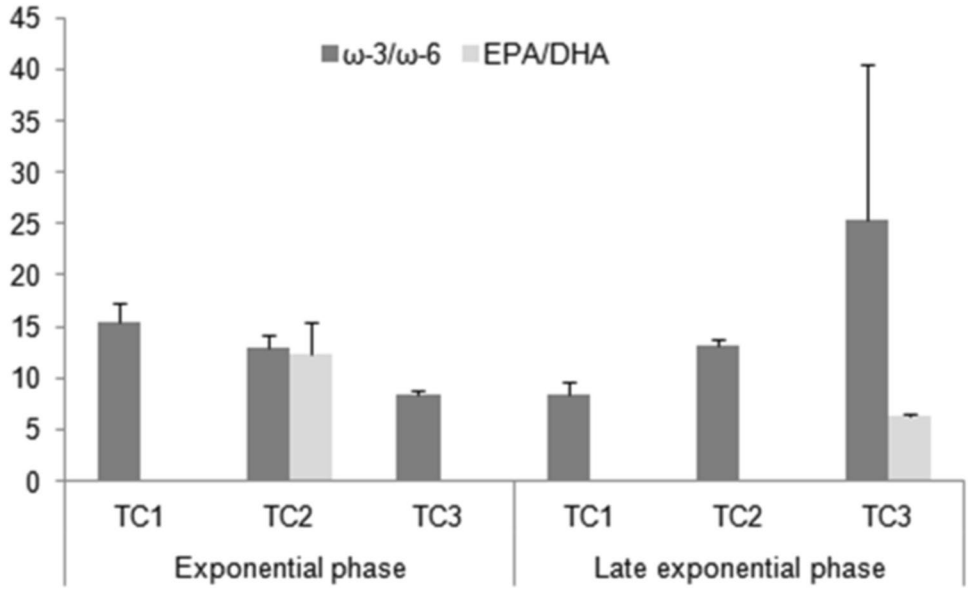

Fig. 4 Omega-3/omega-6 polyunsaturated fatty acids ratio (ω-3/ω-6) and eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA) ratio of the three tested *Tetraselmis chui* strains during both exponential and late exponential growth phases (mean ± SD). EPA/DHA ~ 0 means very low or not DHA content identified in the strain samples

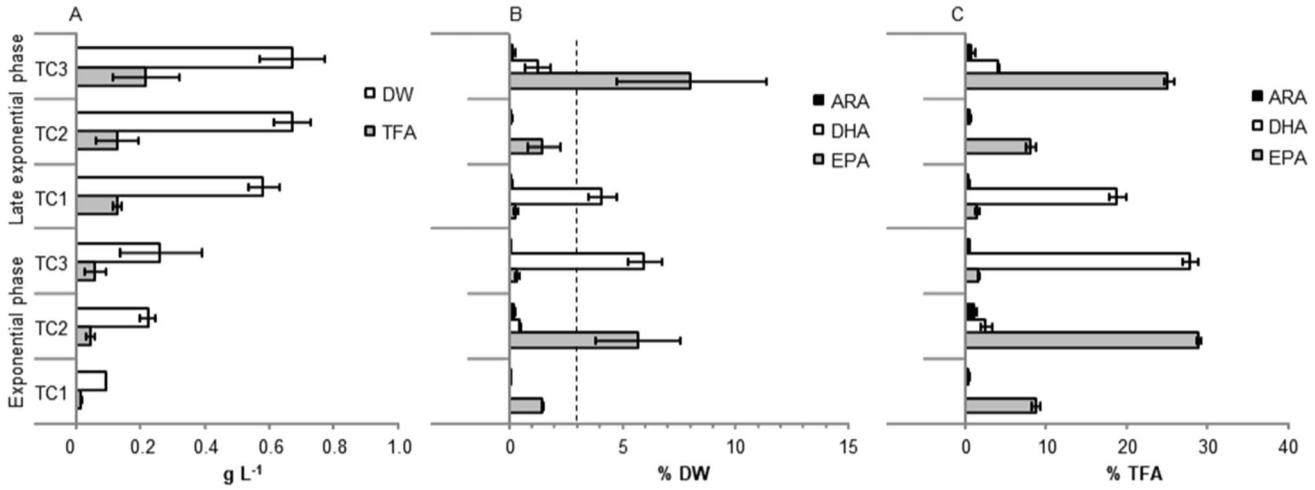

Fig. 5 (A) Dry weight (DW), total fatty acids (TFA); (B) eicosapentaenoic acid (EPA), docosohexaenoic acid (DHA), and arachidonic acid (ARA) content expressed as % DW; and (C) EPA and ARA expressed as % TFA (C) of the three tested *Tetraselmis chui* strains in the exponential and late exponential growth phases. Data are average values with standard deviation (mean ± SD; $n = 3$). The dotted vertical line indicates 3% as a benchmark level in % DW for EPA and DHA

(Adarme-Vega et al. 2014). This could explain different fatty acid profiles in cultures at different growth stages.

Harvesting dry microalgal biomass to produce fatty acids is still expensive when compared to other sources such as fish oil and vegetable oils due to the high energy requirements (Steinrücken et al. 2017). The discovery of new and robust microalgae strains that grow fast and produce naturally high levels of the desired fatty acids is a requirement for improving new technologies (Nogueira et al. 2020). The *T. chui* strains used here exceed the requirements stated by Steinrücken et al. (2017), since they grow fast ($\mu > 0.7$ day^{-1}) and present EPA and DHA higher than 3% (DW) (Fig. 5). The three tested strains showed TFAs ranging from 1.1 to 32.2% DW (Supplementary Table 7), comparable to the upper limits of other *T. chui* strains (Mohammadi et al. 2015), as well as other species commonly used as food sources, such as *Nannochloropsis gaditana* (Nogueira et al. 2020), *Scenedesmus obliquus* (Darki et al. 2017), and *Galdieria* sp. (López et al. 2018) and were generally higher than those reported for diatoms in Steinrücken et al. (2017) (summarized in Supplementary Table 8).

In an extensive review, Bellou et al. (2014) showed that different microalgae taxonomic groups contain higher amounts of specific PUFAs, i.e., diatoms usually have high EPA, dinoflagellates high EPA and DHA, as well as Chrysophyceae, Rhodophyta ARA and EPA, and Chlorophyceae ALA, EPA and DHA. Strains TC1 and TC3 had a higher DHA content (4.1 and 5.9% DW, or 18.8% and 27.9% TFA, respectively; Fig. 3, Supplementary Table 7) than that observed for other *T. chui* strains (AECOSAN 2017) as well as for other *Tetraselmis* species (Patil et al. 2007; Adarme-Vega et al. 2014; Pereira et al. 2018, 2019), comparable with the upper limit observed for some diatom species (Steinrücken et al. 2017) and lower than those observed for *Isochrysis galbana* and *Pavlova* spp. (revised at Supplementary Table 8). Concerning EPA, the higher content of EPA (> 5% of DW and 25% of TFA content) found in strains TC2 and TC3 at different growth phase stages was similar to those observed for other *Tetraselmis* species (< 12% of TFA) (Patil et al. 2007; Adarme-Vega et al. 2014; AECOSAN 2017; López et al. 2018; Malibari et al. 2018; Pereira et al. 2018, 2019; Qazi et al. 2021) and lower than those observed for other microalgae used as food sources and nutraceuticals, such as *Pavlova* spp., *Isochrysis galbana*, and *Nannochloropsis* spp. (Patil et al. 2007; Adarme-Vega et al. 2014; Peltomaa et al. 2018) (other references summarized in Supplementary Table 8). Considering all omega-3 PUFA, TC3 registered higher content (72.6 mg L^{-1} and 59.3% of TFA; Supplementary Table 7, and all the strains presented values in the upper range of those observed for other *Tetraselmis* species (18–68% of TFA; Supplementary Table 8) and for other microalgae (12–64%; Supplementary Table 8). The omega-3:omega-6 ratio of the three strains was always over 1:1, ranging from 7 to 21 (Fig. 4), higher than other *Tetraselmis* species (0.7–1.9) (Mohammadi et al. 2015; Malibari et al. 2018) and higher to those observed for *Nannochloropsis* (2.4–10.0) and *Dunaliella* (4.3–16.9) (Peltomaa et al. 2018) (Supplementary Table 8). The nutritional value of these strains is clear because EPA is essential for several metabolic pathways in humans and animals and for adequate nutrition, in particular for children and infants (Crawford et al. 2003). Also, DHA is usually added as a food supplement for infants since it has a positive effect on visual acuity development (Birch et al. 1998). Moreover,

anti-inflammatory properties have been reported for long-chain PUFAs such as EPA and DHA (Wall et al. 2010; Yates et al. 2014).

In a scenario of rising demand for omega-3 long chain PUFAs, microalgae are a potential supply for higher concentrations of EPA and DHA for supporting more healthy human diets (Birch et al. 1998). Therefore, our results of EPA and DHA as % TFA are comparable to those found in products already on the market, using microalgae as PUFA source, such as AlgalPrime™ DHA, DHAgold™, and ForPlus™, listed by Tocher et al. (2019) in their review about traditional and new sources of omega-3 long chain PUFA.

In the present study, we show for the first time the ability of *Tetraselmis* strains for synthesize PUAs (Table 6). These molecules are oxilipins, derivatives generated from PUFA lypoxidation once a cell is damaged. Ecologically, these molecules have been identified as defense molecules against grazers and infochemicals (Ianora and Miralto 2010). The obtained PUA level was in the range of some diatoms in culture (Wichard et al. 2005). In nature, these PUAs could confer an advantage for outcompeting other species in open systems cultivation since recently, Franzè et al. (2018) demonstrated that dissolved octadienal and heptadienal reduced microzooplankton herbivory on PUA producing diatoms > 5 µm, while enhancing grazing on smaller phytoplankton cells and cyanobacteria.

An additional consequence of PUA production in these strains is that during the last decade, HD and DD have been shown to have antiproliferative activity on human cancer cell lines (Sansone et al. 2014) with valuable properties supporting the use of *Tetraselmis* strains for nutraceutical applications. Moreover, Troncoso et al. (2011) reported the efficacy of 2-*trans*-4-*trans*-decadienal as an antiparasitic ingredient feed for salmon in aquaculture.

In addition to high omega-3 production and derivatives, such as oxilipins, the *Tetraselmis* strains also produced high quantities of total carotenoids (Table 2). The obtained carotenoid contents were twice as high as other microalgae (Goiris et al. 2012). Carotenoids are known as antioxidants and some of them such as astaxanthin or β-carotene constitute high-value market products (Goiris et al. 2012; Sarpal et al. 2019). The characterization of the carotenoids and phenolic content in these strains is desirable in future experiments to define the main types; however, based on these results, they are promising candidates for carotenoid production and antioxidant activities.

Conclusions

This study compares the detailed fatty acid profile of three strains of *Tetraselmis chui* cultured in PBR for biotechnological applications: the model strain CCAP 8/6 (TC1) and two freshly isolated Brazilian strains TCBG-1 and TCBG-2 (TC2 and TC3, respectively). Our findings demonstrate that all the strains produced significant quantities of PUFAs and also that their fatty acid profiles varied among strains and with culture age. At the exponential growth phase, strains TC1 and TC2 are EPA producers, while strain TC3 produces DHA. However, at late exponential phase, strain TC3 is of the three strains the most prolific in the production of EPA. These findings facilitate a continuously production of rich EPA algal biomass using scaled cultivation in PBRs of different strains of *T. chui*. The three strains showed an optimal growth in PBR, and thus we concluded that the tested strains are recommended for upscaling, considering their fast growth and high percentage of PUFAs.

A two-stage growth system based on the simultaneous cultivation of the two Brazilian *T. chui* strains can be an optimal mode of PUFA production for nutraceuticals and show a practical application of our findings.

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Author contribution Gleyci A.O. Moser contributed with the conceptualization, methodology, formal analysis, investigation, and original draft preparation. Jose J. Barrera-Alba contributed with the formal analysis, investigation, and manuscript review and editing. Maria J. Ortega contributed with formal analysis, investigation, resources, and manuscript review and editing. Catharina Alves-de-Souza contributed with formal analysis, investigation, and manuscript review and editing. Ana Bartual contributed with the conceptualization, methodology, formal analysis, investigation, resources, supervision, and original draft preparation. All authors read and approved the final manuscript.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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