Biomass Nutrient Profiles of the Microalga *Nannochloropsis*

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The nutritional composition of the marine eustigmatophyte *Nannochloropsis* spp. cultured in an indoor chemostat under continuous illumination was analyzed. Proximate composition, (moisture, ash, crude protein, available carbohydrates, fiber, lipids, and energy), nitrate, nucleic acid, mineral element (Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Pb, Cd, Cr, Ni, Co, and S), fatty acid, and pigment (carotenoids and chlorophyll) concentrations were determined. On average, the biomass contained 37.6% (w/w) available carbohydrates, 28.8% crude protein, and 18.4% total lipids. Mineral in 100 g of dry biomass were as follows: Ca (972 mg), K (533 mg), Na (659 mg), Mg (316 mg), Zn (103 mg), Fe (136 mg), Mn (3.4 mg), Cu (35.0 mg), Ni (0.22 mg), and Co (<0.1 mg). Toxic heavy metal contents (Cd and Pb) were negligible. Fatty acid content was as follows (on percent dry weight): 0.6% of 14:0, 5.0% of 16:0; 4.7% of 16:1ω7, 3.8% of 18:1ω9, 0.4% of 18:2ω6; 0.7% of 20:4ω6, and 2.2% of 20:5ω3. Nutrient composition of the biomass was highly influenced by residence time in the photobioreactor. The biomass harvested for short residence times was richer in protein and eicosapentaenoic acid than biomass harvested for high residence time.

Keywords: *Nannochloropsis; microalgae; proximate composition; mineral element; fatty acid; eicosapentaenoic acid; arachidonic acid; pigment; carotenoid; nutrient composition*

INTRODUCTION

Algae cultures have been developed principally for feeding marine molluscs used in human nourishment (*1*, *2*). Sometimes the algal biomass is added to foods to supplement the human diet and animal feed (*3*, *4*). Nearly all microalga biomass is a rich source of ω3 and ω6 fatty acids, essential amino acids (leucine, isoleucine, valine, etc.), and carotene (*5*).

Nannochloropsis is a genus that was first described by Hibbered (*6*). Members of this genus are characterized by the absence of chlorophyll *b*, which is a common phenomenon within the class Eustigmatophyceae, and the composition of the cellular xanthophyll pigments (*7*, *8*). The carotenoid composition of *Nannochloropsis* is relatively simple, containing the major xanthophyll pigments β-carotene, violaxanthin, and a vaucheraxanthin-like pigment (*9*, *10*). Zeaxanthin and anteraxanthin can be detected as minor constituents in cultures exposed to high light intensity (*11*). On the other hand, glucose is the dominant sugar in the polysaccharide composition (*12*). In the protein, the amino acids aspartate, glutamate, and proline predominate, whereas methionine, tryptophan, cystine, histidine, and hydroxyproline are found in low concentrations (*12*).

Fatty acids are another characteristic principle of the biomass; eicosapentaenoic acid (20:5ω3, EPA) is found in large amounts. Other fatty acids also found are 14:0, 16:0, 16:1, and 20:4ω6 (*13*−*15*). The triacylglycerols are characterized by a high proportion of saturated and monounsaturated short-chain fatty acids 14:0, 14:1, 16:0, and 16:1 (*14*). The high quality of *Nannochloropsis* biomass for the aquaculture industry is attributed to its high EPA amount (*16*). Among sterol composition, cholesterol, fucosterol, and isofucosterol are the principal constituents in all *Nannochloropsis* species (*17*).

The use of *Nannochloropsis* has been recognized for human diets. Thus, there are several studies about the enrichment of foods such as noodles with this biomass, to improve their nutritional profile (*3*, *4*). To date, feeding experiments have demonstrated the beneficial effect of reducing blood pressure in hypertensive rats (*18*). The bioavailability of EPA from algal sources was evaluated in rats fed with diets supplemented with this biomass. Feeding resulted in an increased ω3/ω6 ratio in liver and blood (*11*).

This paper reports on the nutrient composition of *Nannochloropsis* spp., produced in a bubble column type photobioreactor. Data are reported on proximate composition, nucleic acids, nitrate, fatty acids, mineral elements and several pigments. The objective was to show variations in the nutrient profile of the microalgal biomass as a function of the dilution rate and, hence, to determine the most appropriate conditions for producing biomass for use in human nutrition.

MATERIALS AND METHODS

Microorganism and Growth Conditions. *Nannochloropsis* sp. was supplied by Dr. H. Zmora (National Center for Mariculture, Israel Oceanographic and Limnological Research, Eilat, Israel). Cultures were grown in sterilized seawater enriched with f/2 medium nutrients (*19*). The air injected into the photobioreactor was filtered through 0.22-μm Millipore filters. Culture pH was maintained at 7.6 by automatic injection of pure carbon dioxide. The temperature was kept at 20 °C. The reactor residence time varied from 14.5 to 4.8 days, for an average value of irradiance on surface of 116 μeinsteins m^{-2} s^{-1}.

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Photobioreactor. A cylindrical bubble column (internal diameter = 0.15 m) photobioreactor was used for the indoor cultivation of *Nannochloropsis* spp. The total culture volume in the bioreactor was 29 L. Air was continuously supplied at a flow rate of 0.014 mol s^{-1}. Carbon was added as pure CO_2 directly injected as needed for pH control at a flow rate of 0.0014 mol s^{-1}. An arrangement of six Osram Dulux (36 W) fluorescent lamps with a cylindrical reflector around the bubble column directed the light into the photobioreactor. The irradiance on the culture surface was measured with a 2π sensor (LI-190SA, Li-cor, Inc., Lincoln, NE).

Analytical Methods. After steady states were reached, culture samples were collected for analysis of the biomass. The productivity of the cultures was estimated by daily measurement of the dry biomass concentration. For dry weight determination, duplicate culture samples (50 mL) were diluted (1:10) with distilled water and filtered through preweighed 1.2-μm membrane filters (Whatman). Filtered cells were then quickly washed with 25 mL of 0.9% NaCl, to remove nonbiological material such as mineral salt precipitates, and dried to a constant weight at 105 °C.

The biochemical composition of *Nannochloropsis* sp. was studied in samples collected at different reactor residence times. Biomass was harvested by centrifugation in a batch Selecta centrifuge, at 2200*g* for 5 min. The paste obtained (80% moisture) was washed with 0.5 M NaCl and distilled water and was freeze-dried (Edwards Modulyo-4K freeze-drier) for storage. The dry biomass was stored in Ependorf vials at −18 °C.

The freeze-dried biomass was analyzed for the following:

Moisture was determined by drying a representative 2-g sample in an oven with air circulation at 100−105 °C for 40 h.

Crude protein. Total N was determined in an elemental analyzer (Leco CHNS-932). The carrier gas was He, and as burning gas O_2 was used. Results were compared with that obtained by means of a semimicro Kjeldahl apparatus. Both methods gave similar results. Total protein was calculated from the evaluated nitrogen by multiplying by 6.25, previous deduction of N from nucleic acids and nitrate (*5*).

Sulfur and carbon were determined by the elemental analyzer (*20*).

Total lipids were determined as the extract obtained with chloroform/methanol (2:1) (v/v) (*21*, *22*).

Available carbohydrates were estimated by using the anthrone spectrophotometric method (*23*).

Energy content (kilojoules) of the biomass was determined by multiplying the values obtained of protein, available carbohydrates, and fat by 16.7, 15.7, and 37.6, respectively, and summing the results (*24*).

Dietary fiber was determined according to the neutral detergent fiber method (*25*).

Ash was determined by incineration of a representative 0.5-g sample in an oven at 450 °C for 48 h.

Mineral Metallic Elements. For determining metals, the ash obtained by incineration of the biomass was dissolved in a mixture of HNO_3 (37.5% w/w) and HCl (17.5% w/w) (1:1 v/v), diluted with water, and analyzed for Na, K, Ca, Mg, and Zn in an ionic charge chromatograph (Dionex DX-100). Fe, Cu, and Mn were determined by an atomic absorption spectrophotometer (Perkin-Elmer ASS-1100B), equipped with a graphite chamber (HGA-700) (*24*, *26*).

RNA and DNA. Nucleic acids were determined spectrophotometrically (*27*).

Nitrate was determined according to the brucine method (*28*).

Sulfur and carbon were determined by using the elemental analyzer previously mentioned.

Fatty Acids. Methyl esters were prepared by treatment of the lipid fraction with pure acetyl chloride and absolute methanol (*29*). The fatty acid methyl esters (FAME) of the mixture were analyzed by gas chromatography, being identified by comparing their retention times with those for standards (rapeseed oil mix and PUFAS-1, from Sigma), in a Hewlett-Packard HP5890 series II chromatography provided with a flame ionization detector and an HP3394 integrator. A capillary column of high-polarity fused silica was used (Supelco SP2330; length = 30 m; internal diameter = 0.25 mm; thickness of the film = 0.2 μm). The flow of carrier gas (N_2) was 0.75 L/min, and the split ratio of the injector was 100:1. The injector temperature was 240 °C, and the detector temperature was 260 °C. The starting temperature of the oven was 205 °C, and it was increased at a rate of 6 °C/min to 240 °C (5.83 min). The injection volume was 5 μL, and a blank was run after every two analyses. Peaks were identified by using standard FAME and quantified by using methyl heptadecanoate (17:0) as an internal standard.

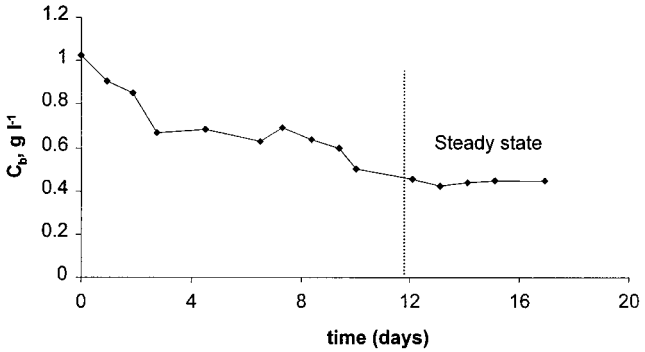

Figure 1. Variation of *Nannochloropsis* spp. biomass concentration with time. The residence time was 5.8 days.

Total carotenoids were determined spectrophotometrically (*30*).

Carotenoids Class. Carotenoids were extracted from the biomass using the method of Mercadante and Rodriguez-Amaya (*31*). The carotenoids in the mixture were analyzed by HPLC−mass spectrometry. Analyses were made with a Hewlett-Packard HP11100. The stationary phase was YMC Carotenoid C-30 5 μm, 4.6 × 250 mm. The drying gas flow was 6 L/min, the nebulizer pressure was 40 psig, the drying gas temperature was 325 °C, the vaporizer temperature was 450 °C, the capillary voltage was 2500 v and the corona current was 3 mA.

The mobile phase was a mixture of methanol:methyl-terbutyl-ether. The gradient began with 15% (v/v) of methanol and finished with 100% of methanol at $t = 60$ min. The eluent flow was 1 mL/min. The interface between the LC and MS was atmospheric pressure chemical ionization (APCI) positive (fragmentor 100 v). The carotenoids were identified by comparing their molecular weights and retention times with those in the literature. The percentages for the different carotenoids were obtained by mean of the areas integration method (*8*, *32*).

Chlorophylls were determined spectrophotometrically (*33*).

Recoveries were determined by the addition of known quantities of the principles to the microalgal biomass at the beginning of the experimental procedures. Mean recoveries ranged from 93% for available carbohydrates to 103% for nitrate.

Statistical Analysis. Principal component analyses were performed with the software package Statgraphics for Windows v. 3.3.

All analyses were performed in triplicate, and variation in any one sample was routinely <5%. Mean values and standard deviations based on these results are shown in the tables.

RESULTS AND DISCUSSION

Growth Conditions. The chemostat culture is a useful tool for obtaining steady-state conditions for the study of microalgal growth. The effect of different residence times was studied under a constant external irradiance (116 μeinsteins m^{-2} s^{-1}) to see the response of the cells to different culture ages. Generally, light availability determines the steady-state biomass concentration achievable in chemostat cultures. Figure 1

Table 1. Effect of Residence Time (Rt) on Steady-State Biomass Concentration (C_b) and Biomass and EPA Output Rate (P_b and P_{EPA}) in *Nannochloropsis* spp. Biomass

steady state	Rt (days)	C_b (g L^{-1})	P_b (g L^{-1} day^{-1})	P_{EPA} (mg L^{-1} day^{-1})
S_A	14.5	0.80 ± 0.02	0.055 ± 0.001	1.05 ± 0.02
S_B	9.7	0.73 ± 0.01	0.075 ± 0.001	1.45 ± 0.13
S_C	7.2	0.61 ± 0.02	0.084 ± 0.003	1.83 ± 0.05
S_D	5.8	0.57 ± 0.02	0.099 ± 0.003	2.53 ± 0.03
S_E	4.8	0.45 ± 0.01	0.093 ± 0.002	2.43 ± 0.02

shows the progress of biomass concentrations (C_b) over culture time. Steady states could be obtained for each residence time (Rt). The behavior pattern was similar to that obtained in a light-limited continuous culture for others microalgae (*34*); that is, at low Rt, a high specific growth rate is attained, requiring a greater light availability, which leads to a low biomass concentration. This effect is better observed in Table 1, where the steady-state biomass concentration (C_b), biomass productivity (P_b), and EPA productivity (P_{EPA}), are shown as functions of Rt. The highest steady-state biomass concentration (0.80 g L^{-1}) was attained at the highest residence time (S_A) and, conversely, the higher P_{EPA} was found for steady-states having lower Rt (S_D and S_E).

These results are instructive, not only for the estimate of attainable peak yields but also for selecting optimum biomass concentration and residence time. Thus, as seen in Table 1, the peak biomass productivity was reached at 5.8 days, giving rise to a 0.57 g L^{-1} optimal cellular density, at which the utilization of irradiance was maximum.

Biochemical Composition. All results of analysis of the biomass are expressed on a 100-g dry weight basis. Data referring to proximate composition are shown in Table 2. Moisture content was low, with a mean of 3.10 g, values in the range of general recommendations for microalgae quality (<10%) (*5*). Protein (prot) content was low (mean = 28.8 g), whereas available carbohydrate (Carb) (35.9 g) and lipid (18.4 g) were higher than other values reported, which can be due to the large Rt (*16*, *35*). Fiber amounts were low, ranging from 1.20 to 3.64 g. Low fiber suggests an easily digestible biomass for human use. Other microalgae have high fiber content, and this has been an argument against use of microalgae in human nutrition (*5*). The biomass had moderate amounts of RNA and DNA, with mean values of 2.02 and 0.35 g, respectively, suggesting an RNA/DNA of 5.8, and a total nucleic acids value of 2.37 g, which is a lower value than 6 g, the recommended maximum daily intake (*5*). Consequently, the biomass provided few purines, similar to levels in commonly consumed vegetables (1−2 g) (*36*). Nitrate was also low (mean = 0.074 g). For a daily biomass consumption of microalgae of 15 g, nitrate intake will be 0.011 g, an amount lower than the established 0.5 g known to cause some adverse effects in humans (*37*).

Ash content was moderate in most cases, ranging from 8.5 to 10.8 g. Higher values have been reported for other marine microalgae used in a human diet, for example, 20.2 g of ash in *Phaeodactylum tricornutum* (*3*) and 21 g in *Tetraselmis chui* (*2*). Freshwater algae show lower figures for ash, for example, 6−15 g in *Scnedesmus* and *Spirulina* (*38*).

Fatty acid profiles are recorded in Table 3. The major fatty acids were palmitic acid (PA, 16:0) at 5.05 g, followed by palmitoleic acid (POA, 16:1ω7) at 4.72 g, oleic acid (OA, 18:1ω9) at 3.79 g, eicosapentaenoic acid (EPA, 20:5ω3) at 2.24 g, arachidonic acid (ARA, 20:4ω6) at 0.69 g, myristic acid (MA, 14:0) at 0.63 g, and linoleic acid (LA, 18:2ω 6) at 0.36 g. This profile was widely affected by culture conditions. The EPA content was higher than that obtained with *Porphyridium cruentum* (*39*) and lower than that reported in outdoor *Nannochloropsis* sp. cultures (*40*). Occurrence of EPA in the biomass increases its nourishment value; EPA is an eicosanoid precursor (*41*).

Pigment concentrations are shown in Table 4. Chlorophylls (0.29 g) constitute the main pigments group, with chlorophyll *a* being the dominant pigment. Total chlorophyll ranged from 0.18 to 0.46 g. Carotenoids are scarce in this microalgae, ranging from 0.04 to 0.10 g. The carotenoid profile for each steady state is shown in Table 5. For all analyzed states, violaxanthin was the main carotenoid, and vaucheraxanthin and β,ϵ-carotene were the most scarce, in agreement with previous reports (*8*, *9*). Mineral element data are given in Table 6. The biomass was rich in Ca (972 mg), K (533 mg), Na (659 mg), and Mg (316 mg). Levels for other mineral elements were as follows: Zn, 103 mg; Fe, 136 mg; Cu, 35.2 mg; Mn, 3.41 mg; Cr, 0.37 mg; Ni, 0.22 mg; Co, <0.1 mg; and S, 529 mg. Toxic heavy metal amounts were lower than the recommended values for quality (*5*). Thus, for the mean of steady states, Pb = 0.38 mg

Table 2. Proximate Composition, Nucleic Acids, and Nitrate in *Nannochloropsis* spp. (g per 100 g of Dry Biomass) (Mean ± SD)

steady state	moisture (g)	crude protein (g)	available carbohydrates (g)	lipids (g)	ash (g)	fiber (g)	RNA (g)	DNA (g)	nitrate (g)	energy (kJ)
S_A	1.95 ± 0.35	22.2 ± 0.46	40.4 ± 0.39	21.0 ± 1.84	8.66 ± 0.09	3.05 ± 0.76	1.71 ± 0.20	0.61 ± 0.03	0.060 ± 0.09	1790 ± 83
S_B	3.40 ± 0.28	22.5 ± 1.01	35.7 ± 1.93	21.7 ± 2.05	8.48 ± 0.14	2.18 ± 0.38	1.71 ± 0.30	0.09 ± 0.02	0.036 ± 0.006	1750 ± 124
S_C	3.00 ± 0.00	28.2 ± 0.25	38.1 ± 1.03	18.0 ± 0.78	9.64 ± 0.64	3.64 ± 0.64	1.83 ± 0.05	0.32 ± 0.05	0.056 ± 0.003	1740 ± 50
S_D	2.70 ± 0.42	33.8 ± 0.85	35.0 ± 0.56	16.0 ± 2.55	10.8 ± 0.14	1.20 ± 0.00	3.21 ± 0.23	0.22 ± 0.08	0.145 ± 0.09	1870 ± 119
S_E	4.45 ± 0.07	37.4 ± 0.28	28.7 ± 0.48	15.1 ± 1.84	9.60 ± 0.42	2.00 ± 0.62	1.64 ± 0.31	0.49 ± 0.19	0.070 ± 0.008	1640 ± 81
mean	3.10 ± 0.24	28.8 ± 0.63	35.9 ± 4.38	18.36 ± 2.18	9.44 ± 0.24	2.41 ± 0.60	2.02 ± 0.22	0.35 ± 0.07	0.074 ± 0.009	1760 ± 91

Table 3. Fatty Acid Content in *Nannochloropsis* spp. (g per 100 g of Dry Biomass) (Mean ± SD)

steady state	14:0	16:0	16:1ω7	18:1ω9	18:2ω6	20:4ω6	20:5ω3
S_A	0.59 ± 0.10	5.70 ± 0.66	5.29 ± 0.90	5.49 ± 0.77	0.29 ± 0.06	0.65 ± 0.09	1.91 ± 0.24
S_B	0.62 ± 0.02	5.84 ± 0.17	5.84 ± 0.13	5.54 ± 0.09	0.34 ± 0.01	0.66 ± 0.01	1.93 ± 0.03
S_C	0.60 ± 0.01	5.41 ± 0.11	4.72 ± 0.11	3.66 ± 0.07	0.27 ± 0.00	0.57 ± 0.01	2.18 ± 0.04
S_D	0.66 ± 0.09	4.46 ± 0.46	3.68 ± 0.38	2.36 ± 0.25	0.44 ± 0.06	0.77 ± 0.06	2.56 ± 0.32
S_E	0.67 ± 0.09	3.82 ± 0.52	4.09 ± 0.55	1.92 ± 0.16	0.46 ± 0.07	0.81 ± 0.09	2.62 ± 0.33
mean	0.63 ± 0.08	5.05 ± 0.38	4.72 ± 0.41	3.79 ± 0.27	0.36 ± 0.04	0.69 ± 0.05	2.24 ± 0.19

Table 4. Pigment Content in *Nannochloropsis* spp. (g per 100 g of Dry Biomass) (Mean ± SD)

steady state	chlorophyll *a*	chlorophyll *c*	total chlorophylls	carotenoids
S_A	0.34 ± 0.04	0.12 ± 0.02	0.46 ± 0.06	0.10 ± 0.01
S_B	0.19 ± 0.01	0.10 ± 0.01	0.29 ± 0.02	0.07 ± 0.01
S_C	0.17 ± 0.03	0.12 ± 0.01	0.29 ± 0.04	0.05 ± 0.01
S_D	0.15 ± 0.02	0.06 ± 0.01	0.21 ± 0.03	0.04 ± 0.00
S_E	0.14 ± 0.02	0.04 ± 0.00	0.18 ± 0.02	0.04 ± 0.00
mean	0.20 ± 0.02	0.09 ± 0.01	0.29 ± 0.03	0.06 ± 0.01

Table 5. Carotenoid Class of *Nannochloropsis* spp. Biomass (Area Percent of Total Detected Area)

steady state	violaxanthin	vaucheraxanthin	zeaxanthin	*β*,*β*-carotene	*β*,*ϵ*-carotene
S_A	73	9	11	6	1
S_B	64	8	9	10	9
S_C	50	15	10	20	5
S_D	56	10	15	12	7
S_E	37	14	14	25	10
mean	56	11	12	15	6

and Cd = 0.028 mg. The seawater used had a low amount of toxic elements, but the situation could be different with water from other sources.

The mineral element contents were consistent with the recommended daily intakes, if they were bioavailable. Thus, a daily consumption of 82 g of microalgae meets the calcium needs of an adult; 133 g of this microalgae fulfills the recommended daily allowance of magnesium (420 mg for an adult of 70 kg), and 9.8 g of algae meets the daily need for zinc.

Statistical Analysis. The correlation coefficients among variables were high and generally significant, indicating that the variations observed in the data could be due to a few related causes. To establish relationships among the variables, a multivariable data analysis was performed for data obtained at various states. Multivariable data analysis is a suitable approach to find underlying structures in complicated biological systems. One of the most powerful and widely used methods is principal component analysis (PCA), which reduces the number of variables to a limited number of principal components (PC) (*42*, *43*). Unlike measured variables, PC are orthogonal linear combinations of the original variables and thereby describe independent variation structures in the data. The first PC always explains the greatest part of the total variance, and the following PC successively explain smaller parts of the original variance. The presence of significant PC indicates structure in the data. Graphic overviews, ideally showing a large part of the variance in the two dimensions of the objects and variables, are obtained by score and loading plots, respectively. Variables found in a similar direction and far from the origin are positively correlated, whereas those found at opposite sides of the plot are negatively correlated. This means that correlated variables are explained by the same PC and less correlated variables by different PC. In the present analysis, the two first PC explained 50.8 and 20.9%, respectively, of the total variance, and most variables had a strong influence on the model. A plot for the two first component weights (Figure 2a) shows that there are groupings of variables. Thus, Rt has a strong influence on component 1, and it is positively correlated with other variables: C_b (r = 0.938; $p < 0.05$), carotenoids (r = 0.995; $p < 0.0005$), chlorophyll *a* (r = 0.966; $p < 0.01$), LA (r = 0.885; $p < 0.05$), violaxanthin (r = 0.894; $p < 0.05$), and Zn (r = 0.925; $p < 0.05$). These variables, with others such as

Table 6. Mineral Element Content in *Nannochloropsis* Spp (mg per 100 g of Dry Biomass) (mean ± SD)

steady state	Na	K	Ca	Mg	Fe	Zn	Cu	Mn	Pb	Cd	Cr	Ni	Co	S
SA	1206 ± 100	608 ± 37	1800 ± 85	435 ± 39	148 ± 0	235 ± 14	57.3 ± 1.77	2.3 ± 0.3	0.44 ± 0.05	0.036 ± 0.004	0.10 ± 0.03	0.22 ± 0.01	<0.1	611 ± 39
S_B	421 ± 20	328 ± 0	415 ± 35	130 ± 11	116 ± 15	74 ± 1	27.5 ± 0.70	5.0 ± 0.0	0.34 ± 0.26	<0.017	0.29 ± 0.07	0.34 ± 0.02	<0.1	384 ± 18
SC	166 ± 33	518 ± 7	1255 ± 64	220 ± 18	122 ± 4	83 ± 0	15.8 ± 1.77	2.0 ± 0.0	0.17 ± 0.02	<0.025	0.30 ± 0.08	0.26 ± 0.03	<0.1	417 ± 20
SD	1324 ± 67	713 ± 37	292 ± 46	567 ± 21	166 ± 45	51 ± 0	23.0 ± 1.41	6.0 ± 0.0	0.30 ± 0.03	<0.025	0.06 ± 0.04	0.17 ± 0.02	<0.1	590 ± 44
SE	178 ± 30	500 ± 10	1100 ± 71	230 ± 20	131 ± 5	72 ± 1	52.3 ± 15.0	1.8 ± 0.0	0.63 ± 0.03	0.036 ± 0.005	1.11 ± 0.11	0.11 ± 0.01	<0.1	641 ± 47
mean	659 ± 50	533 ± 182	972 ± 60	316 ± 22	136 ± 14	103 ± 3	35.0 ± 1.4	3.4 ± 0.1	0.38 ± 0.08	0.028 ± 0.02	0.37 ± 0.07	0.22 ± 0.09	<0.1	529 ± 119

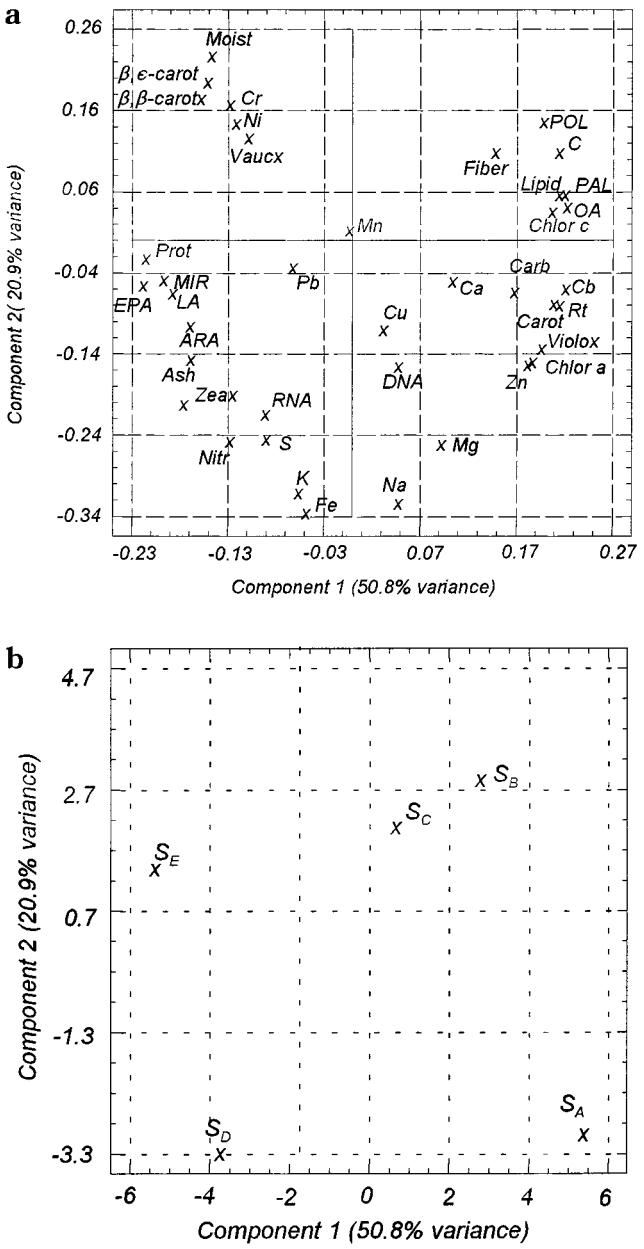

Figure 2. (a) Plot for first two component weights: Moist, moisture; Carot, carotenoid; Chlor a, Chlorophyll *a*; Chlor c, chlorophyll *c*; Cb, biomass concentration; Carb, available carbohydrates; Nitr, nitrate; Prot, crude protein; Violx, violaxanthin; Vaucx, vaucheraxanthin; Zeax, zeaxanthin. (b) Scatterplot for first two component weights.

available carbohydrates and Ca, form a group that increases jointly, for large Rt values. The variables available carbohydrates and C_b placed in the same location suggest that the cell concentration increases with available carbohydrates as the main component. Furthermore, C_b is positively correlated with the variables lipid ($r = 0.940$; $p < 0.05$), PA ($r = 0.920$; $p < 0.05$), carotenoids ($r = 0.915$; $p < 0.05$), and OA ($r = 0.955$; $p < 0.01$), being placed next to the variable fiber and Rt, which can be explained by the fact that for high Rt, the energy is accumulated in reserve lipid form. On the other hand, the variable *C* is positively correlated with the variables PA ($r = 0.899$; $p < 0.05$); POL ($r = 0.991$; $p < 0.01$), and OA ($r = 0.974$; $p < 0.005$), which suggests that the saturated and monounsaturated fatty acids constitute the main carbon storage form for this microalga. The positive correlation between fatty acids POL and OA ($r = 0.942$; $p < 0.05$) may be explained by considering that both biomolecules are biosynthesized from different substrates (PA and stearic acid respectively) by means of the same desaturase enzymatic system.

Another group of variables located at the left of the plot and, consequently, increasing for low Rt, is formed by the variables protein, ash, zeaxanthin, RNA, nitrate, S, MIR, EPA, ARA, and LA. This cluster of variables can be interpreted by considering that low residence times imply younger cells with increasing proteins needs for the cell growth and cell reproduction (*20*, *44*, *45*). On the other hand, fatty acid variation as a function of residence time can be explained by considering that a long culture cycle induces the cells to accumulate reserve lipids that are rich in saturated fatty acids as the storage carbon, and the need for structural biomolecules is lower. Thus, low Rt implies that polyunsaturated fatty acids will increase, as the main components of the cell membrane (*46*). The positive correlation between the variables ARA and LA ($r = 0.977$; $p < 0.05$), both ω6 fatty acids, could be explained by the reasons discussed for the variables POL and OL. The positive correlation found between RNA and nitrate ($r = 0.934$; $p < 0.05$) has been cited elsewhere (*47*, *48*).

If it is considered that an increase in cell concentration reduces light availability in the photobioreactor, the location in the same area of the variables Rt, C_b, chlorophyll *a*, and carotenoids can be explained by considering that low irradiance values cause the cells to increase chlorophyll *a* and carotenoids content; cells exposed to high irradiance will use lower resources for chlorophyll biosynthesis than for ribulose 5-diphosphate synthesis and other enzymes of phototosynthesis (*49*). Furthermore, low irradiance induces a longer cellular cycle and, consequently, a greater lipid accumulation. These observations agree with those that indicate that when growth is slowed by any limiting factor, such as light restriction, lipid and carbohydrate synthesis may be enhanced at the expense of protein synthesis (*50*).

The resulting scatterplot (Figure 2b) provides a conceptual overview of the samples by showing a total of 71.7% of the variance. The relationships between those variables and the PCs are defined as loadings. The pattern of covariation between the stationary phases can be seen in this figure. The component plot and scatterplot can be interpreted together because objects with high scores for a specific PC also have high values for the variables with high loading plots and low values for those with low loadings. This plot indicates that steady states with low residence times are placed to the left of the plain opposed to those with high residence time. Thus, the present scatterplot indicates that steady states can be grouped depending on their residence time values and, hence, depending on their nutrient contents. This confirms the strong influence of the operational variable Rt on the nutrient composition of the biomass.

We believe that if minerals were bioavailable and no toxic or antinutritional factors are detectable, as in this work, *Nannochloropsis* sp. biomass could be used for nutritional purposes because of the amount and diversity of nutrients it contains. Also, the nutritional composition of the biomass can be modified by means of operational variables, principally by using short Rt values to obtain EPA and protein enrichment.

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