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Growth, bioactive compounds and chemical composition of marine microalga *Dunaliella salina* in batch cultures. Crescimento, compostos bioativos e composição química da microalga marinha *Dunaliella salina* em meios de cultura.

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Resumo

Como as microalgas contêm uma grande variedade de componentes químicos e bioativos que podem ser utilizados na alimentação de animais, principalmente espécies destinadas à aquicultura, o presente estudo teve como objetivo avaliar o crescimento, compostos bioativos e a composição química da microalga marinha *Dunaliella salina* em dois meios de cultura: autotrófico (salino) ou mixotrófico (Conway). Assim, curva de crescimento, diâmetro celular, taxa de crescimento (k), produtividade (mg/L/dia), densidade celular máxima (DMC) e composição química foram avaliados através dos macro e micronutrientes e compostos bioativos. A *D. salina* em meio mixotrófico Conway teve uma fase exponencial mais precoce e maior DMC (23.500 células/ml), produtividade e taxa de crescimento ($P < 0,05$) do que o meio autotrófico salino (18.200 células/ml), embora o diâmetro celular da *D. salina* tenha sido maior ($P < 0,05$) em meio autotrófico (25,5 µm ± 2,65) do que em meio mixotrófico (21,25 µm ± 1,26). A biomassa de *D. salina* em meio mixotrófico obteve maiores valores ($P < 0,05$) de proteína (60,43%), nitrogênio (9,67 mg/g) e clorofila a (60,60 mg/L), enquanto o meio autotrófico proporcionou maiores valores de lipídios (6,93%), açúcares (8,09%), energia bruta (3955 kcal/kg), fósforo (8,40 mg/g), potássio (128,66 mg/g), magnésio (29,23 mg/g), zinco (8,23 mg/g), carotenóides totais (34,73 mg/L), vitamina C (87 mg/100g), polifenóis totais (81,00 mg/100 g), antocianinas (30 mg/kg) e flavonóides amarelos (45,30 mg/100g). O uso do meio autotrófico salino para a microalga *D. salina* foi uma alternativa viável ao meio mixotrófico Conway. Devido ao seu valor nutricional e perfil de compostos bioativos, a biomassa da microalga *D. salina* pode ser destinada à nutrição animal e humana.

Palavras-chave: Antioxidantes. Biomassa. Carotenoides. Produtividade da microalga. Taxa de crescimento.

Abstract

Because microalgae contain a wide variety of chemical and bioactive components that can be used to feed animals, especially aquaculture species, the present study aimed to evaluate the growth, bioactive compounds and chemical composition of the marine microalga *Dunaliella salina* in two batch cultures: autotrophic (saline) or mixotrophic (Conway). Thus, growth curve, cell diameter, growth rate (k), productivity (mg/L/day), maximum cell density (MCD), and chemical composition were evaluated through the macro and micronutrients and bioactive compounds. The *D. salina* in mixotrophic Conway medium had an earlier exponential phase and higher MCD (23,500 cells/ml), productivity, and growth rate ($P < 0.05$) than autotrophic saline medium (18,200 cells/ml), although the cell diameter of *D. salina* was greater ($P < 0.05$) in autotrophic (25.5 μm ± 2.65) than in mixotrophic medium (21.25 μm ± 1.26). The biomass of *D. salina* in mixotrophic medium obtained greater values ($P < 0.05$) of protein (60.43%), nitrogen (9.67 mg/g), and chlorophyll a (60.60 mg/L), whereas autotrophic medium provided greater values of lipids (6.93%), sugars (8.09%), gross energy (3955 kcal/kg), phosphorus (8.40 mg/g), potassium (128.66 mg/g), magnesium (29.23 mg/g), zinc (8.23 mg/g), total carotenoids (34.73 mg/L), vitamin C (87 mg/100g), total polyphenols (81.00 mg/100 g), anthocyanins (30 mg/kg), and yellow flavonoids (45.30 mg/100g). The use of the autotrophic saline medium for the D. salina microalgae was a viable alternative to the mixotrophic Conway medium. Due to its nutritional value and bioactive compound profile, *D. salina* microalgae biomass can be destined to animal and human nutrition.

Keywords: Antioxidants. Biomass. Carotenoids. Microalgae productivity. Growth rate.

Introduction

Microalgae contain a wide variety of chemical components such as carotenoids, fatty acids, polysaccharides, vitamins, minerals and other bioactive compounds that have been used for a long time as animal and human food (OLIVARRÍA et al., 2010). In this context, *Dunaliella salina* is one of the most widely used microalgae for obtaining these compounds.

D. salina is a marine microalga distributed around the world (DERNER et al. 2006). It grows as motile bi-flagellated green cells under favorable growth conditions. However, during extreme environmental conditions such as nutrient limitation, low temperature, increased light intensity or high salt concentrations these cells undergo morphological and biochemical changes concomitant with their survival strategies (GÓMEZ; GONZÁLEZ, 2005). These survival strategies include lipid-soluble or water-soluble antioxidant compounds such as carotenoids, polyphenols, flavonoids, tocopherols, ascorbic acid, glutathione, mycosporine-like amino acids, and antioxidative enzymes (DUFOSSÉ et al., 2005; RAJA et al., 2007; LAMERS et al., 2008; SAHA et al., 2013).

In view of the aforementioned, and the scarcity of information on ideal growing conditions for obtaining higher biomass and concentration of nutrients and bioactive compounds, the objective was to evaluate the growth, chemical composition and bioactive compounds of the marine microalga *D. salina* in batch cultures.

Material and methods

D. salina microalgae were collected in saline tanks in the State of Rio Grande do Norte, Brazil (Latitude 5º 4'S; Longitude 37º 16 'W), with the aid of plankton netting with a 20 μm mesh opening. We used horizontal trawls in the sub-surface of the water column (BICUDO; MENEZES, 2006). The collected samples were immediately conditioned in Falcon tubes and kept alive for further

identification and isolation (GUEVARA et al., 2005). To obtain the supernatant the centrifuge was used at 4000 rpm for 10 min.

For culture of the *Dunaliella salina* microalga, two different culture media were tested, with three replicates each. It was used the Conway mixotrophic medium indicated for this type of microalga (WALNE, 1966), with maximum salinity of 10%, absence of the silicate solution, and addition of molasses of sugarcane as carbon source. As an alternative, a saline autotrophic medium (mean salinity of 26%) was developed from the water collected along with the microalga in saline tanks, containing 0.25g/L of Ca, 25.16g/L of SO4, 17.63g/L of Mg, 153.3g/L of Cl, 5.27g/L of K, 74.96g/L of Na, and 0.95g/L of Br.

The method used was batch, where there is no nutrient renewal after addition of the culture medium and the inoculum (CARVALHO et al., 2004), for both treatments. The culture was developed in one liter Erlemeyer flasks with constant aeration and initial pH of 7.5 and 7.8 for the Conway and saline media, respectively. The temperature was monitored (25ºC) and the photoperiod used was 12 hours, provided by fluorescent lamps of 40 W.

The Conway was composed of 400 mL of culture medium, 4 mL of the inoculum (*D. salina*), and 40 mL of sugarcane molasses as a carbon source, while the saline medium was composed of 400 mL of saline water (originating at the microalga collection site) and 4 mL of the inoculum (*D. salina*). The initial cell density for the two media was 6 x 103 cells/mL (GÓMEZ et al., 2003), and the inoculation period lasted 14 days.

Sterile “Pasteur” pipettes were used to collect the samples. The cell counts were performed daily with the aid of the Neubauer camera and optical microscope with a 40x objective. The parameters analyzed in both treatments included the growth curve (estimated using the polynomial regression equation), the maximum cell density (measured at the time when the number of cells per mL attained its maximum value), productivity (obtained using the equation established by LOURENÇO, 2006), the daily growth rate (K, represented by the number of cell divisions per day and determined by the equation reported by STEIN (1973), and the cell diameter (measured by microscopic photographic images with a 40x objective. We processed our data using the Cheese® program and IMAGE J software.

At the end of the inoculation period, the biomass of *D. salina* was obtained by centrifugation and subsequent drying (AOAC, 2011) of the culture medium to determine the nutritional value of each medium. Analysis of the sugar content was performed in using near-infrared spectrometry (NIRS) with a wavelength of 1200–2400 nm and intensity of 0–55,000. Was performed gross energy (GE) analyzing using an adiabatic calorimetric pump. Was analyzed macro and micronutrients following the methodology of Baccan et al. (1995). The levels of nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) were quantitatively determined using graphite furnace atomic absorption spectrophotometry.

It was carried out an analysis of total carotenoids, total extractable polyphenols, anthocyanins, yellow flavonoids and vitamin. The determination of total carotenoids was conducted according to the methodology of Higby (1962); to determine the total extractable polyphenols we followed the methodology proposed by Larrauri et al. (1997). Our analyses of anthocyanins and yellow flavonoids followed the methodology of Francis (1982). We determined the vitamin C content according to the method of Strohecker and Henning (1967). The analysis of chlorophyll a was performed using the method proposed by Lorenzen (1967), and this work was performed with a fluorescence spectrophotometer.

Statistical analysis

Data normality and homogeneity of all variances were tested with Cramer-von Mises and Levene tests, respectively. Subsequently, the data referring to the growth curve were submitted to ANOVA and polynomial regression at 5% of probability, while data referring to chemical composition and bioactive compounds were submitted to ANOVA and Fisher Test at 5% of probability, using the R – DEVELOPMENT CORE TEAM (2011) software.

Results and discussion

The growth of the *D. salina* microalga differed in the two culture media after 24 hours of inoculation and in the period between the 8th and 14th day, with the exponential growth phase for the Conway medium between the 2nd and 6th day and, for the saline medium between the 2nd and 7th day (Figure 1).

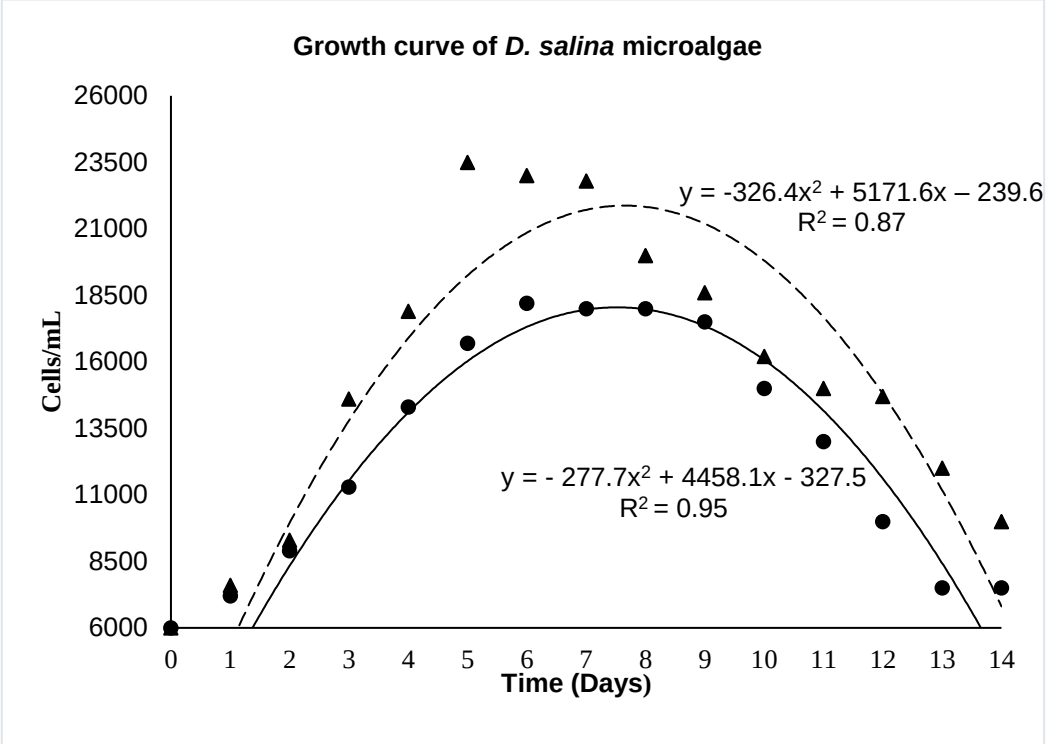

Figure 1 - Growth curve of *Dunaliella salina* microalgae in Conway and Saline culture media. Each triangle represents the mean of three replicates ± standard error of the number of cells per day of culture in Conway medium. The dashed line represents the trend to polynomial regression of order 2. The regression equation y = -326.4x2 + 5171.6x - 239.6 (R2 = 0.87) represents the growth curve of *D. salina* microalga per day growing in Conway medium. Each circle represents the mean of three replicates ± standard error of the number of cells per day of culture in Saline medium. The continuous line represents the trend to polynomial regression of order 2. The regression equation y = - 277.7x2 + 4458.1x - 327.5 (R2 = 0.95) represents the growth curve of the *D. salina* microalga per day of culture in Saline medium.

Olivarría et al. (2010) evaluated the growth of *Dunaliella* sp. for 10 days in culture medium (control, with nitrates, and medium with nitrogen limitations) and observed a maximum cell density of 1.2 x 106 cells/mL for the control medium after 6 days of culture. These authors observed that although the highest cell density occurred in the control medium, which contained nitrates as a source

of nitrogen, the medium with increasing nitrogen limitations exhibited a significant increase in the biomass production of this microalga.

The saline medium yielded *D. salina* cells with a larger mean diameter of 25.5 μm (± 2.65; P < 0.05), while the Conway medium yielded microalgal cells with a mean diameter of 21.25 μm (± 1.26) (Figure 2, a-b).

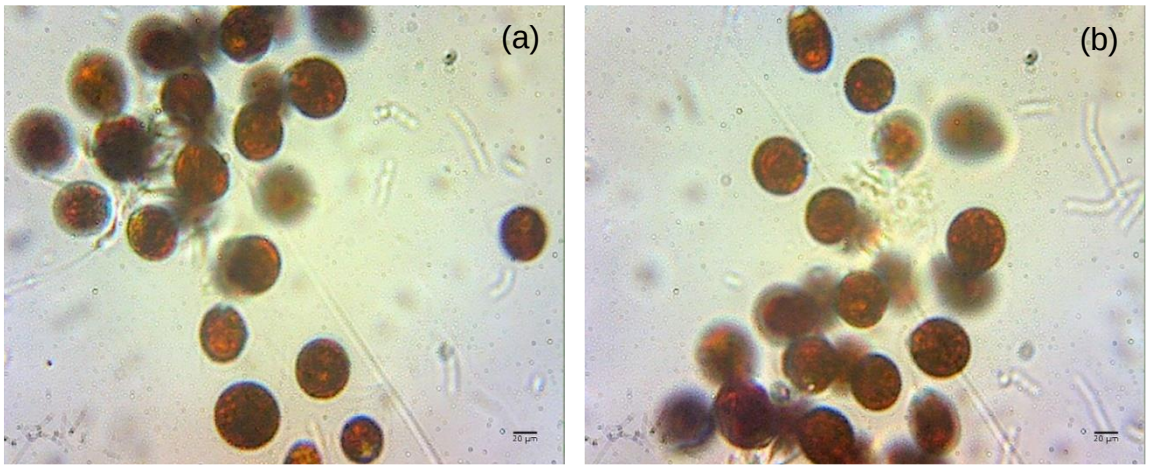

Figure 2 - Photomicrographs in 40x objective of *D. salina* in culture medium: (a) – Conway and, (b) – Saline. The Saline medium yielded *D. salina* cells with a larger mean diameter of 25.5 μm (± 2.65); the Conway medium yielded microalgal cells with a mean diameter of 21.25 μm (± 1.26). The means differed by the F test (P < 0.05). Micrograph scale: 20 μm.

Corroborating with the mean diameter of microalgal cells, was found that some microalgal species underwent morphological changes, such as increased cell size, to survive in a nutrient-limited environment (SAID, 2009).

The maximum cell density (MCD), productivity and daily growth rate (k) differed (P < 0.05) between the two media: the Conway medium attained the highest values for all of the parameters evaluated (Table 1).

Table 1 - Productivity (mg/L/day), growth rate (k) and maximum cell density (MCD; cells/mL) of the *D. salina* microalga

Time (days)	Productivity (mg/L/day)			Growth rate (k)		
	Conway medium	Saline Medium	P	Conway medium	Saline medium	P
1	16.00 ± 0.25	12.00 ± 0.41	0.604	0.34 ± 0.03	0.26 ± 0.01	0.001*
2	16.50 ± 0.05	14.50 ± 0.25	0.006*	0.31 ± 0.03	0.28 ± 0.01	0.954
3	28.66 ± 0.12	17.66 ± 0.06	0.001*	0.42 ± 0.01	0.30 ± 0.01	0.005*
4	29.75 ± 0.07	20.75 ± 0.16	0.001*	0.39 ± 0.01	0.31 ± 0.01	0.108
5	35.00 ± 0.50	21.40 ± 0.10	0.002*	0.39 ± 0.01	0.29 ± 0.01	0.179
6	28.33 ± 0.08	20.33 ± 0.11	0.036*	0.32 ± 0.01	0.26 ± 0.01	0.817
7	24.00 ± 0.25	17.14 ± 0.02	0.439	0.27 ±0.01	0.22 ± 0.01	0.111
8	17.50 ± 0.18	15.00 ± 0.05	0.832	0.21 ± 0.01	0.19 ± 0.01	0.007*
9	14.00 ± 0.20	12.77 ± 0.06	0.143	0.18 ± 0.01	0.17 ± 0.02	0.001*
10	10.20 ± 0.05	9.00 ± 0.05	0.033*	0.14 ± 0.01	0.13 ± 0.01	0.001*
11	81.81 ± 0.06	6.36 ± 0.02	0.008*	0.12 ± 0.01	0.10 ± 0.01	0.002*
12	7.25 ± 0.07	3.33 ± 0.01	0.001*	0.10 ± 0.01	0.06 ± 0.02	0.006*

13	4.61 ± 0.03	1.15 ± 0.01	0.001*	0.07 ± 0.01	0.02 ± 0.02	0.001*
14	2.85 ± 0.07	1.07 ± 0.01	0.001*	0.05 ± 0.01	0.02 ± 0.02	0.007*

MCD (cells/mL) Conway medium	MCD (cells/mL) Saline medium	SD	P
23,500	18,200	±3.74	0.002*

Each value is a mean of three biological replicates and the results are presented with ± SD. * Significant effect by the F test (P < 0.05) on the line. SD- Standard Deviation. P- Probability.

Greater biomass yield (mg/L/day) was observed for the microalga cultivated in Conway medium during the periods between the 2nd and 6th day, as well as, 10th and 14th day after inoculation of *D. salina*. The maximum productivity point was observed at 5th day for the two culture media. On the other hand, the highest daily growth rate (k) was observed for the Conway medium on the 3rd day after inoculation, and the Saline medium was later, on the 4th day, and also presented a lower value for growth rate.

The method of batch cultivation adopted for the two culture media ensures that from the inoculation in the chosen media, until the end of the experiment, the media does not receive any nutrient replacement (CARVALHO et al., 2004). However, the Conway mixotrophic medium has nitrogen, copper, cobalt, zinc, sodium and B vitamins sources in its composition (WALNE, 1966), besides the addition of cane molasses as a source of carbon at the time of inoculation, in proportions that allow a better performance and biomass production (COÊLHO et al., 2013). Antagonistically, autotrophic culture media, which deplete some nutrients such as nitrogen, trigger a series of metabolic reactions that assure the survival of the *D. salina* microalga, to the detriment of its exponential growth (SAHA et al., 2013), as observed in the Saline medium.

The nutrient composition observed for the *D. salina* biomass differed (P < 0.05) between the two media: Conway and saline medium (Table 2).

Table 2 - Chemical-energetic composition (based on dry matter) of *D. salina* biomass in two culture media

Nutrients	Conway medium	Saline medium	P	SEM	CV(%)
Moisture (%)	1.13	1.40	0.3891	0.34	7.07
Protein (%)*	60.43	55.36	0.0017	0.82	1.43
Lipids (%)*	4.43	6.93	0.0002	0.13	2.36
Fiber (%)	1.73	1.89	0.0181	0.04	2.32
Sugars (%)*	7.59	8.09	0.0370	0.20	2.57
Ashes (%)	15.23	14.90	0.4199	0.45	3.01
Gross Energy (kcal/kg)*	3873	3955	0.0004	9.60	1.24

* Significant effect by the F test (P < 0.05) on the line; P- Probability; SEM- Standard error of means; CV (%) Coefficient of variation.

The proteins observed in the two biomass culture media were the most abundant components of the centesimal composition, as also observed in literature by Becker (2007) and Campos et al. (2010). On the other hand, the concentrations of sugars in the two media and lipids in the Conway medium were lower than the levels reported by Muhaemin and Kaswadji (2010). This finding may be a consequence of the high availability of nitrogen in the Conway culture medium (WALNE, 1966) nitrogenous compounds are used by microalgae for protein and amino acid biosynthesis (HORNES et al., 2010), which may have propitiated to *D. salina* high protein production and lower synthesis of carbohydrates and lipids. Relatively low concentrations of carbohydrates and lipids reflect the

coupling between carbon and nitrogen metabolism since the accumulation of nitrogen and proteins naturally leads to the reduction of reserve substances (CAMPOS et al., 2010). On the other hand, the inverse relation was observed in the saline environment, where the low contents of the nitrogen compounds yielded higher lipid and sugar contents.

Gross energy is the starting point for energy evaluation of food and nutrients, and there is an inverse relationship between fat and protein in gross energy (SIPAÚBA-TAVARES et al., 2009). This inversely proportional relation was verified in this study, and the saline medium yielded a biomass with a higher energy value.

The macro and micronutrient contents of the *D. salina* biomass in the two culture media (Table 3) were higher than those reported by Tang et al. (2010). These authors noted values between 1 and 10 mg/g for Ca, K, Fe, Mg and Na and between 0.1 and 1 mg/g for Zn, Cu, and Mn. There was a statistically significant difference between the biomasses of the culture media in terms of N, P, K, Mg, and Zn.

Table 3 - Macro and micronutrient contents of the *D. salina* biomass (based on dry matter) in the two culture media

Nutrients (mg/g)	Conway medium	Saline médium	P	SEM	CV(%)
Nitrogen (N)*	9.67	8.86	0.0321	0.26	1.76
Phosphor (P)*	6.66	8.40	0.0020	0.24	2.79
Potassium (K)*	123.66	128.33	0.0004	0.24	1.07
Sodium (Na)	83.66	84.10	0.5624	0.44	1.49
Calcium (Ca)	61.23	60.70	0.4190	0.73	1.19
Magnesium (Mg)*	28.97	29.63	0.0421	0.27	1.93
Copper (Cu)	2.36	2.50	0.6507	0.33	13.73
Zinc (Zn)*	6.56	8.23	0.0040	0.34	4.64
Iron (Fe)	48.03	45.50	0.0008	0.34	1.73
Manganese (Mn)	24.43	24.26	0.5593	0.39	1.58

* Significant effect by the F test ($P < 0.05$) on the line; P- Probability; SEM- Standard error of means; CV (%) - Coefficient of variation.

Nitrogen is a basic component of proteins, nucleic acids, and photosynthetic pigments, and it is a constituent of several substances of primary metabolism and can be found in varying concentrations within algal cells in its inorganic form and as nitrite, nitrate and ammonium (BOUGARAN et al. 2010). The concentrations of proteins and chlorophylls in the cells are directly proportional to the nitrogen supply (LOURENÇO 2006), and the highest content of this macronutrient in the biomass obtained by the Conway culture medium is justified because it has a higher content of nitrogen compounds compared with the saline medium.

The phosphorus content in the biomass cultured in saline was higher than that of the biomass cultured in the Conway medium. Like nitrogen, phosphorus is considered to be one of the primary limiting elements for microalgae. It is important to regulate cell metabolism (lipid and carbohydrate synthesis), and the phosphate supply is critical for energy generation and the constitution of structural molecules such as ATP, phosphate sugars, nucleic acids and phosphoenzyme (SIPAÚBA-TAVARES et al., 2009). Therefore, this result is perfectly correlated with the lipid content observed for the saline medium, which was also higher than that of the Conway medium.

Potassium is a regulator of osmotic pressure, and it stimulates respiration at reduced pH. It is also a cofactor of several enzymes and responsible for the co-formation and stability of proteins. Magnesium, on the other hand, is an essential element for microalgae because it is a constituent of the chlorophyll molecule. It is a cofactor of several enzymes and participates in the activation of glycolytic enzymes. Magnesium also stimulates the synthesis of essential fatty acids and regulates cellular ionic levels. When there is a deficiency of magnesium, loss of the pigment content of the cell occurs, a process called chlorosis (LOURENÇO 2006). Zinc is a structural component of carbonic anhydrase (transport and binding of CO2), enzymes involved in transcription of DNA and alkaline phosphatase (SAHA et al. 2003). The presence of these minerals in a larger quantity in the microalgal biomass inoculated in the saline medium may be associated with the initial concentrations of these macro and micronutrients naturally present in this culture compared with the Conway medium, which only provides a low amount of zinc (WALNE 1966).

The contents of the primary bioactive compounds present in the *D. salina* biomass in the two culture media are presented in Table 4.

Table 4 - Levels of bioactive compounds present in the *D. salina* biomass (based on dry matter) in the two culture media

Bioactive compounds	**Conway medium**	**Saline medium**	**P**	**SEM**	**CV(%)**
Chlorophyll a (mg/L)*	60.60	40.93	7.916^{-08}	0.26	1.07
Total carotenoids (mg/L)*	28.56	34.73	$8.54e^{-06}$	0.26	1.83
Vitamin C (mg/100g)*	490.50	503.13	$3.25e^{-06}$	0.42	1.08
Total polyphenols (mg/100g)*	70.96	81.00	$9.09e^{-06}$	0.43	1.57
Anthocyanins (mg/100g)*	27.70	30.87	0.0022	0.56	1.91
Yellow flavonoids (mg/100g)*	41.40	45.30	$9.09e^{-06}$	0.43	1.99

* Significant effect by the F test ($P < 0.05$) on the line; P- Probability; SEM- Standard error of means; CV (%) - Coefficient of variation.

The concentrations of proteins and chlorophylls in the cells are directly proportional to the nitrogen supply. As a result, a decrease in the protein concentration results in a significant increase in the percentage of sugars, and a decrease in chlorophyll increases the concentration of carotenoids, which tend to appear yellowish (LOURENÇO, 2006). Therefore, it is possible to establish a correlation between the nitrogen content present in the microalgal biomass of the culture medium studied and the concentrations of chlorophyll a and total carotenoids. The Conway medium yielded a biomass with higher concentrations of nitrogen and chlorophyll a, and the saline medium yielded a biomass with a higher sugar content and total carotenoids.

The concentrations of chlorophyll a and total carotenoids in the two culture media were higher than the values reported in the literature. It was found (SAHA et al., 2013) concentrations of 18.10 and 13.50 mg/L for chlorophyll a and total carotenoids, respectively. These authors also observed that the ratio between chlorophyll a and carotenoid content was 1:1.34; we found a ratio of 1:1.83. These authors affirmed that this relation is indicative of ideal stress conditions and triggering of the carotenogenesis process for some microalga species; the lower the value obtained through this relation, the more intense the process.

It was Guevara et al. (2005) noted a maximum chlorophyll a value of 7.00 mg/L and a maximum total carotenoid value of 40.00 mg/L when evaluating five different strains of *D. salina* in

saline lagoons of Venezuela. On the other hand, it was Gómez et al. (2003) reported a minimum concentration of 6.90 and maximum concentration of 29.50 mg/L of total carotenoids in *D. salina* biomass when they tested increasing salinities in the culture medium. This result reveals that factors such as high salinity, temperature, radiation and nutritional limitations during cultivation trigger and intensify the production of carotenoids, which, because of their antioxidant function, act as a cellular defense mechanism for this species (GOMÉZ et al. 2003).

The *D. salina* biomass from the saline medium exhibited higher concentrations of vitamin C, polyphenols, anthocyanins and yellow flavonoids compared with the biomass from the Conway medium ($P < 0.05$). The levels of these compounds in microalgae of the genus *Dunaliella* sp. have not yet been reported in the literature. However, it is known that the depletion of some nutrients in the culture medium, particularly nitrogen in the saline medium, can trigger the formation of reactive oxygen species in microalgal cells via the production of various antioxidants and enzymes such as superoxide dismutase (SAHA et al. 2013).

Although interest in microalgae has increased significantly within the last decade, there have been no results of research evaluating the microalga *D. salina* under the same conditions of cultivation or the same parameters presented in this study. This situation is evidence of the relevance of the development of future research as a way of fomenting the production of microalgae biomass for animal and human nutrition.

Conclusion

The use Saline autotrophic medium for the *D. Salina* microalgae resulted in a viable alternative to the Conway mixotrophic medium, because it provided a biomass with higher levels of carotenoids and antioxidants. Due to its nutritional value and profile of bioactive compounds, *D. Salina* microalgae biomass can be destined to animal and human nutrition.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Raimunda Thyciana Vasconcelos Fernandes - original idea, reading and interpretation of works and writing; Alex Augusto Gonçalvez - guidance, corrections and revision of the text.; Alex Martins Varela de Arrusa - guidance, corrections and revision of the text.

References

AOAC. **Official Methods of Analysis of AOAC International**. 18th ed. Gaithersburg: AOAC, 2011, 1505p.

BACCAN, N.; GODINHO, O. E. S.; ALEIXO, L. M.; STEIN, E. **Introdução à Semimicroanálise Qualitativa**. 6ª edição. Editora da Unicamp: Campinas, 1995.

BECKER, E. W. Micro-algae as a source of protein. **Biotechnology Advances**, v. 25, n. 2, p. 207-210, 2007. doi: http://doi.org/10.1016/j.biotechadv.2006.11.002

BICUDO, C. E. M.; MENEZES, M. **Gêneros de algas de águas continentais do Brasil: chave para identificação e descrições**. 2nd ed. São Carlos: Rima, 2006.

BOUGARAN, G.; BERNARD, O.; SCIANDRA, A. Modeling continuous cultures of microalgae collimated by nitrogen and phosphorus. **Journal of Theoretical Biology**, v. 265, n. 3, p. 443-454, 2010. doi: http://doi.org/10.1016/j.jtbi.2010.04.018

CARVALHO, J. C. M.; FRANCISCO, F. R.; ALMEIDA, K. A.; SATO, S.; CONVERTI, A. Cultivation of *Arthrospira* (Spirulina) *platensis* (Cyanophyceae) by fed-batch addition of ammonium chloride at exponentially-increasing feeding rates. **Journal of Phycolology**, v. 40, n. 3, p. 589-597, 2004. doi: http://doi.org/10.1111/j.1529-8817.2004.03167.x

CAMPOS, V. B.; BARBARINO, E.; LOURENÇO, S. D. O. Growth and chemical composition of ten species of marine microalgae in batch cultures. **Ciência Rural**, v. 40, n. 2, p. 339-347, 2010. doi: http://doi.org/10.1590/S0103-84782010005000009

COÊLHO, A. C.; BARROS, M. U. G.; BEZERRA, J. H. C.; SILVA, J. W. A.; MOREIRA, R. L.; FARIAS, W. R. L. Growth of the microalgae Tetraselmis tetrathele and nitrate depletion in culture medium Guillard f/2 and Conway. **Acta Scientiarum Biological Sciences**, v. 35, n. 2, p. 163-168, 2013. doi: http://doi.org/10.4025/actascibiolsci.v35i2.13971

DERNER, R. B.; OHSE, S.; VILELA, S.; CARVALHO, S. M.; FETT, R. Microalgae, products and applications. **Ciência Rural**, v. 36, n. 6, p. 1959-1567, 2006. doi: http://doi.org/10.1590/S0103-84782006000600050

DUFOSSÉ, L.; GALAUP, P.; YARON, A.; ARAD, S. M.; BLANC, P.; KOTAMBALLI, N.; MURTHY, C.; RAVISHANKAR, G. A. Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? **Trends in Food Science & Technology**, v. 16, n. 9, p. 389-406, 2005. doi: http://doi.org/10.1016/j.tifs.2005.02.006

FRANCIS, F. J. Analysis of anthocyanins. *In*: MARKAKIS, P. (Ed.). **Anthocyanins as food colors**. New York: Academic Press, p. 181-207, 1982.

GÓMEZ, P.; BARRIGA, A.; CIFUENTES, A. S.; GONZÁLEZ, M. Effect of salinity on the quantity and quality of carotenoids accumulated by *Dunaliella salina* (strain CONC-007) and *Dunaliella bardawil* (strain ATCC 30861) Chlorophyta. **Biological Research**, v. 36, n. 2, p. 185-192, 2003. doi: http://doi.org/10.4067/S0716-97602003000200008

GÓMEZ, P. I.; GONZÁLEZ, M. A. The effect of temperature and irradiance on the growth and carotenogenic capacity of seven strains of *Dunaliella salina* (Chlorophyta) cultivated under laboratory conditions. **Biological Research**, v. 38, n. 2-3, p. 151-162, 2005. doi: http://doi.org/10.4067/S0716-97602005000200005

GUEVARA, M.; LODEIROS, C.; GOMEZ, O.; LEMUS, N.; NUÑES, P.; ROMERO, L.; VÁSQUEZ, A.; ROSALES, N. Carotenogenesis of five strains of the algae Dunaliella sp. (Chlorophyceae) isolated from Venezuelan hypersaline lagoons. **Revista de Biologia Tropical**, v. 53, n. 3-4, p. 331-337, 2005. doi: http://doi.org/10.15517/rbt.v53i3-4.14593

HIGBY, W. K. A simplified method for determination of some the carotenoid distribution in natura and carotene – fortified orange juice. **Journal of Food Science**, v. 27, n. 1, p. 42-49, 1962. doi: http://doi.org/10.1111/j.1365-2621.1962.tb00055.x

HORNES, M.; SILVA, A. G.; MITTERER, M. L.; QUEIROZ, M. I. Influence of nitrogen compounds on the protein concentration of the cyanobacterium Aphanothece microscopica Nägeli. **Food Science and Technology**, v. 30, n. 2, p. 364-371, 2010. doi: http://doi.org/10.1590/S0101-20612010000200012

LAMERS, P. P.; JANSSEN, M.; DE VOS, R. C. H.; BINO, R. J.; WIJFFELS, R. H. Exploring and exploiting carotenoid accumulation in *Dunaliella salina* for cell-factory applications. **Trends in Biotechnol**, v. 26, n. 11, p. 631-638, 2008. doi: http://doi.org/10.1016/j.tibtech.2008.07.002

LARRAURI, J. A.; RUPÉREZ, P.; SAURA-CALIXTO, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. **Journal of Agricultura and Food Chemistry**, v. 45, n. 4, p. 1390-1393, 1997. doi: http://doi.org/10.1021/jf960282f

LORENZEN, C. J. Determination of chlorophyll and pheopigments: spectrophotometric equations. **Limnology and Oceanography**, v. 12, n. 2, p. 343-346, 1967. doi: http://doi.org/10.4319/lo.1967.12.2.0343

LOURENÇO, S. O. **Cultivo de Microalgas Marinhas: princípios e aplicações**. 1st ed. São Carlos: Rima, 2006, 587p.

MUHAEMIN, M.; KASWADJI, R. F. Biomass nutrient profiles of marine microalgae *Dunaliella salina*. **Jurnal Penelitian Sains**, v. 13, n. 3, p. 64-67, 2010. doi: https://doi.org/10.26554/jps.v13i3.142

OLIVARRÍA, D. F.; CASTILLO, L. R. M.; LÓPEZ, A. M.; ELÍAS, J. A. L. Crecimiento y biomasa de Dunaliella sp. cultivada en medios limitantes en nitrógeno. **Biotecnia**, v. 12, n. 3, p. 58-66, 2010. doi: https://doi.org/10.18633/bt.v12i3.104

RAJA, R.; HEMAISWARYA, S.; BALASUBRAMANYAM, D.; RENGASAMY, R. Protective effect of *Dunaliella salina* (Volvocales, Chlorophyta) against experimentally induced fibrosarcoma on wistar rats. **Microbiological Research**, v. 62, n. 2, p. 177-184, 2007. doi: http://doi.org/10.1016/j.micres.2006.03.009

R DEVELOPMENT CORE TEAM. **R: A language and environment for statistical computing.** R Foundation for Statistical Computing, Vienna, Austria, 2011. https://www.r-project.org/

SAHA, S. K.; HAYES, J.; MOANE, S.; MURRAY, P. Tagging of biomolecules with deuterated water (D_2O) in commercially important microalgae. **Biotechnology Letters**, v. 35, p. 1067-1072, 2013. doi: http://doi.org/10.1007/s10529-013-1176-8

SAHA, S. K.; UMA, L.; SUBRAMANIAN, G. Nitrogen stress induced changes in the marine cyanobacterium *Oscillatoria willei* BDU 130511. **FEMS Microbiology Ecology**, v. 45, n. 3, p. 263-272, 2003. doi: http://doi.org/10.1016/S0168-6496(03)00162-4

SAID, H. S. Changes in levels of cellular constituents of *Dunaliella parva* associated with inorganic phosphate depletion. **Middle East Journal of Scientific Research**, v. 4, n. 2, p. 94-99, 2009. https://www.cabdirect.org/cabdirect/abstract/20103225168

SIPAÚBA-TAVARES, L. H.; IBARRA, L. C. C.; FIORESI, T. B. *Ankistrodesmus gracilis* (reisch) korsikov (chlorophyta) laboratory cultured in CHU12 and macrophyte with NPK media. **Boletim do Instituto de Pesca**, v. 35, n. 1, p. 111-118, 2009. https://www.pesca.sp.gov.br/boletim/index.php/bip/article/view/842

STEIN, J. R. **Handbook of Phycological Methods: culture methods and growth measurements**. Cambridge: University Press, 1973, 460p.

STROHECKER, R.; HENNING, H. M. **Análises de vitaminas: métodos comprovados**. Madrid: Paz Montalvo, 1976, 428p.

TANG, Y.; WANG, C. H.; HUANG, D. Analysis of the mineral element contents of axenic and natural *Dunaliella salina*. **Guang Pu Xue Yu Guang Pu Fen Xi**, v. 30, n. 7, p. 1956-1959, 2010. doi: http://doi.org/10.3964/j.issn.1000-0593(2010)07-1956-04

WALNE, P. R. Experiments in the large scale culture of the larvae of *Ostrea edulis*. **Fishery investigations (Great Britain. Ministry of Agriculture, Fisheries and Food)**, v. 25, n. 4, p. 1-53, 1966. https://www.worldcat.org/title/experiments-in-the-large-scale-culture-of-the-larvae-of-ostrea-edulis-l/oclc/954380

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