# Assessment of the Nutraceutical Potential of the Biochemical Profile of *Dunaliella salina* Biomass

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**Microalgae are recognized as promising sources of bioactive compounds for human health; however, gaps remain in the characterization and functional application of their biomass in dietary supplements. *Dunaliella salina*, despite its high biotechnological value, still lacks integrated studies addressing its nutraceutical potential and applicability in food products. Therefore, this study evaluated *D. salina* biomass as a sustainable food ingredient. The cultivation exhibited a well-defined exponential growth phase ($\mu = 0.0113\ h^{-1}$), allowing the identification of an optimal harvesting window (~10 days), which supported the efficient production of nutritionally rich biomass containing high levels of proteins (25.7 ± 0.42%), lipids (18.5 ± 0.06%), carbohydrates (28.5 ± 0.71%), and ash content (26%), along with phenolic compounds (2.16 ± 0.21 mg GAE 100 $g^{-1}$ DW) associated with antioxidant activity and oxidative stress reduction. These findings highlight the biotechnological potential of *D. salina* biomass as a sustainable nutraceutical supplement.**

**Keywords: Microalgae. *Dunaliella salina*. Biochemistry.**

Microalgae are recognized as reliable sources of biochemically active compounds, with potential applications as raw material for various products, due to their high biodiversity and the wide variability in the chemical composition of their biomass, combined with their sustainable and renewable nature [1].

In this context, the use of microalgae extends across multiple industrial sectors, not limited to the pharmaceutical and cosmetic areas, but also encompassing the dietary supplementation industry [2]. This versatility results from their capacity to co-produce different biomolecules, such as pigments, proteins, polyunsaturated fatty acids, and antioxidant compounds, which has increased their interest as therapeutic agents as well as in the form of nutraceuticals [3].

Among biotechnologically relevant microalgae, *Dunaliella salina* is the focus of this study, being widely applied in the production of high-value compounds with industrial relevance, with research on the topic showing a growing trend [4].

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The present study evaluates whether the biomass of *D. salina*, obtained under the established conditions, exhibits adequate quality regarding its macromolecular and bioactive compound profile for application as a nutraceutical.

## Materials and Methods

The study evaluated proteins, lipids, ash and carbohydrates, as well as total phenolic content as an indicator of bioactive compounds. Experiments were conducted at the Didactic Biotechnology Laboratory of SENAI CIMATEC and at the Didactic Undergraduate Research Laboratory of Serviço Social da Indústria - SESI Djalma Pessoa.

### Cultivation of the Microalga *D. salina*

The microalgal strain was obtained from the Iracema Nascimento Microalgae Bank, Institute of Biology, Federal University of Bahia, Salvador, Brazil. The inocula were cultivated following a methodology adapted from Fonseca and colleagues [5], under closed batch culture conditions, using artificial seawater (ASW) [6] composed of distilled water and salts (g $L^{-1}$) at the following concentrations: 30.07 NaCl, 4.08 $Na_2SO_4$, 0.72 KCl, 0.17 $NaHCO_3$, 1.16 $CaCl_2$, 5.20 $MgCl_2$, and 0.72 $KNO_3$. The culture medium was supplemented

with 1 mL of Conway medium containing macro- and micronutrients and 0.5 mL of vitamin solution [7]. Microalgal growth was maintained under continuous illumination using fluorescent lamps at a light intensity of 40.5 μE $m^{-2}$ $s^{-1}$, with aeration provided by atmospheric air compressors with a nominal airflow rate of 795 L $h^{-1}$ (210 GPH).

Cell Growth Kinetics

Microalgal growth was monitored for 13 days by optical density measurements [8]. 60 mL of microalgal culture were inoculated into 140 mL of artificial seawater (ASW) in 250 mL culture flasks, supplemented with 0.2 mL of Conway medium and 0.2 mL of vitamin solution. Optical density was measured at 740 nm using a UV–Vis spectrophotometer [8], and the values obtained were used to construct the growth curve.

The Δ parameter [9] was calculated as the absolute difference between the initial and final optical density values of each growth phase. Δ was used to quantify the magnitude of biomass accumulation during the exponential and stationary phases.

$$\Delta = X_2 - X_1 \quad (1)$$

$X_1$: corresponds to the optical density value at the initial time ($t_1$).

$X_2$: corresponds to the optical density value at the final time ($t_2$) of the analyzed interval.

The specific growth rate (μ) [9] was determined from the slope of the linear regression of the natural logarithm of optical density values ($OD_{740}$) as a function of time, considering only the exponential growth phase.

$$\mu = \frac{ln\, X_2 - ln\, X_1}{t_2 - t_1} \quad (2)$$

$X_1$ and $X_2$: correspond to the optical density values measured at 740 nm ($OD_{740}$) at times $t_1$ and $t_2$, respectively;

$t_1$ and $t_2$: represent the initial and final times of the selected interval within the exponential growth phase;

ln: denotes the natural logarithm.

The doubling time (td) was calculated to estimate the time required for the microalgal biomass to double during the exponential growth phase. The calculation was based on the specific growth rate (μ), assuming exponential growth, according to the following equation:

$$td = \frac{ln(2)}{\mu} \quad (3)$$

td: corresponds to the cellular doubling time;

μ: represents the specific growth rate;

ln(2): is the natural logarithm of 2.

Biomass Extraction by Flocculation

Biomass was extracted using NaOH as a coagulant at 2 g $L^{-1}$ [5]. Wet biomass was dried in an oven at 70 °C for 4 h, then macerated, sieved, and weighed.

Total Lipid Determination

For total lipid analysis [10], 50 mg of dried biomass were weighed and 25 mL of a chloroform–methanol solution (2:1, v/v) was added. The mixture was heated at 60 °C for 15 min to extract the lipids. The resulting extract was filtered, and 10 mL were collected, followed by the addition of 2 mL of distilled water and centrifugation at 4,000 rpm for 10 min. The organic phase was transferred to pre-treated aluminum containers and heated until complete solvent evaporation to obtain the lipid content, which was calculated using equation:

$$\text{Lipid (\%)} = \frac{m1 - m2}{m3} \times 100 \quad (4)$$

$m_1$: mass of the container with lipids (g) after solvent evaporation;

$m_2$: mass of the empty container (g);

$m_3$: mass (g) of the biomass after dilution in chloroform–methanol solution.

Total Carbohydrates Determination

Total carbohydrates were quantified using the phenol–sulfuric acid method [11,12]. A 0.002 g

sample of dried biomass was weighed, followed by the addition of 1 mL of $H_2SO_4$ solution (1.0 mol/L) and 5 min of ultrasonic bath. Then, 4 mL of $H_2SO_4$ (1.0 mol/L) were added, and the mixture was heated in an oven at 100 °C for 1 h, followed by centrifugation at 4,000 rpm for 15 min and simple filtration. One milliliter of 5% phenol was added to the acid filtrate, and after 40 min, 5 mL of $H_2SO_4$ were added. Total carbohydrate content was calculated using equation:

$$\text{Carbohydrates (\% m/m)} = \frac{\left[\left(\frac{C}{Va}\right) x\, Ve\right]}{m} \text{ x } 100 \quad (5)$$

C: carbohydrate concentration (mg/mL);
$V_a$: sample volume (L) used;
$V_e$: volume (L) of the acid extract used in the treatment;
m: amount of dried biomass (g).

Total Protein Determination

Total proteins were quantified following the method described by Lowry and Colleagues and Fachet and colleagues [13,14]. Solutions were prepared as follows: A (1 mol/L NaOH), B (5% $Na_2CO_3$), C (0.5% $CuSO_4$ and 1% $KNaC_4H_4O_6 \cdot 4H_2O$), D (2 mL of solution C and 50 mL of solution B), and E (Folin–Ciocalteu reagent). Five milligrams of dried biomass were weighed and dissolved in 0.5 mL of distilled water and 0.5 mL of solution A. The mixture was heated at 60 °C for 10 min in an oven, followed by the addition of 2.5 mL of solution D and incubation for 10 min in the dark. Then, 0.5 mL of solution E was added, followed by 30 min incubation in the dark. Absorbance was measured at 580 nm using a UV–Vis spectrophotometer. Total protein content was calculated according to the following equation:

$$\text{Protein (\% m/m)} = \frac{C\, x\, 100}{m} \quad (6)$$

C: protein concentration (mg/mL);
m: weight of dried biomass (g).

Total Total Ash Determination

Total ash analysis was performed following Freitas and colleagues [15]. Crucibles were preheated at 100 °C for 1 h in an oven. One gram of dried biomass was weighed and subjected to muffle furnace calcination at 525–550 °C for approximately 5 h. The ash content (%) was calculated using the following equation:

$$\text{Ash (\%)} = \frac{(Pa - Pc)}{m} \text{ x } 100 \quad (7)$$

$P_a$: weight of the crucible with ash;
Pc: weight of the empty crucible;
m: weight of the sample.

Total Phenolic Determination

Total phenolic content was determined following Fonseca and colleagues, and Singleton and Rossi [5,16]. 5 mL of microalgal culture were mixed with 500 µL of 10% (v/v) Folin–Ciocalteu reagent, followed by the addition of 1,000 µL of 7.5% (w/v) sodium carbonate and 3.5 mL of distilled water. The mixture was incubated in the dark at room temperature for 2 h. Absorbance was measured at 580 nm using a UV–Vis spectrophotometer. Results were expressed as gallic acid equivalents per 100 g of dry weight (mg GAE 100 $g^{-1}$ DW).

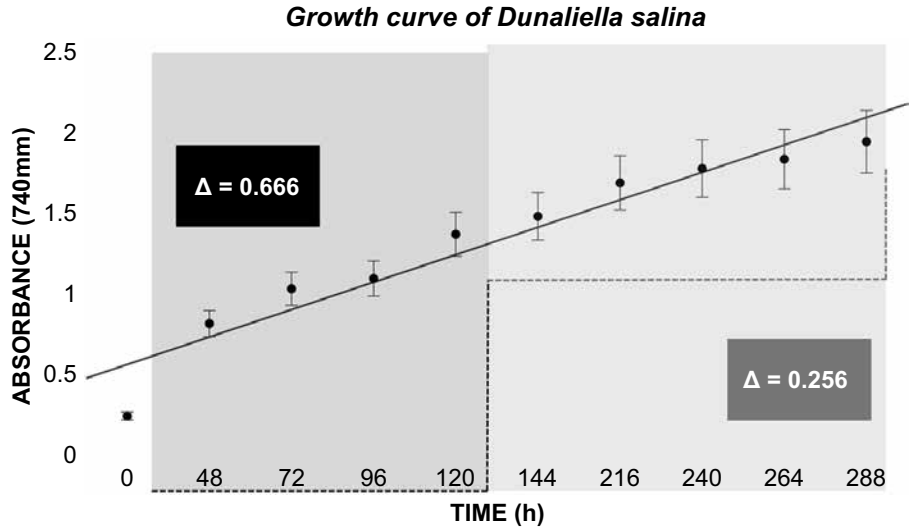
## Results and Discussion

Study Related to Cell Growth

The evaluation of microalgae cell growth in the ratio 30:70 (chopped culture/culture medium) proved to be effective, where it was possible to observe (Figure 1) the exponential growth until reaching high cell density, defined as ideal for biomass harvesting.

The Δ value was used as a comparative growth descriptor to highlight differences in cellular behavior throughout the cultivation phases. The calculation considered two time intervals: from

**Figure 1.** Growth curve of *Dunaliella salina* during a 13-day cultivation period.




day 2 (~48h) to day 7 (~144h), with Δ = 0. 666 corresponding to the exponential growth phase, and from day 8 (~168h) to day 13 (~288h), with Δ = 0.256, corresponding to the stationary phase.

The higher Δ observed during the first interval indicates more intense cell growth, characteristic of the exponential phase, marked by rapid biomass accumulation and low cell mortality, which favors efficient metabolic activity. In contrast, the reduction in Δ during the second interval reflects the transition to the stationary phase, associated with decreased nutrient availability, metabolite accumulation, and physiological adjustments of the microalga [17].

Thus, the Δ values clearly demonstrate the occurrence of physiologically distinct growth phases during the cultivation of *Dunaliella salina*. The cultivation system combined with the adopted peaking regime proved effective for the rapid production of viable biomass, allowing the identification of an optimal harvesting window within 10 days (~216h) of cultivation and supporting the biotechnological optimization of the produced biomass.

The specific growth rate (μ) obtained during the exponential phase was 0.0113 $h^{-1}$, indicating a moderate and sustained growth pattern. This value is consistent with typical growth rates reported for *Dunaliella* species under non-limiting cultivation conditions [18]. The calculated doubling time (~61 h) further supports the occurrence of a well-defined exponential growth phase prior to growth stabilization.

Yield Obtained from the Extraction of Biomass by Flocculation with NaOH

The extraction of *Dunaliella salina* biomass using sodium hydroxide as a chemical coagulant showed promising results, obtaining an average yield of 3.100 g of dry biomass per 1L of culture. In the studies directed by Andrade [19], the yield is 1.194 g $L^{-1}$, in which he highlights the effectiveness of the approach adopted.

Determination of Lipids, Carbohydrates and Total Proteins

The results corresponding to the levels of proteins, lipids and total carbohydrates are presented in Table 1, in which the values were promising for a potential supplement.

When we analyze the results obtained with other studies with *D. salina*, such as carried out by Sui and Vlaeminck [20] in a constant photoperiod regime, a rate of 54% of proteins,

**Table 1.** Proximate biochemical composition of *Dunaliella salina* biomass (proteins, carbohydrates, and lipids).

| Compounds | Content (%) |
|---|---|
| Proteins | 25.7 ± 0.42 |
| Carbohydrates | 28.5 ± 0.71 |
| Lipids | 18.5 ± 0.06 |

however, the carbohydrate rates registered values below 20%. In another study carried out by Gharajeh et al. in a light and dark phase regime, 40% of proteins and 32% of carbohydrates were obtained, but values of less than 6% of lipids. As for the lipid content, in a study directed by Gharajeh and colleagues [21], the lipid content present in dry biomass.

Therefore, the cultivation regime used has shown potential in obtaining a rich biomass, evidenced by the concentrations of the three main biochemical compounds in a balanced manner, highlighting their efficacy and adaptability for the production of biomolecules of interest through microalgae.

Determination of Total Ashes

The ash value obtained from the dry biomass was 26%, which is higher than the value reported in the literature (approximately 11%) [17]. This value may be related to the precipitation of calcium carbonate ($CaCO_3$) in the culture medium, which can be influenced by the concentration of calcium ions ($Ca^{2+}$), dissolved inorganic carbon (DIC), elevated pH, and availability of nucleation sites. According to the literature, this process is known as biocementation and has been used in microalgae for the development of biocement [22]. In principle, the salts present can be interpreted as possible mineral salts available in the biomass composition; however, for this confirmation, it will be necessary to identify these salts as well as their respective concentrations.

Determination of Total Phenolic

The total phenolic content found in the biomass was 2.16 ± 0.21 gallic acid equivalents per 100 g of dry weight (mg GAE 100 $g^{-1}$ DW), thus confirming the presence of antioxidant activity in the microalgal biomass content, in accordance with the literature [17]. It is important to emphasize that, despite the lower concentration observed, the microalga still demonstrates antioxidant potential, since phenolic compounds are associated with the neutralization of reactive oxygen species and the reduction of oxidative stress, processes related to the prevention of chronic diseases and the promotion of human health, reinforcing its potential application as a nutraceutical [23,24].

## Conclusion

The results of this study demonstrate the potential of *Dunaliella salina* biomass for application in supplementation aimed at human health. Moreover, the analyses revealed an adequate biochemical composition and the presence of bioactive compounds, particularly phenolic compounds, which consequently contribute to its antioxidant activity and functional value. Therefore, these characteristics support the potential use of *D. salina* biomass in the development of nutraceuticals and functional ingredients for human consumption. Finally, future studies should focus on optimizing biomass yield, standardizing the production process, and validating its application through appropriate models for human use.

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