

## Sunscreen Production from *Chlorella Vulgaris*

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Sunscreen use has increased due to climate change and greater widespread awareness of the risk of acquiring skin diseases, including cancer, due to ultraviolet radiation from the sun (Hiatt & Beyeler, 2020; Alli et al., 2023). This has had a negative impact on water due to the increased release of components from these cosmetic products (Couselo-Rodríguez et al., 2022). In this sense, product formulation should consider aspects of environmental and social sustainability. Microalgae capture CO<sub>2</sub> from the atmosphere, remove contaminants from wastewater, including traces of emerging pollutants (Ávila-Cújar et al., 2021), and give an alternative for environmentally friendly sunscreens. The initiative becomes a first step towards the possible development of a business based on sunscreen production. Microalgal biomass production was carried out under laboratory conditions. Subsequently, the biomass was separated from the culture medium, and the components responsible for the protective function, such as beta-carotenes and mycosporines, were quantified by spectrophotometry. Next, the sunscreen formulation was developed, and finally, the sun protection factor (SPF) was determined, in addition to verifying some organoleptic properties related to its application. The product obtained achieved an SPF of 8 with in vitro tests, and the use of environmentally friendly components in its formulation makes it an attractive alternative from an environmental and social perspective.

### 1. Introduction

In the global context, cosmetic industry generates around \$262.21 billion, with skin care products representing 38% of the market value. Sunscreens, categorized as part of the skincare product range, have increased their market share, serving as preventive measures against aging, sunburns, and the development of keratinocyte cancers and melanoma due to prolonged exposure to UV radiation (Grand View Research, 2020; Narla & Lim, 2020). Consequently, these products are considered pharmaceuticals and emerge as an alternative for skin cancer prevention, because 1.5 million new cases of skin cancer were reported worldwide in 2020, caused for the increased ultraviolet radiation on Earth (World Health Organization, 2022).

Currently, among various cosmetic products, sunscreens give the most effective protection against UV radiation damage. Following Ambruso et al. (2023), sunscreens are "any cosmetic product containing UV filters in its formulation with the aim of protecting the skin from harmful solar ultraviolet light, preventing or minimizing the damage this radiation can cause to human health". Their formulation includes organic compounds (aminobenzoate, benzophenones, and dibenzylmethanes) and inorganic compounds (titanium dioxide and zinc oxide) with absorption and reflection properties for solar radiation. However, the environmental impact caused by the organic UV filters used in the process is alarming, as their low solubility and high lipophilic properties hinder their elimination in wastewater, disrupting aquatic ecosystems and biodiversity. According to the environmental organization Green Cross, 25,000 tons of sun creams reach the oceans annually (Crespo, 2022). Microalgae offer advantages in terms of biomass production, rapid growth times, and tolerance to wide ranges of pH, salinity, and temperature. Besides, they can convert solar energy into chemical energy, producing high-value-added natural bioactive compounds (Ariede et al., 2017). As a result, they have gained attention in the cosmetic industry, for the development of pharmaceutical and cosmetic products. Thus, microalgae and their secondary metabolites are a more sustainable alternative for manufacturing ecological ingredients and products. *Chlorella vulgaris* is a spherical microalga, 2-10 µm in diameter, non-motile cell, with a green coloration, which reproduces through binary fission via asexual spores, growing rapidly and generating an average of one cell every 24 hours (Raiesie et al., 2021). The main components of its algal biomass are proteins, lipids, and

carbohydrates, constituting up to 90% of the total dry weight. The remaining 10% includes pigments, vitamins, and other minor components (Ahmad et al., 2021). These secondary metabolites result from amphibolic or anabolic pathways when supplied with nitrogen and phosphorus, as well as atmospheric CO<sub>2</sub>. Among these compounds are β-carotenoids and mycosporines-like amino acids (MAAs), known for their photoprotective activity in absorbing UV radiation at short wavelengths, making them effective protective agents against UV radiation (Amador-Castro et al., 2020; Yarkent et al., 2020). In this context, the overall objective of this study was to produce a sunscreen from the microalga *Chlorella vulgaris* on a laboratory scale.

## 2. Methodology

This section is divided in five parts: Microalgae cultivation, microalgae growth, biomass separation, metabolites extraction and quantification; and sunscreen formulation.

### 2.1 Microalgae cultivation

*Chlorella vulgaris* was supplied by microbiology laboratory at the Francisco de Paula Santander University in Cúcuta, Colombia. It was cultivated in two glass photobioreactors, one under white, and the other under red LED light lamps to study the effect of exposure to different light spectra on cell growth and the production of secondary metabolites. In both photobioreactors, Bold Basal Medium (BBM) was used as culture medium (Bertoloni, 2022). It was sterilized at 120 °C in a DENTOMAT brand autoclave, model Automat 3000, for 15 minutes. Both photobioreactors, sealed with Parafilm to avoid contamination, had the same total volume of 150 mL, comprising 77 mL of BBM, 5 mL of microalgae, and 68 mL of deionized water. Continuous aeration and agitation, to ensure effective light and nutrients dispersion, was given by a fan and hoses connected to each photobioreactor. The first photobioreactor (culture 1) was placed inside a polystyrene incubator with red light tape on its walls to provide illumination to the culture. And the second photobioreactor (culture 2) was outside the incubator and illuminated by a white LED light lamp, with an average light intensity of 125.85 Lux to the front of the culture. Culture 1 was maintained at a temperature above ambient, specifically at 19 °C, while culture 2 at 17 °C, the average ambient temperature of Bogotá. pH measurements were taken weekly, staying within the range of 8-9 for both experiments (Arredondo & Voltolina, 2007).

### 2.2 Microalgae growth

Microalgae growth was monitored by measuring the optical density of each culture at a wavelength of 640 nm using a UV/VIS spectrophotometer, THERMOSPECTRONIC model Genesis 20. This wavelength was chosen for its proximity to the chlorophyll absorption peak (675 nm), allowing measurements even at minimal cellular concentrations (Zhang et al., 2023). These measurements were taken, by triplicate, every two days. The specific growth rate in the exponential growth phase was calculated by equation (1) (Metsoviti et al., 2019):

$$\mu_{exp} = \frac{\ln(\alpha_2/\alpha_1)}{(t_2 - t_1)} \quad (1)$$

$\alpha_1$  and  $\alpha_3$  are the optical densities at the beginning and at the end of the exponential growth phase.

### 2.3 Biomass separation

Solid biomass was separated from the liquid medium, in a TECNAL centrifuge model 206-baby I for 15 minutes at 3000 rpm. Supernatant was discarded, and the precipitate was collected. A further drying process was necessary. The samples were placed in an oven BINDER model IP20 at 65 °C for 2 hours. The recovered dry biomass was milled and stored at -15 °C (Outon, 2019) and divided into different quantities for use in dry weight determination, extraction, quantification of target metabolites, and sunscreen production. Dry weight (in g L<sup>-1</sup>) and productivity (in g L<sup>-1</sup> d<sup>-1</sup>) were calculated using the following equations (Corzo et al., 2019) where  $m_1$  is the weight of filled container,  $m_0$  is the weight of empty container,  $V_{culture}$  is the total volume of microalgae culture, including medium, at the end of the cultivation; and,  $t$  is the cultivation time:

$$Dry\ weight\ (g\ L^{-1}) = \frac{m_1 - m_0}{V_{culture}} \quad (2)$$

$$Productivity\ (g\ L^{-1}d^{-1}) = \frac{Dry\ weight}{V_{culture} * t} \quad (3)$$

### 2.4 Metabolites extraction and quantification

Beta-carotenoids were extracted and quantified 45 days after the initiation of the culture and following the acquisition of dry microalgal biomass, following the standardized protocol by Ibanez (2018). The dry biomass

used for this process was obtained from the culture exposed to white LED light, as the quantity obtained from the red LED culture was insufficient for analysis. Initially, using an analytical balance (RADWAG model AS-220/C/2), 0.1 g of dry microalgal biomass was weighed and added to a 15 mL Falcon tube containing 7 mL of the extraction solution (90% acetone). The mixture underwent manual agitation for 30 seconds followed by ultrasonic bath treatment for 10 minutes (Chronis et al., 2021). After this, it was centrifuged using a TECNAL centrifuge model 206-baby I for 20 minutes at 3200 rpm. The supernatant was collected in a 25 mL volumetric flask, and the process was repeated twice until the matrix decolorization was achieved (Ibanez, 2018). The total concentration of beta-carotene was determined by spectrophotometry using acetone as a blank. In addition, the 25 mL volumetric flask containing the supernatant was levelled with acetone. Following this, the absorbance of the supernatant was measured at a wavelength of 450 nm to calculate the concentration of beta-carotene in  $\mu\text{g mL}^{-1}$  using the following equation (Ibanez, 2018)

$$C = \frac{A_{MAX} * M * 1000}{\varepsilon * \delta} \quad (4)$$

Where  $A_{MAX}$  is the absorbance at 450 nm;  $M$  is the molecular weight of carotene ( $537 \text{ g mol}^{-1}$ );  $\varepsilon$  is the molar extinction coefficient of the  $\beta$  - carotenoid in acetone ( $140663 \text{ L mol}^{-1} \text{ cm}^{-1}$ ) and  $\delta$  is the optical path in cm. For mycosporines (MAAs) extraction methanol and acetone were used as polar extraction solvents to evaluate their effectiveness. Acetone was used as extraction solvent for four samples of which two came from the Culture 1 (white light) and the other two from the Culture 2 (red light). Each of the samples were crushed, after mixed with silica gel, in a mortar for 3 minutes and added to test tubes containing 4 mL of each solvent. Following this, all samples were stored at a temperature of  $-20 \text{ }^\circ\text{C}$  for 22 h in a dark place (Outon, 2019). Finally, the samples were centrifuged for 20 minutes at 4200 rpm, precipitate was separated and the supernatant went to another test tube (Outon, 2019). Optical density of the supernatant at a wavelength between 326-704 nm were measured. General equation to quantify mycosporines was

$$\text{Total MAAs Content} = \frac{C_1 * A_{\lambda_1} - C_2 * A_{\lambda_2}}{\text{Dry weight}} * \frac{A_{MAAS}}{A_{chla}} * 1000 \quad (5)$$

$A_{MAAS}$  is the area under the curve at wavelengths from 326 to 356 nm;  $A_{chla}$  is the area under the curve at wavelengths from 621 to 704 nm, corresponding to chlorophyll – a. The exact parameters  $C_1, C_2$  and the wavelengths  $\lambda_1, \lambda_2$  depend on which solvent was used: acetone or methanol (Huang et al., 2016; Outon, 2019).

## 2.5 Sunscreen formulation

A semi-solid formulation was prepared by means of an oil-in-water (O/W) emulsion, intended for topical application with a protective effect (Rios, 2020). The compounds used were separated in PHASE A (oily) and PHASE B (aqueous). All compounds were added according to limits allowed by the FDA (U.S. Food & Drug, 2023). Finally, protection factor (SPF) was evaluated by an *in vitro* method, specifically applying UV spectrophotometry to samples onto a 1X2 cm strip of a tape in a wavelength range from 290 to 320 nm, with intervals of 5 nm, where the determination of the SPF, which is the correlation between the erythema effect (EE) and the radiation intensity ( $I$ ) at each wavelength ( $\lambda$ ) adapts to the following equation (Widsten et al., 2020) where CF is the correction factor which values 10, and  $Abs$  is the optical absorbance at each wavelength in the range (Martínez-Inda et al., 2023):

$$FPS = CF * \sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda) \quad (6)$$

## 3. Results

### 3.1 Effect of light on Microalgae growth

Red light LED, used in Culture 1, led to a specific growth rate in the exponential phase of  $0.22 \text{ d}^{-1}$ , a productivity of  $0.52 \text{ g L}^{-1} \text{ d}^{-1}$ , and a dry weight of  $14.47 \text{ g L}^{-1}$ . As for Culture 2, the white LED light intensity led to a specific growth rate in the exponential phase of  $0.31 \text{ d}^{-1}$ , a productivity of  $1.24 \text{ g L}^{-1} \text{ d}^{-1}$ , and a dry weight of  $34.61 \text{ g L}^{-1}$ . The highest cellular growth rate, dry weight, and productivity for the Culture 2, with white LED light, is the same behaviour found in literature (Martinez et al., 2022).

### 3.2 Metabolites extraction and quantification

The amount of biomass obtained in Culture 1 was not sufficient to perform carotenoid extraction, only for mycosporines, the first procedure made under four different conditions: with two solvents and evaluating the use of silica gel in sample drying. The results are shown in Table 1.

Table 1: Quantification of mycosporines

Solvent	Culture 1		Culture 2	
	With silica gel	Without silica gel	With silica gel	Without silica gel
Methanol	6.61 $\mu\text{g g}^{-1}$	6.22 $\mu\text{g g}^{-1}$	2.98 $\mu\text{g g}^{-1}$	1.93 $\mu\text{g g}^{-1}$
Acetone	5.10 $\mu\text{g g}^{-1}$	3.96 $\mu\text{g g}^{-1}$	2.12 $\mu\text{g g}^{-1}$	1.98 $\mu\text{g g}^{-1}$

A higher amount of mycosporines was obtained from the microalgal biomass of Culture 1 compared to Culture 2, which is consistent with Llewellyn et al. (2020) in their work with *Chlorogloeopsis fritschii*, where they found that the impact of exposure to UVB radiation and red light on MAA biosynthesis was greater compared to white light. Based on these results, a factorial regression "MAAs concentration versus extraction solvent; desiccant" was applied by means of free Minitab 17®, resulting in a variance analysis in which it was found that the solvent (effect A) and desiccant (Effect B) had an effect of -1.15 and -0.68 on mycosporines extraction, respectively. Furthermore, it was found that the effect of both factors is not statistically significant at a significance level of 0.05 because the P-values are higher, being 0.53 for factor A and 0.70 for factor B. Finally, the concentration of  $\beta$ -carotenoids for Culture 2 was 0.094  $\mu\text{g mL}^{-1}$ .

### 3.3 General and protective features of the sunscreen

In Figure 1 the final product is shown. However, the formulation included all the separated biomass of *Chlorella vulgaris* instead of only the metabolites. From the picture, it is evident its green color and viscous appearance. Besides, its consistency was creamy-gel, smooth to the touch, and had a light and fluid texture, facilitating its application. Additionally, it was free from lumps and particles, indicating that the compounds used in the formulation exhibited compatibility with each other, ensuring skin safety when applied.



Figure 1: Sunscreen produced from the microalgae *Chlorella vulgaris* on a laboratory scale

Then, pH and sun protection factor, SPF, were determined to assess security and effectiveness of the sunscreen formulation, obtaining values of 6 and 7.74, respectively, from which it is considered that the sunscreen is secure for skin care and presented a medium level of photoprotection according to the standard of the European cosmetic and perfumery association, COLIPA, (Schneider et al., 2020). Finally, it is also important to consider that the SPF depends not only on the concentration of the pigments, but also on the quantity used and on the specific method used.

#### 4. Conclusions

The white LED light cultivation showed the highest growth rate: 0.31 d<sup>-1</sup>, dry weight: 34.61 g L<sup>-1</sup>, and productivity: 1.24 g L<sup>-1</sup> d<sup>-1</sup> compared to the red LED light cultivation with 0.22 d<sup>-1</sup>; 14.47 g L<sup>-1</sup> and 0.52 g L<sup>-1</sup> d<sup>-1</sup> respectively. However, the mycosporines concentration was higher in the cultivation under red LED light, with a value of 6.61 µg g<sup>-1</sup>, while for the cultivation under white LED light, it was 3.38 µg g<sup>-1</sup>. As a possible strategy to increase mycosporines production, microalgae growth should be made with white LED light until stationary phase, and then red LED light should be used. In the sunscreen, the dry biomass of *Chlorella vulgaris* was used as a new UV filter, not only mycosporines and carotenoids, resulting in a product with a pH of 6, a characteristic green color of the microalga, a creamy-gel consistency, and a light and fluid texture. The SPF of the sunscreen was 7.74, classifying it as a medium protection photoprotector. The results obtained in this work demonstrate the potential of *Chlorella vulgaris* to be used in the production of biological sunscreens. It is also needed to make an economical evaluation in future research to improve the profitability of the process.

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