UNIVERSIDADE DE SÃO PAULO
Faculdade de Ciências Farmacêuticas
Programa de Pós-Graduação em Fármacos e Medicamentos
Área de Produção e Controle de Fármacos

In vitro photoprotective attributes from the *Chlorella vulgaris* cultivated in photobioreactor

Bruna Bertoloni dos Santos

Dissertação para obtenção do Título de
MESTRE

Orientador:
Prof. Dr. André Rolim Baby

São Paulo
2022

UNIVERSIDADE DE SÃO PAULO
Faculdade de Ciências Farmacêuticas
Programa de Pós-Graduação em Fármacos e Medicamentos
Área de Produção e Controle de Fármacos

In vitro photoprotective attributes from the *Chlorella vulgaris* cultivated in photobioreactor

Bruna Bertoloni dos Santos

Versão original

Dissertação para obtenção do Título de
MESTRE

Orientador:
Prof. Dr. André Rolim Baby

São Paulo
2022

Bruna Bertoloni dos Santos

In vitro photoprotective attributes from the *Chlorella vulgaris* cultivated in photobioreactor

Comissão julgadora da
Dissertação para obtenção do Título de Mestre

Prof. Dr. André Rolim Baby
Orientador/ Presidente

_____
1º Examinador

_____
2º Examinador

_____
3º Examinador

São Paulo, _____ de _____2022.

FICHA CATALOGRÁFICA

Ficha Catalográfica elaborada eletronicamente pelo autor, utilizando o programa desenvolvido pela Seção Técnica de Informática do ICMC/USP e adaptado para a Divisão de Biblioteca e Documentação do Conjunto das Químicas da USP

Bibliotecária responsável pela orientação de catalogação da publicação:
Marlene Aparecida Vieira - CRB - 8/5562

Santos, Bruna Bertoloni
S237a Atributos fotoprotetores in vitro da Chlorella vulgaris cultivada em fotobiorreator / Bruna Bertoloni Santos. - São Paulo, 2022.
61 p.

Dissertação (mestrado) - Faculdade de CiênciasFarmacêuticas da Universidade de São Paulo.
Departamento de Farmácia.
Orientador: Baby, André Rolim

1. photoprotect. 2. microalgae. 3. Chlorella vulgaris. 4. ultraviolet radiation. I. T. II. Baby, André Rolim , orientador.

ACKNOWLEDGEMENTS

Firstly, thanks to my family for always supporting me and believing in my dreams. Because of you I was able to reach at this point. You were my foundation and everything I've built so far belongs to us.

Thanks to my friends Gabriela, Doku, Victória, Tragédia, Ricardo, Romário, Mariana, Felipe, Inácio and Luiza who are also my family, who have helped me in so many ways, thank you all for always supporting me and for all the good times.

I'm grateful for the friendships I've found along the way. Thanks to Amanda, Maira and Fernanda for all their help and the friendship.

Thank you to my boyfriend Endrew for all the love, care, patience and support.

Finally, I would like to thank my advisor André Baby and Professor João Carlos Monteiro.

Professor André, you welcomed me from the first moment and believed in me and in my potential as a researcher. This encouraged and motivated me to get here. Thank you for the trust and all the learning. You are a fantastic professional and an inspiration to me as a researcher and person.

Professor João Carlos, my learning in your laboratory went beyond research. Thank you for welcoming me and for your companionship. I learned with you from the hard work to the discussion of results and also as a person. You showed me that knowledge and learning is something free and omnipresent, we can learn and transmit knowledge all the time and in different ways, just make the best out of the opportunities.

This study was financed, in part, by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001; the FAPESP (Fundação de Apoio à Pesquisa do Estado de São Paulo), grants n. 2019/16169-0 and n. 2016/24360-4; and the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), grant (project) n. 830634/19991.

My sincere thanks to everyone who somehow supported me during this period.

Para ser grande, sê inteiro: nada

Teu exagera ou exclui.

Sê todo em cada coisa. Põe quanto és

No mínimo que fazes.

Assim em cada lago a lua toda

Brilha, porque alta vive

Ricardo Reis

ABSTRACT

SANTOS, B.B. ***In vitro* photoprotective attributes from the *Chlorella vulgaris* cultivated in photobioreactor**. 2022. 61p. Dissertation (Master) – Faculty of Pharmaceutical Sciences, University de São de Paulo, São Paulo, 2022.

Chlorella vulgaris, a species of green microalgae, is an eukaryotic microorganism with high photosynthetic ability. *C. vulgaris* is found in different environments, predominantly in fresh and salt water, where there is exposure to high levels of UV radiation and extreme survival conditions. In response to these challenges, *C. vulgaris* produces several secondary metabolites that may have antioxidant properties. Considering the field of Cosmetology, through studies carried out so far, *C. vulgaris* showed potential to develop attributes, like preventing skin blemishes, repairing skin damage and collagen, inflammation inhibition, hydration and protection against damage caused by solar radiation, among other features. Due to these, the genus *Chlorella* has been gaining visibility scientific and commercially. The use of microalgae biomass uses to be seen as ecologically correct mainly due to the possibility of using it in various fields of application, such as in foods, source of biodiesel, pharmaceuticals and cosmetics. Besides, there are the possibility of directing the production of substances of interest through different cultivation strategies. Thus, microalgae extract can be a relevant type of constituent in products considered natural for sun protection. Based on this, this project evaluated employability of *C. vulgaris* as a photoprotective and photostabilizing ingredient. Thus, different conditions of cultivation were evaluated with different concentrations of nitrogen, obtaining better results in the 3N (8.82 mM) condition. After obtaining, preparing and characterizing the *C. vulgaris* extract, photoprotective formulations were prepared with 1.0, 5.0% and 10.0% extract. The photoprotective efficacy of the formulation was verified through a significant increase in SPF when using the *C. vulgaris* extract it at 10.0%, however, the extract did not show a photostabilizing efficacy in the formulation at any of the concentrations tested.

Keywords: photoprotection, microalgae, *Chlorella vulgaris*, ultraviolet radiation.

RESUMO

SANTOS, B.B. **Atributos fotoprotetores *in vitro* da *Chlorella vulgaris* cultivada em fotobiorreator**. 2022. 61p. Dissertação (Mestrado) – Faculdade de Ciências Farmacêuticas, Universidade de São de Paulo, São Paulo, 2022.

Chlorella vulgaris, uma espécie de microalga verde, é um microrganismo eucariótico com alta capacidade fotossintética. *C. vulgaris* é encontrada em diferentes ambientes, predominantemente em água doce e salgada, onde há exposição a altos níveis de radiação UV e condições extremas de sobrevivência. Em resposta a esses desafios, *C. vulgaris* produz vários metabólitos secundários que podem ter propriedades antioxidantes. Considerando o campo da Cosmetologia, através dos estudos realizados até o momento, *C. vulgaris* mostrou potencial para desenvolver atributos, como prevenção de manchas na pele, reparação de danos na pele e colágeno, inibição de inflamações, hidratação e proteção contra danos causados pela radiação solar, entre outros aspectos. Devido a estes, o gênero *Chlorella* vem ganhando visibilidade científica e comercial. O uso da biomassa de microalgas costuma ser visto como ecologicamente correto principalmente pela possibilidade de utilização em diversos campos de aplicação, como em alimentos, fonte de biodiesel, farmacêutico e cosmético. Além disso, por meio de diferentes estratégias de cultivo é possível direcionar a produção de substâncias de interesse. Assim, o extrato de microalgas pode ser um tipo relevante de constituinte em produtos considerados naturais para proteção solar. Baseado nesses aspectos, este projeto avaliou a empregabilidade de *C. vulgaris* como ingrediente fotoprotetor e fotoestabilizador. Com isso, diferentes condições de cultivo foram avaliadas com diferentes concentrações de nitrogênio, obtendo-se melhores resultados na condição 3N (8,82 mM). Após a obtenção, preparação e caracterização do extrato de *C. vulgaris*, foram preparadas formulações fotoprotetoras com 1,0, 5,0% e 10,0% de extrato. A eficácia fotoprotetora da formulação foi verificada através de um aumento significativo do FPS ao utilizar o extrato de *C. vulgaris* a 10,0%, porém, o extrato não apresentou eficácia fotoestabilizadora na formulação em nenhuma das concentrações testadas.

Palavras-chave: fotoproteção, microalga, *Chlorella vulgaris*, radiação ultravioleta.

SUMMARY

1. INTRODUCTION

Human skin is constantly exposed to the ultraviolet (UV) radiation, as well as many organisms, and this stressor induces various types of acute and chronic disorders (DE JAGER; COCKRELL; DU PLESSIS, 2017). Also, when in excess, this stressor can promote lipid peroxidation, damaging cell membranes; activate or inactivate enzymes and, again, DNA damage that can lead to cancer, among other pathologies (HIRATA; SATO; SANTOS, 2004; BROINIZI et al., 2007; MAMBRO, V.M.D.; FONSECA, 2007; GOLDSTEIN, 2008; GOMES; DAMAZIO, 2009; MONTAGNER; COSTA, 2009; COSTA, 2010; STEINER, 2011; AVADHANI et al., 2017). To reduce damage caused by radiation, the use of sunscreens is highly recommended. However, there has been an increase in demand for dermocosmetics developed with natural and sustainable ingredients, offering opportunities for new investigations of natural, sustainable and renewable sources of biocompounds and bioactive molecules for the development of products that can be considered *eco-friendly* (CHRAPUSTA et al., 2017; SHOW et al., 2017; MCINTOSH et al., 2018).

In search of their survival, many organisms, such as microalgae, develop strategies and mechanisms to defend themselves against the challenges of the environment. The production of secondary metabolites by various organisms plays a fundamental role in their survival, acting as defense in response to environmental challenges (DELGODA; MURRAY, 2017). The environmental challenges faced by organisms include predation, competition with other plants and organisms for resources, forms of occupation, establishment and propagation in the environment, and solar radiation. In the case of exposure to UV radiation, its effects can cause damages to the physiological and biochemical processes of these organisms, such as growth, survival, buoyancy, photosynthesis, pigmentation, CO_2 absorption, structural changes of proteins and DNA, among others (RASTOGI; INCHAROENSAKDI, 2013; KUMAR et al., 2016; DELGODA; MURRAY, 2017; BACOVA et al., 2019).

Chlorella vulgaris, a species of green microalgae, is a eukaryotic microorganism with high photosynthetic ability. *C. vulgaris* is found in different environments, predominantly in fresh and salt water, where there is exposure to high levels of UV radiation and extreme survival conditions. In response to these challenges, *C. vulgaris* produces several secondary metabolites that may have antioxidant properties (CHA et

al., 2010; PRIYADARSHANI; RATH, 2012; WANG et al., 2015; HYNSTOVA et al., 2017; JIANG; KALODIMOS, 2017; KHAN; SHIN; KIM, 2018).

Cultivation of *C. vulgaris* can be performed in different production systems, both in the open and in more sophisticated and specific systems. When the goal is to achieve high productivity, strains are grown in a system called closed-system photobioreactor, where it is possible to control cultivation conditions, such as temperature, light and pH value. This implies a high production scale, enabling the commercial production of a series of desired compounds and greater biomass yield, in addition to the advantage of its unicellular nature to ensure biomass with a unique composition (DERNER, 2006; DURAN; KUMAR; SANDHU, 2018).

Considering the field of Cosmetology, knowledge about ingredients derived from microalgae is still scarce and needs further investigations. However, through studies carried out so far, *C. vulgaris* showed potential to develop attributes, like preventing skin blemishes, repairing skin damage, treating seborrhea, conditioning of hair, collagen repair, inflammation inhibition, hydration and protection against damage caused by solar radiation, among other features (MOURELLE; GÓMEZ; LEGIDO, 2017; BHALAMURUGAN; VALERIE; MARK, 2018).

Due to these circumstances, the genus *Chlorella* has been gaining visibility in the scientific and commercially. According to Mourelle and coleagues (2017), the cultivation of microalgae is becoming one of the modern biotechnologies, in addition, the use of microalgae biomass is seen as ecologically correct mainly due to the possibility of using it in various fields of application, such as in foods, source of biodiesel, pharmaceuticals and cosmetics (SHOW et al., 2017; MCINTOSH et al., 2018; ARIEDE et al., 2017; KHAN; SHIN; KIM, 2018). Another advantage that can be highlighted is the possibility of directing the production of substances of interest, according to the intended objective, through different cultivation strategies (SHOW et al., 2017). Thus, microalgae extract can be a relevant type of constituent in products considered natural for sun protection (STAGES, 2010; LIU; CHEN, 2014; FONSECA, 2016; BHALAMURUGAN; VALERIE; MARK, 2018).

2. LITERATURE REVIEW

2.1 Microalgae

Microalgae are eukaryotic and photoautotrophic beings, consisting of a simple structure. They require water, CO_2, light and inorganic nutrients for growth and reproduction. Microalgae can be found in all ecosystems, including marine waters and, generally, they live in environments with limiting and stressful conditions, such as exposure to heat, cold, high salinity, osmotic pressure, different light sources, solar radiation (MOROCHO JÁCOME et al., 2012; SKJÅNES; REBOURS; LINDBLAD, 2013; MARKOU; VANDAMME; MUYLAERT, 2014; PANAHI et al., 2019) *etc*.

They are identified as photosynthetic microorganisms that convert CO_2 into energy and bioactive compounds, such as pigments, carbohydrates, proteins, polyunsaturated fatty acids and vitamins. Microalgae are, mostly, autotrophic, but they can also be heterotrophic or mixotrophic. Autotrophs are those that carry out photosynthesis as main metabolic process, using sunlight and CO_2 as a carbon source. Heterotrophics need an organic carbon source and do not need light, as this group does not carry out photosynthesis. Mixotrophs have both strategies (SHOW et al., 2017).

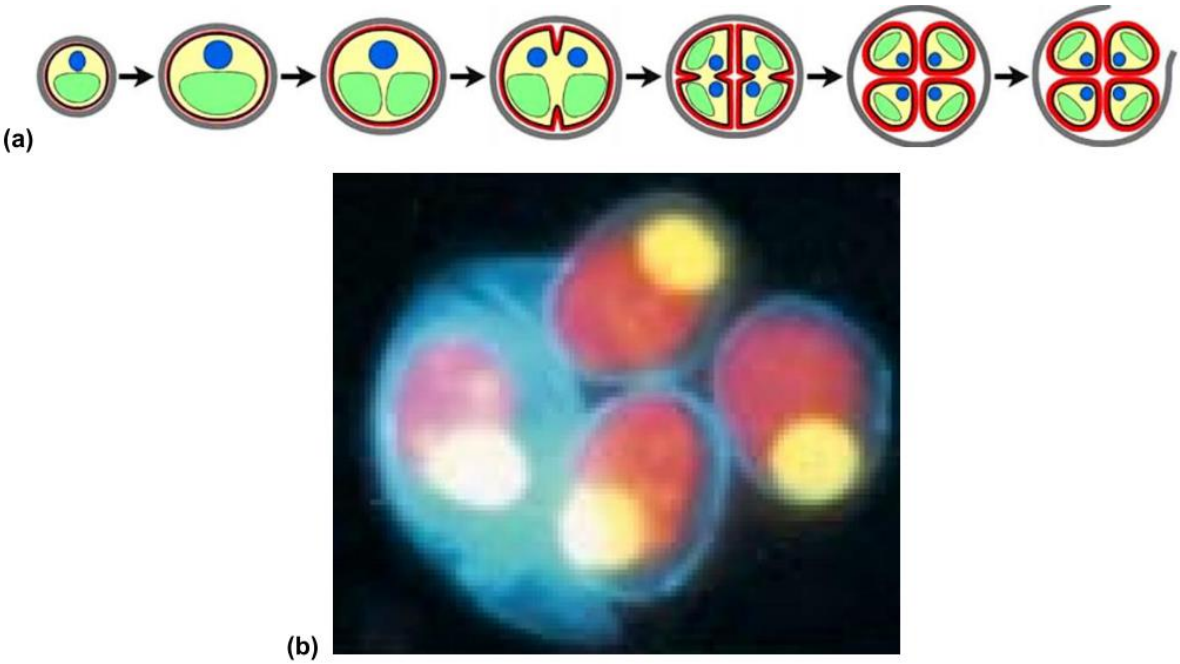
In relation to superior plants, microalgae have advantages for having fast growth, low consumption of water for production, easy harvesting, high CO_2 fixation, helping to reduce the concentration of greenhouse gases in the atmosphere, production of O_2, production of non-seasonal biomass and the possibility of being cultivated in waste or non-potable water (POHNDORF et al., 2016; MARINO, 2018; CEZARE-GOMES et al., 2019). However, it is essential to control the cultivation conditions to obtain uniform biomass composition (ARIEDE et al., 2017). Therefore, there is wide commercial interest for photosynthetic microorganisms in several areas, like for the development of nutraceutical, pharmaceutical and cosmetic products, as they are able to convert CO_2 into biomass that is a source of natural bioactive compounds and they present factors that aid to the balance of Earth's ecosystem, finally, contributing with the demand for eco-friendly, renewable and sustainable ingredients and products (ARIEDE et al., 2017; SHOW et al., 2017; CEZARE-GOMES et al., 2019).

2.1.1 *Chlorella vulgaris*

The taxonomic classification of *C. vulgaris* (C. VAN DEN HOEK, D.G. MANN, 1995) is Phylum Chlorophyta > Class *Trebouxiophyceae* > Order *Chlorellades* > Family of *Chlorellaceae* > Genus *Chlorella* > Specie *C. vulgaris*.

C. vulgaris is a microalgae that has a spherical shape sizing from 2-10 µm in diameter; there is no flagellum, being an immobile cell; its cytoplasm is composed of water, proteins and minerals and only one chloroplast that has photosynthetic pigments, such as chlorophyll a and b in its interior; it has a greenish color and its reproduction occurs by binary partition through asexual spores that grow quickly, originating an average of one cell every 24 hours, when under optimal cultivation conditions (Figure 1 a and b) (RAVEN; EVERT; EICHHORN, 2007; ARAÚJO, 2015b; MARINO, 2018). Its wall is rigid due to the presence of glucosamine in its basic composition, however, its thickness and composition vary according to the growth phase (SAFI et al., 2014; MARINO, 2018).

Figure 1: (a) Cell growth stages, chloroplast division, protoplast division, daughter cell maturation stage and cell hatch stage. (b) Cell hatching and daughter cells leaving the interior of the mother cell.



Source: SAFI et al., 2014

C. vulgaris, as well as other species of microalgae, have unique culture characteristics, showing adequate growth in nutrient-rich medium. However, studies are needed to be carried out to understand better its metabolism, so the necessary adjustments would be adopted regarding the cultivation conditions to achieve high productivity of biomass and biocompounds of interest (ARAÚJO, 2015b).

2.2 Microalgae Composition

The composition of microalgae can vary according to the cultivation conditions to which they were subjected. However, basically, the microalgae biomass is composed, in greater amounts, of proteins, carbohydrates and, in smaller amounts, of vitamins and antioxidants (PANAHI et al., 2019).

Proteins are essential for the composition of microalgae and are involved in various physiological processes of the cell, such as growth, repair and maintenance, and also act as chemical messengers, regulators of cellular activities and even in defense against invaders (SAFI et al., 2014). The amino acids found in the species of *C. vulgaris* in greater amounts are alanine and glutamic acid (URSU et al., 2014).

Lipids are a group of compounds defined as being insoluble in water, which mainly include glycolipids, waxes, hydrocarbons, phospholipids and fatty acids. These compounds can be modulated according to the amount of nitrate and light intensity available during their cultivation (LIAO et al., 2017). They are synthesized by chloroplasts and are also found in cell walls and in some organelles, such as mitochondria (SAFI et al., 2014; MARINO, 2018). **Table 1** shows the fatty acid profile accumulated by *C. vulgaris* cultivated under different light intensities.

Table 1: Accumulation of fatty acids in the microalgae *C. vulgaris* at different degrees of light intensity.

Fatty acid composition	Luminous intensity (μmol.m^{-2}.s^{-1})					
	160	**260**	**360**	**460**	**560**	**660**
C16:0	30.77	23.25	27.22	27.89	26.19	31.03
C16:1	5.36	5.67	2.22	2.89	2.28	2.15
C16:2	0.79	0.53	0.51	0.57	0.28	0.33
C16:3	4.20	4.81	4.59	4.09	4.45	4.08
C18:0	4.06	5.96	1.56	1.62	0.13	0.34
C18:1	21.34	24.80	35.88	35.37	39.00	35.87
C18:2	23.03	22.00	16.03	15.55	16.75	15.30
C18:3	6.64	8.08	8.12	7.45	8.16	8.33
Saturated fatty acid	34.83	29.21	28.78	29.51	26.32	31.37
Monounsaturated fatty acid	26.70	30.47	38.10	38.26	41.28	38.02
Polyunsaturated fatty acid	34.66	35.42	29.25	27.66	29.64	28.04

Source: LIAO et al., 2017

Carbohydrates represent a group of reduced sugars and polysaccharides, such as starch and cellulose. Starch is the most abundant polysaccharide in the microalgae *C. vulgaris*, located in the chloroplast and which, in addition to sugars, act as a store of energy for the cell. Cellulose is located in the cell wall and is a highly resistant structural polysaccharide that works as a protective barrier. The amount of carbohydrates in *C. vulgaris* species can vary from 12-55% of its total composition, depending on the growing conditions (LORDAN; ROSS; STANTON, 2011; SAFI et al., 2014).

The most abundant pigments in *C. vulgaris* are chlorophylls and carotenoids. Chlorophylls are located in the chloroplast thylakoids in about 1-2% of the dry biomass of the microalgae, and carotenoids, such as β-carotene, perform some vital cell functions, such as capturing light and transferring it to the photosystem, which are complex proteins involved in cellular photosynthesis, and even protecting chlorophyll molecules from degradation during exposure to intense solar radiation (SOLOMON; BERG; MARTIN, 2011; SAFI et al., 2014; FERNÁNDEZ LINARES et al., 2017; CEZARE GOMES et al., 2019).

2.3 Types of Cultivation

The cultivation of microalgae can occur in open or closed systems according to the intended cultivation objectives and both have advantages and disadvantages. When cultivation is carried out in an open system, the main advantage is the lower cost of the process, however, there is no contamination control. In closed system cultivation, on the other hand, there is the advantage of less contamination and the possibility of controlling the conditions, favoring greater biomass production, however, the cost of this process is higher due to the costs of the needed infrastructure (BOROWITZKA, 1999; UGWU; AOYAGI; UCHIYAMA, 2008; BRENNAN; OWENDE, 2010; SINGH; SHARMA, 2012; CARVALHO et al., 2014; DURAN; KUMAR; SANDHU, 2018).

2.3.1 Closed Cultivation System

The closed cultivation system was developed with the intention of reducing the influences and limitations that affect the open system cultivation, promoting the opportunity to control the conditions of the environment and obtain high cell concentration, as well as the substances of interest produced by the microalgae that are widely used in production of pharmaceuticals, nutraceuticals and cosmetics (SAFI et al., 2014; CEZARE-GOMES et al., 2019). The low risk of contamination in these systems is one of the advantages that allows the commercialization of microalgae biomass, called "clean" algae culture, being a type of crop suitable for species more sensitive to competition with other algae and growing conditions (SAFI et al., 2014). With this, several models of closed system were created, such as the flat plate, tubular and column photobioreactors (SHOW et al., 2017; CEZARE-GOMES et al., 2019). Furthermore, regarding the *C. vulgaris*, a comparative study carried out by Lam (2012) showed its most efficient growth when cultivated in a closed system.

2.3.2 Tubular Photobioreactor

Among the photobioreactor models, the tubular one presents itself as the most suitable for large-scale biomass production, as it enables greater productivity, permitting its commercialization (CEZARE-GOMES et al., 2019). The tubular photobioreactor can be built with plastic or glass tubes with a maximum diameter to ensure greater light penetration and ensure its absorption by the cells. The length must

also be optimized and the homogenization of the medium is usually performed by air pumps, promoting agitation and preventing cell sedimentation (SHOW et al., 2017; MARINO, 2018).

2.4 Factors Related to Cultivation

The microalgae cultures of the *Chlorella* genus need for their cell growth, basically, macronutrients, micronutrients and some factors, such as light intensity, pH and CO_2 that are regulated and optimized according to the selected species (LIU; CHEN, 2014; ARAÚJO, 2015b; SHOW et al., 2017).

Among the main macronutrients needed for the cultivation of microalgae of the *Chlorella* genus are carbon, nitrogen and phosphorus. (LIU; CHEN, 2014). Carbon constitutes about 40-50% of the total content of cells, in addition to being responsible for the basis of their central metabolism, with carbon dioxide being the main source of carbon for *Chlorella*. Right after carbon, we have nitrogen as the second main macronutrient which represents about 1-10% of the total content of cells, being the main constituent of amino acids and nucleic acids and can be used by the *Chlorella* genus in the form of nitrate, ammonium or urea. Micronutrients include elements such as Fe, Mg, Na, K, Ca, Cl and B, Cu, Mn, Zn, V, Co and Se that ensure cell function, acting as cofactors, while others act in biosynthesis processes. Vitamins are also added to culture (LIU; CHEN, 2014; SHOW et al., 2017).

In **Table 2** are some of the essential components and their functions for the cultivation of photosynthetic microorganisms (REBOLLOSO-FUENTES et al., 2001; LIU; CHEN, 2014; ARAÚJO, 2015b).

Table 2: Nutrients, sources, functions and concentrations in culture medium for photosynthetic microorganisms, including microalgae of the genus *Chlorella*.

Nutrient source	Components	Function
Nitrogen	NO_3^-, N_2, urea *etc*.	Supply of nitrogen to the cell, regulation of cell metabolism, composition of chemical structure of molecules, etc
Carbon	CO_2, HCO_3^-, CO_3^{2-} *etc*.	Supplying carbon to the entire cell, etc
Phosphor	Phosphate, hydrophosphate *etc*.	Supply of phosphate for cell reactions, etc
Vitamins	B, C, E *etc*.	Assistance in cell division, etc
Inorganic salts	Na, K, Mg *etc*.	Assistance in cell structural maintenance, cell activity, etc

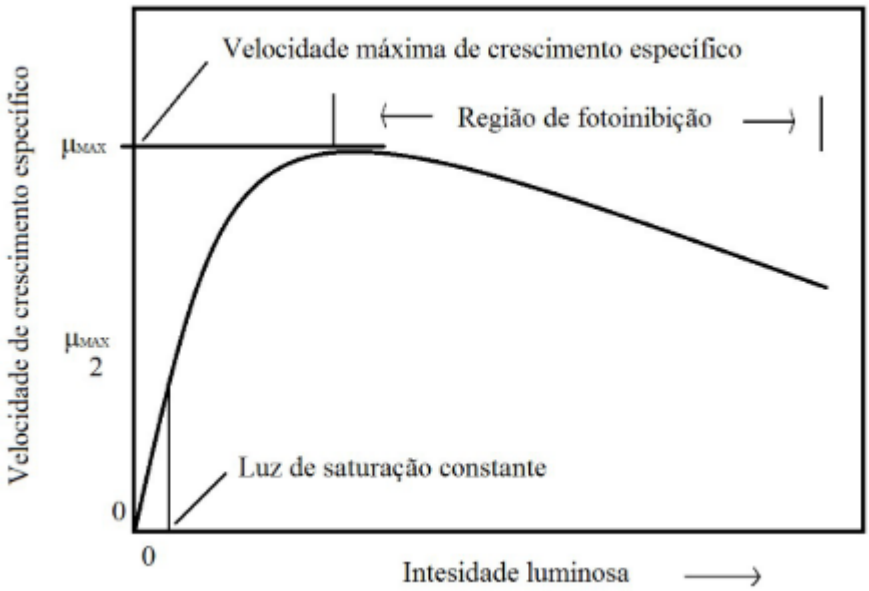
Source: REBOLLOSO-FUENTES et al., 2001; LIU; CHEN, 2014; ARAÚJO, 2015b

2.4.1 Light Intensity

The light intensity and photoperiod directly influence the growth rate of microalgae and their composition, being an important factor in the cultivation of photosynthetic organisms (ANDRADE, 2014; AHLUWALIA et al., 2016). Light can affect cell growth and metabolism under three possible conditions: light limitation, saturation and inhibition (SHOW et al., 2017). According to Andrade (2013) and Baiee (2016), the limitation of light in cultivation causes an increase in protein production, while the adequate availability of light, according to the need of each microalgae, can cause a greater accumulation of polysaccharides, carbon fixation for energy reserve, greater growth and cell concentration (HAMEDI; MAHDAVI; GHESHLAGHI, 2016). The light saturation constant is the luminous intensity that corresponds to half of the maximum specific growth rate. The increase in light intensity from a certain value reduces the cellular photosynthetic capacity, a phenomenon called photoinhibition, inducing the production of reactive oxygen species, causing irreversible damage to cellular structures (CHISTI, 2007; ANDRADE, 2014). In the **Figure 2**, it is showed the behavior of cell growth and its specific growth rate as a function of light intensity. In Figure 3, the same phenomenon of photoinhibition is demonstrated from a study that

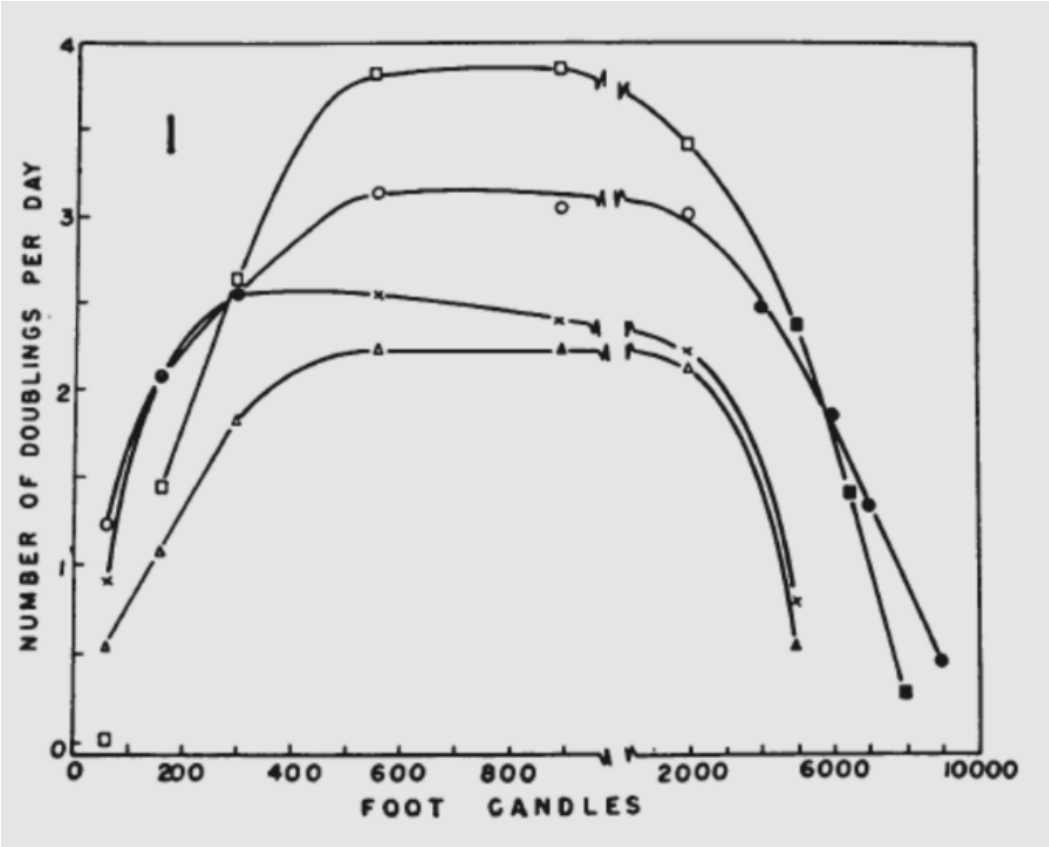
used several species of algae, including *C. vulgaris* (author used “x” to identify it in the graph). However, it is worth noting that this figure only represents the point of photoinhibition of *C. vulgaris* under specific conditions in which the study in question was carried out (SOROKIN, 1958). A drop in cell growth of *C. vulgaris* can be noted when light intensity reached values from 2000 ft-cd, which corresponded to 259.2 $\mu mol.m^{-2}.s^{-1}$. **Table 3** shows the light intensities and photoperiod used in *C. vulgaris* cultivations:

Figure 2: Growth curve and photoinhibition effect as a function of light intensity.



Source: Adapted from CHISTI, 2007

Figure 3: Photoinhibition phenomenon in different algae species.



Legend ○: *Chlorellapyrenoidosa* (van Niel); X: *Chlorella vulgaris*; Δ: *Scenedesmus obliquus*; □: *Chlamidomonas reinharditi.* Open symbols show growth under fluorescent light and closed symbols under incandescent light. **Source:** SOROKIN, 1958

Table 3: Light intensity and photoperiods used in *Chlorella vulgaris* microalgae cultivations.

Light intensity ($\mu mol.m^{-2}.s^{-1}$)	Photoperiod	Reference
60-70	Continuous	ADAMAKIS et al., 2018
60		LV et al., 2010
50		MARINO, 2018
72		MONTOYA et al., 2014

Source: AUTHOR

2.4.2 pH

The pH value is related to the concentration of H+ ions and the solubility of CO_2 and minerals present in the medium that can direct or indirectly affect the cellular

metabolism of microalgae. In photoautotrophic cultivation, change in pH medium is directly related to the CO_2 that acidifies it, so an acidic medium has greater availability of CO_2 (AHLUWALIA et al., 2016). However, due to the low solubility of CO_2 in aqueous media, CO_2 supply must occur throughout the entire cultivation (KLASSON et al., 1991). During the cultivation in response to the biological activities of the cells, the pH value tends to increase, which can be considered as an indicator of microalgal growth, being natural the growth of microalgae in an alkaline medium (ANDRADE, 2014). However, in the specialized literature, *C. vulgaris* precipitated when the pH value of the culture was above 9.5, which consequently caused a decrease in biomass production. The ideal pH range can vary among microalgae species. For the *C. vulgaris*, the pH interval could be between 7-8, when the specific growth rate can reach 2.03 d^{-1}. The pH control can be performed by injecting CO_2 into the medium or buffering agents (AHLUWALIA et al., 2016)

2.4.3 Temperature

In microalgae growth, temperature is also an important factor. Raising the temperature can increase biomass production and even double the antioxidant activity, as shown by Miranda and Sato (2001) using the *C. vulgaris* specie. However, the temperature limit varies according to each species, which can cause growth inhibition by inactivation and heat shock in cell proteins (ANDRADE, 2014). For *C. vulgaris,* Chinnasamy and coleagues (2009) verified that the optimal temperature for its high growth was around 30 ºC. On the other hand, Serra-Maia and coleagues (2016) noticed that the highest specific growth velocity occurred at 25 ºC. Bashan and coleagues (2015) working with *C. vulgaris* UTEX 2714, verified that the temperature of greatest cell growth was 27 ºC, with a decrease in growth at a temperature of 35 ºC. Considering this information, the condition for the cultivation of the genus *Chlorella* at temperature values of 27 ± 2°C would be adequate. However, this value can vary for each species (Table 4), with tolerance for growth of some strains at temperatures of up to 37ºC.

Table 4: *Chlorella* production, yield and cultivation temperature for closed systems.

Microalgae	Temperature (ºC)	Maximum cell concentration ($g.L^{-1}$)	References
C. zofingiensis	25	10.63	SUN et al., 2008
C. pyrenoidosa	25	6.80	KOTHARI et al., 2012
C. vulgaris	30	4.83	MA et al., 2014
C. sorokiniana	37	11.20	ZHENG et al.,2013

Source: Adapted from AHLUWALIA et al., 2016

The temperature can also vary according to the light intensity received, which can influence other parameters, such as the CO_2 solubility and salts present in the culture medium (**Table 5**).

Table 5: Solubility of salts ($g.Kg^{-1}$) influenced by temperature.

Temperature (°C)	NaCl	KCl	$CaCl_2$	Na_2SO_4	$CaSO_4$	$MgCl_2$	$MgSO_4$
20°	264.4	255	745	160	2	351	262
30°	265.5	272	501	291	2.09	357	290

Source: SCHOELLER, 1962; FREITAS, 2016

2.4.4 Nitrogen

Nitrogen is one of the most important macronutrients in the culture medium, as it is part of the chemical structure of various molecules, such as proteins, photosynthetic pigments, amino acids, vitamins and nucleic acids (ACIOLI, 2014; ARAÚJO, 2015b). The main sources of nitrogen added to cultivations are inorganic, such as nitrate, nitrite, ammonium, as well as other organic nitrogen compounds, such as urea (LÚCIO, 2013; ARAÚJO, 2015b). Nitrogen sources in microalgae cultures, including those of the genus *Chlorella*, are capable of influencing the biomass composition and growth of microalgae when in greater or lesser concentration. According to the literature, the high concentration of nitrogen can induce, in the species *C. vulgaris*, the increase of proteins and chlorophyll in the cells, rising their photosynthetic capacity, however there will be a decrease in the concentration of

carbohydrates (LOURENÇO, 2006; VENCKUS; KOSTKEVIČIENĖ; BENDIKIENĖ, 2017; MARINO, 2018). When at low concentrations, it can induce lipid accumulation in up to 70% of dry biomass and carbohydrate accumulation, however, it inhibits cell division, causing a decrease in protein levels, biomass productivity, photosynthetic capacity of cells, number of photosynthetic pigments and also chlorosis (LIU; CHEN, 2014; LI et al., 2016; VENCKUS; KOSTKEVIČIENĖ; BENDIKIENĖ, 2017; ADAMAKIS et al., 2018). According to Li and colleagues (2016), nitrogen limitation affects photosynthetic capacity, which in turn compromises lipid yield, and it is essential to maintain a balance between these factors so that an optimal yield is achieved.

2.5 Application of Microalgae in Cosmetology

In Cosmetology, microalgae have been used as raw material in product development due to their ability to produce biologically bioactive compounds of interest to this industry. Generally, they are used as antioxidants, anti-inflammatory, photoprotectors, moisturizers, thickening agents, actives against abrasions, tanning products, hair care products, anti-aging, anti-wrinkle, skin and whitening products, among others (MOURELLE; GÓMEZ; LEGIDO, 2017; JOSHI; KUMARI; UPASANI, 2018).

Among the different species of photosynthetic microorganisms, *Spirulina* and *Chlorella* have metabolites and pigments that perform various cosmetic functions. Amino acids and proteins have moisturizing capacity and are generally used in skin and hair care products. Other biocompounds, such as lipids, phycobiliproteins, terpenoids and pigments, contain anti-inflammatory and antioxidant activities and can act as stabilizers (MOURELLE; GÓMEZ; LEGIDO, 2017; JOSHI; KUMARI; UPASANI, 2018). In **Table 6**, it is presented biocompounds derived from microalgae and some of their functions.

Table 6: Different species of microalgae, biocompounds, uses and activities in cosmetics.

Microalgae	Biocompounds	Uses and activities in cosmetics	References
Chlorella sp.	Polysaccharides	Moisturizing and thickening action	MOURELLE; GÓMEZ; LEGIDO, 2017 JOSHI; KUMARI; UPASANI, 2018
Chlorella sp.	Chlorophyll	To mask odors in toothpastes and deodorants, pigment, anti-carcinogenic property	MOURELLE; GÓMEZ; LEGIDO, 2017
Chlorella vulgaris	Extract of *Chlorella vulgaris*	Collagen repair, tissue regeneration and antiaging	SKJÅNES; REBOURS; LINDBLAD, 2013 KOLLER; MUHR; BRAUNEGG, 2014 WANG et al., 2015 FONSECA, 2016 ARIEDE et al., 2017
Chlorella vulgaris	Chlorella	Antimicrobial action	FONSECA, 2016
Chlorella vulgaris	Astaxanthin	Suppresses skin hyperpigmentation, inhibits melanin production	SKJÅNES; REBOURS; LINDBLAD, 2013 WANG et al., 2015
Chlorella sp.	Lutein	Photoprotective and antioxidant action	CHA et al., 2010; WANG et al., 2015 LI et al., 2016 HYNSTOVA et al., 2017 MUSZYNSKA et al, 2018
Chlorella vulgaris	Chlorophyll a, chlorophyll b and β-carotene	Anti-aging, moisturizing, thickening and depigmenting action	CHA et al., 2010 LI et al., 2016 HYNSTOVA et al., 2017 JOSHI; KUMARI; UPASANI, 2018
Chlorella vulgaris	Vitamins	Toning the skin, curing dark circles, hair growth and dandruff treatment	JOSHI; KUMARI; UPASANI, 2018
Chlorella vulgaris	α-carotene	Antioxidant and anti carcinogenic action	HYNSTOVA et al., 2017
Chlorella vulgaris	Palmitic acid, Palmitoleic acid	Anti-aging, depigmentation, moisturizing and thickening agent	JOSHI; KUMARI; UPASANI, 2018

Chlorella sp.	β-1,3 glucane	Anti-inflammatory action, immune system booster and free radical scavenger	MOURELLE; GÓMEZ; LEGIDO, 2017
Chlorella vulgaris	Complex B vitamins	Help maintain skin and hair health	SAFI et al., 2014
Chlorella vulgaris	Xanthophylls	Antioxidant action, help to stabilize the membrane and protect cells from intense radiation	PANAHI et al., 2019

Source: AUTHOR

Microalgae extracts have a wide variety of biocompounds recognized for adding value to cosmetic products. Therefore, studies aim to optimize its production and cultivation so that, consequently, there will the optimization of biomass in the production of the compounds of interest. These studies are relevant for the cosmetic industry, since the composition of biomass directly influences the effect of the final product, in addition to representing a promising and profitable source of raw materials for industries, as a new biotechnology to be included in the market (WANG et al., 2015; ARIEDE et al., 2017; MOURELLE; GÓMEZ; LEGIDO, 2017).

2.5.1 Sunscreens

Ultraviolet (UV) filters available on the market are divided into chemical (oxybenzone, octyl methoxycinnamate, aminobenzoic acid, avobenzone *etc*.) and physical (titanium dioxide, zinc oxide). Sunscreens are widely used to protect against damage caused by exposure to UV radiation, however, they can cause skin irritation, photosensitivity and contact dermatitis. Thus, there is a need to search for new compounds that can be as effective as the protectors already used, being from natural, sustainable sources and causing fewer adverse events with chronic use (AVADHANI et al., 2017).

Among the compounds and substances available on the market for formulating cosmetics, there are biocompounds produced by microalgae. Some are capable of synthesizing substances that absorb UV radiation and promote other benefits to the skin. Some species of microalgae that have potential as a raw material for sunscreens are *Chlorella* spp. which already have visibility for presenting benefits such as anti-aging, antioxidant, moisturizing action, ability to prevent the formation of wrinkles and blemishes on the skin, among others (ARIEDE et al., 2017).

3. OBJECTIVES

The main objective of this research was to evaluate the employability of *C. vulgaris* as a photoprotective ingredient.

3.1 Specific Objectives

- Cultivation of the microalgae in a photobioreactor and evaluation of its growing in different cultivation conditions.
- Obtain biomass and prepare the *Chlorella vulgaris* microalgae extract.
- Characterize the biomass and the extract.
- Develop a photoprotective prototype formulation followed by its functional in vitro characterization.

4. EXPERIMENTAL

4.1 Microalgae

Strains of *C. vulgaris* specie were used in the cultures, being identified as UTEX 2714 and originating from the wastewater treatment pond in Santa Fé de Bogotá, Colombia. These were kept in 500 mL *Erlenmeyer's* on a rotary shaker at 100 rpm, temperature of 25 ± 2°C (VONSHAK, 1997) and luminous intensity of 6 klux (72 µmol fótons m^{-2} s^{-1}) to obtain the inoculum (CARVALHO et al., 2004).

4.2 Culture Medium

Culture medium used for cell growth was the basal Bold (**Table 7**) (SILVA, 2020). It was also used with higher $NaNO_3$ concentrations, with values 3 (Bold 3N medium) and 6 times higher (Bold 6N medium). Culture media were autoclaved at 121 ºC for 30 minutes before use.

Table 7: Composition of Bold basal medium (1N) per liter of distilled water.

Components	Concentration (g L^{-1})	Quantity (mL) of component solution per liter of prepared medium
$NaNO_3$	8.33	30
$CaCl_2.2H_2O$	2.50	10
$MgSO_4.7H_2O$	7.50	10
K_2HPO_4	7.50	10
KH_2PO_4	17.50	10
NaCl	2.50	10
Metal solution PIV *	-	6
Vitamin solution **	-	1

* Solution consisting of (in 1 L of distilled water): Na_2EDTA 750 mg; $FeCl_3.6H_2O$ 97 mg; $MnCl_2.4H_2O$ 41 mg; $ZnCl_2$ 5 mg; $CoCl_2.6H_2O$ 2 mg; $Na_2MoO_4.2H_2O$ 4 mg.

** Vitamin solution (in 100 mL of distilled water): cyanocobalamin 13.5 mg; Biotin 110 mg; Thiamine 2.5 mg.

4.3 Evaluation of Cell Growth and Concentration

For the calibration curves of each strain, a concentrated suspension sample was collected. Ten different dilutions of the suspension were made and these were

analyzed in a spectrophotometer (FEMTO 600 PLµS versão 2.4) in the wavelength of 720 nm (DO_{720}) with 1 cm optical path cuvette (LI et al., 2018). Filtration membranes with porosity of 0.45 µm Millipore™ were placed in a Petri dish, dried in an oven at 60 ºC for 1 hour, left for another 1 hour in a desiccator for cooling and then weighed. Twenty mL of a concentrated suspension were vacuum filtered on one of the membranes of 0.45 µm previously dried and weighed. After filtering, the membrane containing the cells was placed in a Petri dish, left in an oven at 60 ºC for 24 hours to remove all humidity, left for another hour in a desiccator for cooling, and then weighed. Cell mass was obtained by the difference in membrane weight with and without cells (PEREIRA, 2013).

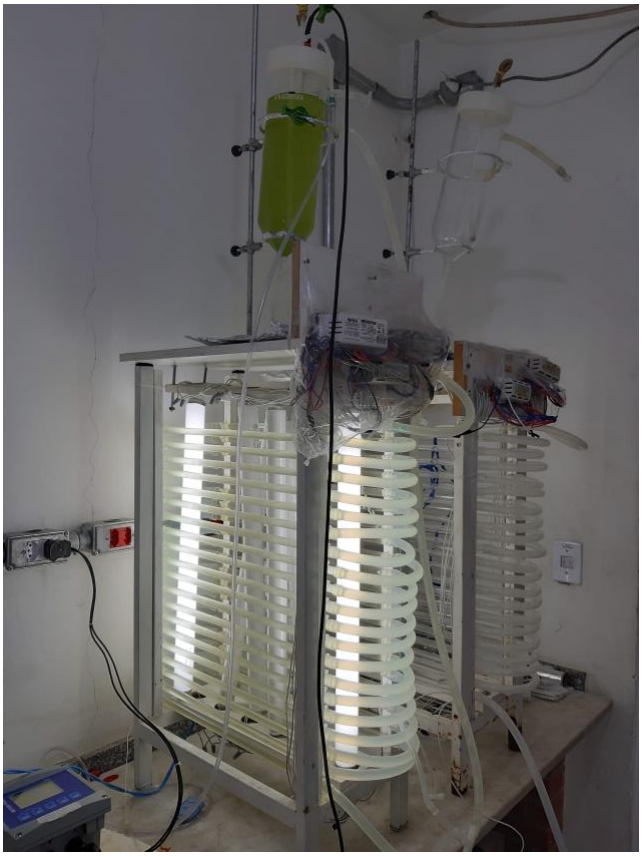
4.4 Inoculum

An aliquot of 350 mL (equivalent to 10% of the photobioreactor volume) of a cell suspension (cell fraction and culture medium) of each strain was kept in the conditions described in the item **4.1** (VONSHAK, 1997; CARVALHO, et al., 2004) until reached the cellular concentration to obtain the inoculum, which was 0,1 $g.L^{-1}$ (LI et al., 2018).

4.5 Cultivation Conditions

The strains were cultivated in a tubular air-lift photobioreactor, developed at the Microalgal Biotechnology Laboratory of the Department of Biochemical-Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of São Paulo (**Figure 4**), consisting of transparent tubes with diameter internal volume of 1.0 cm and total system volume of 3.5 L. The illuminated volume corresponded to 2.0 L and culture flow of 40±1 $L.h^{-1}$ (JÁCOME, 2014). The photobioreactors underwent chemical asepsis before the beginning of the experiments with sodium hypochlorite with active chlorine content between 2.0-2.5% w/w, sodium thiosulfate at a concentration of 12.5 g/L to neutralize the action of chlorine, and deionized water (ANDERSEN, 2005; AHLUWALIA et al., 2016).

Figure 4: Photograph of the photobioreactor (3.5L).



Source: AUTHOR

The temperature was maintained at 27 ± 2 °C (BASHAN et al., 2016) and the pH value of the medium was monitored and kept at 7.5 ± 0.3 (MONTOYA, et al., 2014). The light intensity was maintained between 60 and 70 µmol fótons.m^{-2}. s^{-1} (ADAMAKIS et al., 2018).

The UTEX 2714 strain of the microalgae *C. vulgaris* was cultivated under three different concentrations of sodium nitrate: 1N (2.94 mM), 3N (8.82 mM) and 6N (17.64 mM). The medium used in the cultures was the Bold basal medium, varying in the concentrations of sodium nitrate, proposed as the main source of nitrogen for the culture.

4.6 Biomass Productivity and Specific Growth Rate

Biomass productivity (mg.L^{-1}.d^{-1}) was obtained by the dried biomass concentration (mg.L^{-1}) divided by the specified time range (days) based on the **Equation 1** (TOLEDO-CERVANTES; MORALES; NOVELO, 2013; SANTOS, 2013).

$$P = \frac{Xm - X_i}{Tc}$$

Equation 1: Biomass productivity P ($mg.L^{-1}.d^{-1}$).

Legend:

Xi: inoculum cell concentration;

X_m : maximum cell concentration;

Tc: time needed to obtain X_m.

Specific growth rate (d^{-1}) of *C. vulgaris* was calculated using the **Equation 2** (CLESCERI; GREENBERG, 1989; SANTOS, 2013).

$$\mu_{MÁX} = \frac{\ln\left(\frac{X}{X_0}\right)}{(t - t_0)}$$

Equation 2: Specific growth rate, $\mu_{MÁX}$ (d^{-1}).

Legend:

$\mu_{MÁX}$: slope of the lnX line as a function of time in its linear stretch;

X: final biomass concentration;

X_0: initial biomass concentration;

t: final time;

t_0: initial time.

4.7 Dry Biomass and Extract

At the end of cultures, the microalgal biomass was centrifuged and washed with deionized water to remove excess salts from the culture medium. To obtain the dry biomass, samples were dried with ventilation at 65 ºC for 12 hours (PELIZER et al., 1999), macerated and frozen for storage. To obtain the extract, samples of wet biomass of *C. vulgaris* strains were suspended and shaken with glass spheres under agitation at 1000 rpm for 55 minutes, with microscopic observation to verify cell lysis. After lysis, liquid fraction was separated from the glass spheres by filtration and centrifuged at 4500 rpm for 30 minutes, obtaining the extract, which was the supernatant (BUONO et al., 2012). The extract was lyophilized for better storage conditions. Dry biomass and extract were subjected to the following techniques.

4.9 Total Lipid Content

A mixture of apolar and polar solvent was used to determine the total lipid fraction (PELIZER et al., 1999). Approximately, 1.0 g of the samples was placed in a Soxhlet extractor and refluxed with chloroform and methanol in the proportion of 2:1 v/v until the extracting liquid became clear (PIORRECK, 1984; OLGUÍN et al., 2001). Then, the solvent mixture containing the total lipid fraction was recovered in a rota-evaporator. The lipid content of the samples was calculated by the ratio between the mass of the total lipid fraction and the mass of the samples used in the extraction process. The calculation of the percentage of total lipids was performed using **Equation 3** (PELIZER et al., 1999).

$$\text{Total lipids (\%)} = [(Mf - Mi)/ Ma] \cdot 100$$

Equation 3: Calculation of the percentage of total lipids.

Legend:

Mf: weight of the balloon with the sample;

Mi: empty balloon weight;

Ma: amount of sample used to perform the test.

4.10 Fatty Acid Composition

The determination was performed after converting the fatty acids into their corresponding methyl esters (HARTMAN; LAGO, 1973). Aliquots of 200-250 mg of the lipid fraction (item 5.10) were weighed and they were added with 3.0 mL of methanol solution of 0.5M KOH. The mixture was heated to reflux for 5 minutes. Afterwards, 9.0 mL of the esterification reagent (2 g NH_4Cl + 3 mL H_2SO_4 in 60 mL methanol) were added and the mixture was left to rest for 10 minutes until the reaction occurred. Then it was heated to reflux for another 5 minutes and transferred to a separatory funnel together with 15.0 mL of petroleum ether and 30.0 mL of deionized water. After stirring and separating the phases, the aqueous phase was discarded. In the organic phase, 15.0 mL of deionized water were added and after stirring and phase separation, the aqueous phase was discarded, being the procedure repeated. The organic phase was collected, the solvent was evaporated in a rotary evaporator in a water bath at 40 °C and the residues were removed under nitrogen flow. Finally, the analysis of fatty acid methyl esters was performed according to Rodrigues-Ract (2008), with adaptations, for later injection into the gas chromatograph, model 7890 CX (Agilent®, EUA). The

identification of fatty acid methyl esters was performed according to the comparison of the retention time of the sample constituents with individual standards

4.11 Total Protein Content

To determine the protein content of the biomass and the extract, the Kjeldhal method was used, using 6.25 as the nitrogen-to-protein conversion factor (AOAC, 1984). About 20 ± 0.5 mg of the defatted samples (resulted from the lipid determination) were sprayed, weighed and placed in the test tubes to which 2.0 g of the catalyst (1.9 g of potassium sulfate and 10.0 mg of copper sulfate) and 3.0 mL of sulfuric acid were added, and subjected to digestion at 350 ºC. In this process, the organic nitrogen in the sample was converted to ammonia, which was kept in the system in the form of ammonium sulfate. This sample, which went through the acid digestion process, was then alkalinized by adding 10.0 mL of 60% sodium hydroxide and heated in a still with steam drag, and the ammonia was received in a saturated solution of boric acid. This was displaced in titration in solution with 0.02N HCl, using as indicator the mixture of methyl red and methylene blue. The percentage of total protein was calculated with **Equation 4** (AOAC, 1984):

$$PT\ (\%) = [(V \cdot f \cdot mEq \cdot 6.25)/\ P(g)] \cdot 100$$

Equation 4: Calculation of the percentage of total protein.

Legend:

V: HCl spent volume;

f: HCl factor;

mEq: milliequivalent gram of nitrogen;

6.25: general conversion factor of nitrogen to protein;

P: sample weight in grams.

4.12 Photoprotective Formulation

Semi-solid formulations, oil-in-water (O/W) emulsions, were prepared, being this type of vehicle widely used as cosmetic and dermatological products (ECCLESTON, 1997). To evaluate the potential of the extract of *C. vulgaris* as an ingredient for sunscreens, it was associated at different concentrations (1%, 5% and 10% w/w) with a mixture of UV filters. Octyl *p*-methoxycinnamate and avobenzone

were used to achieve a sunscreen/photostability model (RUSCINC, 2018). The formulations are described in **Table 8**.

Table 8: Photoprotective formulations – qualitative and quantitative composition.

		Proportion % (w/w)			
Composition	**Function**	**F0**	**F1**	**F2**	**F3**
Crodafos™ CES	Emulsifier	4.0	4.0	4.0	4.0
Isopropyl Myristate	Emollient	5.0	5.0	5.0	5.0
Octyl *p*-methoxycinnamate	UVB filter	10.0	10.0	10.0	10.0
Avobenzone	UVA filter	5.0	5.0	5.0	5.0
Glycerin	Humectant	5.0	5.0	5.0	5.0
EUXYL® K 300	Preservative	0.75	0.75	0.75	0.75
Aristoflex® AVC	Emulsifier polymer	1.0	1.0	1.0	1.0
Extract of *C. vulgaris*	Active	-	1.0	5.0	10.0
Citric Acid	pH corretor	q.s.p.	q.s.p.	q.s.p.	q.s.p.
Triethanolamine	pH corretor	q.s.p.	q.s.p.	q.s.p.	q.s.p.
Purified Aqua	Vehicle	q.s.p.	q.s.p.	q.s.p.	q.s.p.

To handle the photoprotective formulations, the raw materials were separated into two phases: aqueous and oily. The UV filters and Crodafos™ CES were mixed with the isopropyl myristate, composing the oil phase. Aristoflex® AVC, a polymer with stabilizing properties used in a wide pH range, was mixed with glycerin and the preservative system in water at 60ºC, forming the aqueous phase. Then, the bioactive compound was incorporated into the purified water. The oil phase was heated to 55°C and then the samples were stored so that the oil phase reached a temperature of up to 35°C and the aqueous phase to 40°C, then the oil phase was added to the aqueous phase to obtain the emulsion. The pH value of the formulations was adjusted between 5.0-6.0 with triethanolamine or citric acid at room temperature (24.0 ± 2.0°C). The pH value of the samples was defined to obtain a pH close to the pH of the skin (4.6 – 5.8)

(LEONARDI; GASPAR; CAMPOS., 2002). The samples were stored in glass containers, at room temperature.

4.13 Physicochemical Characterization and Organoleptic Properties

The samples were characterized by pH value and organoleptic properties. The pH value was estimated with an universal pH strip (Sigma-Aldrich), by direct immersion into the samples, at room temperature (24 ± 2 °C). The pH estimations were performed in replicates of three (BABY, 2007). Appearance, color and odor (organoleptic properties) of the samples were evaluated macroscopically and subjectively (ANVISA, 2004).

4.14 *In vitro* Photoprotective Activity

The evaluation of the *in vitro* efficacy against UVB and broad-spectrum protection of the formulations was carried out using diffuse reflectance spectrophotometry with integration sphere (Labsphere® UV2000S *Ultraviolet Transmittance Analyzer*). Samples were weighed and applied as a uniform thin film at 0.75 mg/cm^2 (BABY, 2007; VELASCO et al., 2008; PERES et al., 2017) over the substrate (polymethylmethacrylate plate, PMMA; Heliplates®). After application, the plates were kept at rest, protected from light for 15 minutes and at room temperature (24 ± 1 °C). Subsequently, the plates were submitted to a spectrophotometric reading, using a PMMA plate treated with glycerin as a blank, in a spectral wavelength interval (λ) of 290-400 nm and at a progression rate of 1,0 nm. The following parameters were obtained by the equipment software UV2000®: *in vitro* sun protection factor (SPF) and critical wavelength (nm) (VELASCO et al., 2008). The experiment was performed in triplicate (SPRINGSTEEN et al., 1999; COSMETICS EUROPE, 2006; UNITED STATES, 2011).

4.15 Evaluation of Photostability

The evaluation of photostability in photoprotective formulations allows to obtain information about possible degradations that the UV filters can suffer when exposed to artificial UV radiation emitted by a controlled source of radiation (UNITED STATES, 2011; BRASIL, 2012). After reading the absorbances of the samples in the *in vitro* photoprotective activity assay, the same PMMA plates were irradiated for 2 h using a

UV irradiation simulator (CPS+, Atlas Suntest®) equipped with a xenon lamp and irradiance of 580 $W.m^{-2}$ (DIFFEY et al., 2000; COSMETICS EUROPE, 2006; UNITED STATES, 2011). After irradiation, the *in vitro* SPF and critical wavelength were obtained and compared with those of the pre-irradiation. The test was performed in triplicate (COSMETICS EUROPE, 2006).

4.16 Statistical Analysis

Data were treated using Minitab® program, version 19, with significance level equal to 5.0% ($p \leq 0.05$). In comparisons between the two variables, the results were treated according to the *t-Student* test. In comparisons among three or more variables, the One-Way ANOVA test was used, followed by the *Tukey* test.

5. RESULTS AND DISCUSSION

5.1 Cell Concentration

To determine the growth and cellular concentration, a calibration curve was constructed from a concentrated suspension of UTEX 2714 strain. The result is illustrated in **Figure 5**. The calibration curve was used to maintain the inoculum, through absorbance (DO_{720nm}) reading, until reaching the appropriate cell concentration fixed at 0.1 $g.L^{-1}$. After, this inoculum was prepared to initiate the microalgae cultivation (LI et al., 2018).

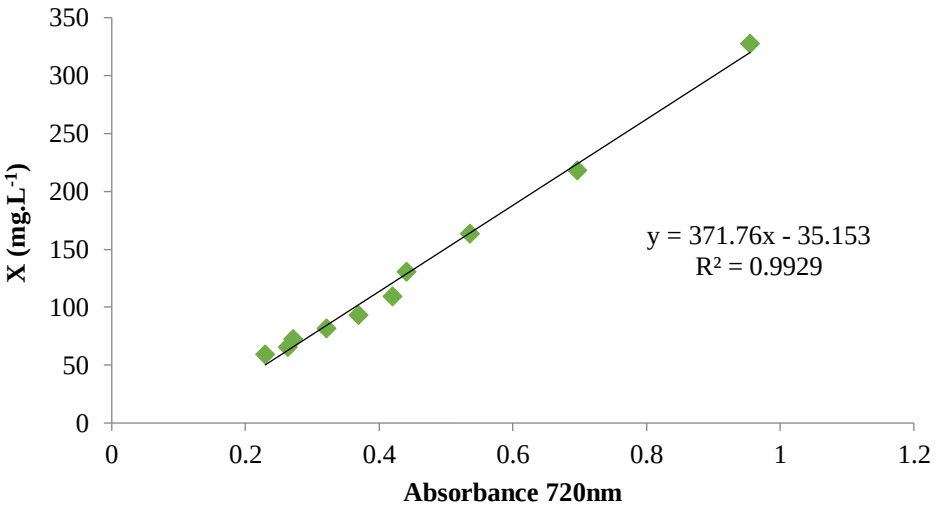


Figure 5: Calibration curve and cell concentration (x) of UTEX 2714 strain (absorbance at 720nm). In the equation, y is coefficient and x is the sample absorbance.

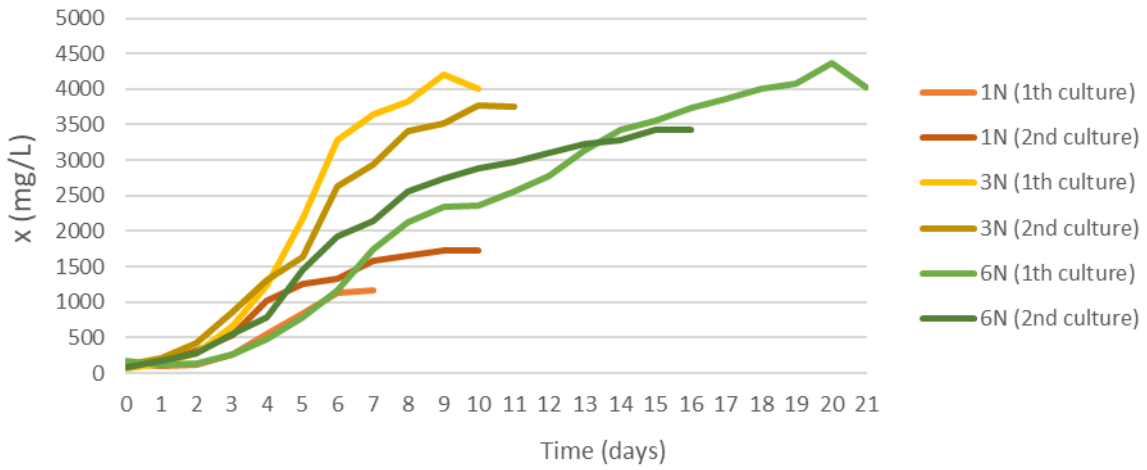
5.2 Cultivation

The cultivation of the strains started after the inoculum acquisition with duration between 6 and 19 days, varying according to the growth of each strain. The growth of strains was monitored and the cultivations were interrupted when stabilized, preventing cell death and a biochemical cell alteration (MARTINS; CAETANO; MATA, 2010). The biomass harvest was performed by centrifugation, an effective method in which 95% of biomass is recover without damaging the cell structure, besides being a cheap and fast process (SAFI et al., 2014).

5.3 Growth Curve

Daily absorbance reading (DO_{720nm}) was taken to follow the strain growth. It was used to monitor the growth phases indicating the best time to interrupt the cultivations without cells entering in the death phase. Microalgae growth is composed of five phases: adaptation or lag phase, exponential or log phase, end of exponential phase or phase of decreased cell growth, stationary phase and senescence phase or cellular death (MARTINS; CAETANO; MATA, 2010). Being, according to Santos (2013), the exponential phase the range of interest to establish the biomass production and its productivity. **Figure 6** shows the growth curve of the UTEX 2714 strain grown in Bold culture medium with sodium nitrate concentration of 2.94 mM (1N), 8.82mM (3N) and 17.64mM (6N).

Figure 6: Cell concentration (X) of *C. vulgaris* UTEX 2714 *versus* cultivation time in different sodium nitrate concentration.



The nitrate is the main source of nitrogen and an essential nutrient for microalgae. Nitrogen composes their cellular structure and it is part of physiological and biological processes (ACIOLI, 2014; ARAÚJO, 2015b). It is possible to affirm that under limiting condition of this nutrient, the microalgae growth will be proportional to the amount of nitrate available, especially when it comes to a discontinuous type of cultivation in which the cells consume the nutrients available in the medium, being are not replenished (LI et al., 2016; ADAMAKIS et al., 2018; MARINO, 2018).

In the 6N condition, the maximum cell concentration was 4366.48 mg L^{-1} in 19 days, while the maximum cell concentration of 3N was 4207.62 mg L^{-1}, reached in 8 days. From an industrial point of view, the 3N cultivation would be the best option regarding the yield and cultivation time. Also, the parameters about cell productivity and maximum specific growth rate were obtained. The values of cell productivity achieved by the UTEX 2714 strain in each nitrogen concentration are in **Table 10**.

Table 10: Cell productivity of UTEX 2714 strain for different nitrogen concentrations.

Nitrogen concentration	Cell productivity (mg L^{-1} d^{-1})
1N	1428.82
3N	3978.67
6N	3886.47

1N = 2.94 mM; 3N = 8.82 mM; 6N = 17.64 mM

As shown through the productivity results, a high cell density and biomass productivity could not be reached under nitrogen limitation whereas the content of chlorophyll, an important photosynthetic pigment, would be negatively affected under such conditions (GEIDER et al. 1993; HERRIG; FALKOWSKI 1989; ZHILA et al. 2005) due to the decreased biosynthesis of crucial chlorophyll proteins complexes (BEARDALL et al., 2005; IKARAN et al., 2015; ADAMAKIS et al., 2018). The results were statistically treated and there was a different between 1N and 3N/6N cultivations ($p<0,05$). There was no difference between 3N and 6N conditions.

The maximum specific growth rate ($\mu_{máx}$) represents the number of cell division per unit of time and it is constant during the exponential phase (ANDRADE, 2014). This parameter was calculated for the cultivations (**Table 11**). The results of maximum specific growth rate from cultivations 1N, 3N and 6N were compared and did not demonstrate statistical differences ($p<0,05$) when compared to each other.

Table 11: Maximum specific growth rate ($\mu_{máx}$) in differents nitrogen concentration.

Strain	Nitrogen concentration	$\mu_{máx}$ (d^{-1})
UTEX 2714	1N	0.67
	3N	0.71
	6N	0.61

1N = 2.94 mM; 3N = 8.82 mM; 6N = 17.64 mM

In our experiment condition, the nitrogen was able to limit the cell growth and cell productivity under low concentration, but it did not influence the maximum specific growth rate. The medium and high concentrations (3N and 6N) of nitrogen demonstrated similar behavior among the analyzed parameters, but with a difference in cultivation duration. The 3N cultivation was able to obtain the same results of 6N, but in the shortest cultivation period. For the preparation of the cosmetic formulation, we choose to use the extract from 3N cultivation, according to its performance in the last tests.

5.4 Biomass and Extract Composition

The biomass and extract of *Chlorella vulgaris* is fundamentally composed of proteins, carbohydrates and lipids. According to cultivation conditions, these composition proportions can vary.

5.4.1 Lipid

The values of lipid content accumulated by UTEX 2714 strain cultivated in different nitrogen concentrations and the 3N extract are presented in the **Table 12**.

Table 12: Lipid content of UTEX 2714 cultivated in different concentrations of nitrogen and 3N extract.

UTEX 2714 strain	Lipid content (% dry cell weight)
1N	30.86
3N	23.50
6N	24.15
3N Extract	43.17

1N = 2.94 mM; 3N = 8.82 mM; 6N = 17.64 mM

The amount of accumulated lipids on microalgae cells is directly linked and inversely proportional to the amount of nitrogen available in the cultivation, being part of a mechanism practiced by many microalgae to deal with nutrient deprivation (SKJÅNES; REBOURS; LINDBLAD, 2013). The microalgae, in contrast with plants, store their primary energy in lipid materials instead of saccharides, so when important nutrients, like nitrogen which is essential for cell structure, are limited, the microalgae stops to multiply and produce some proteins, carbohydrates and other structural components, affecting their growth (SKJÅNES; REBOURS; LINDBLAD, 2013; PANAHI et al., 2019). **Table 13** shows the lipid accumulation potential of *Chlorella vulgaris* in relation to nitrogen available during the cultivation.

Table 13: Lipid accumulation (% dry cell weigh) of *C. vulgaris*, according to specialized literature.

Condition	Lipid % (dry cell weigh)	Reference
N-replete N-deprivation	18 40	ILLMAN; SCRAGG; SHALES, 2000
N-replete N-deprivation	5-40 68	SAFIA et al., 2014
N-replete N-deprivation	16.5 51.8	SCARSELLA et al., 2009
N-replete N-deprivation	25 52	WIDJAJA; CHIEN; JU, 2009
N-replete N-deprivation	5.90 16.41	CONVERTI, 2009
N-replete N-deprivation	21.67 36.60	ADAMAKIS, 2018

Source: AUTHOR

The accumulation of lipids has a wide variation among the cultivations reported at the **Table 13**, however, our results were within the expected. They were corroborated with the above-mentioned authors since we observed that the stress caused by N-deprivation raised the lipid content. As shown in **Table 12**, the lipid content of 3N extract was higher than biomass at 3N cultivation condition. The process

of lipid extraction has a large influence in the range of recovery lipids from a biomass or extract. The difference in the process of lipid extraction between dry biomass and the extract was the breaking of the walls of the cells which, according to Dvoretsky and coleagues (2016), it is one of the variables that could influence in the recovering of the lipid rate. For the dry biomass lipid extraction, the biomass was macerated before the extraction and to obtain the extract, the biomass went through a mechanical process of breaking the cell wall, which may have helped to release lipids from the membranes and cytoplasm.

5.4.2 Fatty Acids

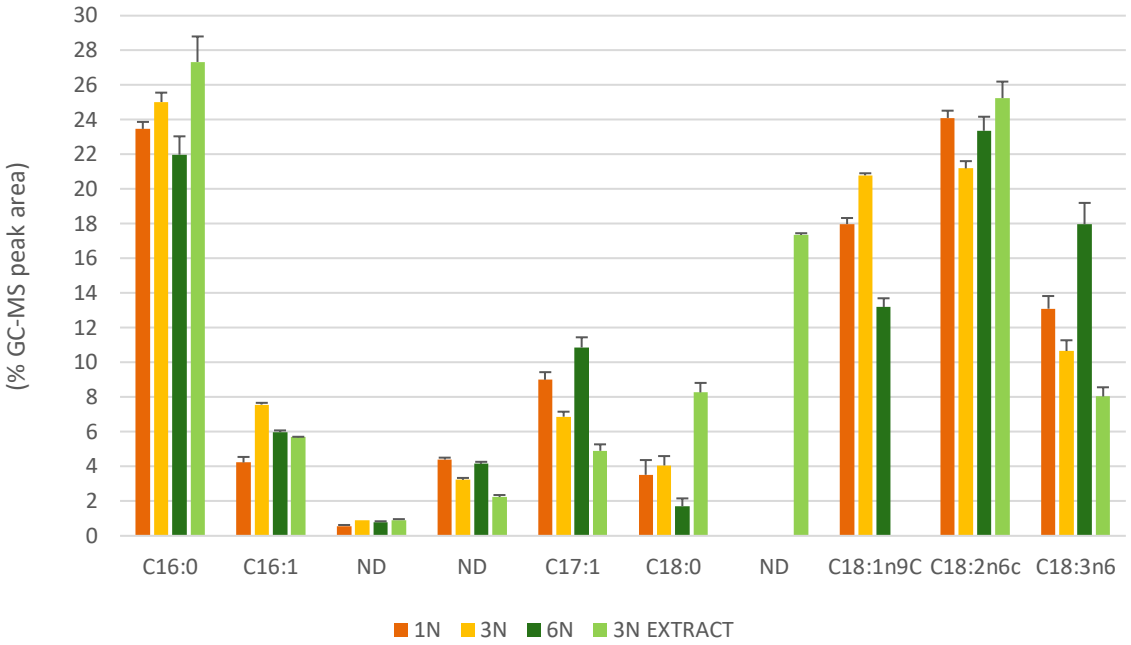
The total lipid content of a microalgae can reach 1 to 70% of its dry weight and can be divided, basically, in two groups: storage (nonpolar lipids, mainly triacyl glycerides) and structural lipids (polar lipids, like phospholipids and sterols) (SHARMA; SCHUHMANN, 2012; HAMED, 2016). The profile of fatty acids of the *C. vulgaris* cultivation under three different nitrogen concentrations and the 3N extract was determined by CG-MS, being the results **Table 14** and **Figure 7**.

Table 14. Fatty acid concentration (% GC-MS peak area) of the lipid/oil fraction of the *C. vulgaris* samples grown under different nitrogen levels (1N, 3N, 6N) and 3N extract.

Fatty acids	Classification	1N	3N	6N	3N extract
C16:0 (palmitic acid)	Saturated	23.47	25.02	21.98	27.33
C16:1 (palmitoleic acid)	Monounsaturated	4.23	7.57	5.98	5.69
ND	-	0.57	0.90	0.80	0.91
ND	-	4.40	3.23	4.15	2.24
C17:1 (cis-10-heptadecenoic acid)	Unsaturated	9.00	6.87	10.86	4.90
C18:0 (stearic acid)	Saturated	3.52	4.06	1.70	8.27
C18:1n9C (oleic acid)	Monounsaturated	17.98	20.79	13.20	-
C18:2n6c (linoleic acid)	Polyunsaturated	24.10	21.19	23.36	25.24
C18:3n6 (γ-linolenic)	Polyunsaturated	13.10	10.66	17.97	8.03

ND = not detected; 1N = 2.94 mM; 3N = 8.82 mM; 6N = 17.64 mM; values are the average of three replicates.

Figure 7. Fatty acid concentration of the cultivations (%) and the 3N extract.



Legend: C16:0: palmitic acid; C16:1: palmitoleic acid; C17:1: cis-10-heptadecenoic acid; C18:1n9C: oleic acid; C18:2n6c: linoleic acid; C18:3n6: γ-linolenic

According to literature, the major components of the lipid content of *C. vulgaris* are oleic, palmitic and linolenic acids, corroborating with our results (MENDES et al., 1995; HAMED, 2016). The palmitic acid can be used as coemulsifier and opacifying agents in the cosmetic industry and, also, it is used to improve the texture of products and help to maintain the skin moisture (KHAN; GAWAS; RATHOD, 2018; “Is Palmitic Acid Bad for the Environment and Your Skin?”, 2020). The oleic acid is similar to those lipids found in the hair and epidermis and its use in cosmetic formulations could help to improve the compatibility of the formulation with cutaneous tissue and, also, it can promote the maintenance of skin hydration (VIOLA; VIOLA, 2009; MONTE, 2013; DARIO et al., 2018). Linoleic acid, when used in cosmetics, accumulates on the stratum corneum, contributing with a better skin barrier, cutaneous regeneration and moisture, being mainly used in products for aged population (DARIO et al., 2018). In cosmetic products, it functions as an emollient, thickening agent and presents anti-inflammatory, acne reductive and skin-lightening properties (“Linoleic Acid”, 2018).

5.4.3 Proteins

Accumulation of proteins is directly proportional to the available nitrogen in the culture medium, as well as the growth rate. **Table 15** describes our results for the protein content for the *C. vulgaris* cultivation, being the data corroborated by Venckus and coleagues (2017).

Table 15. Protein concentration of the *C. vulgaris* samples grown under different nitrogen levels (1N, 3N, 6N) and 3N extract.

UTEX strain	Protein content (%dry cell weight)
1N	13.82
3N	19.31
6N	26.03
3N Extract	19.78

1N = 2.94 mM; 3N = 8.82 mM; 6N = 17.64 mM

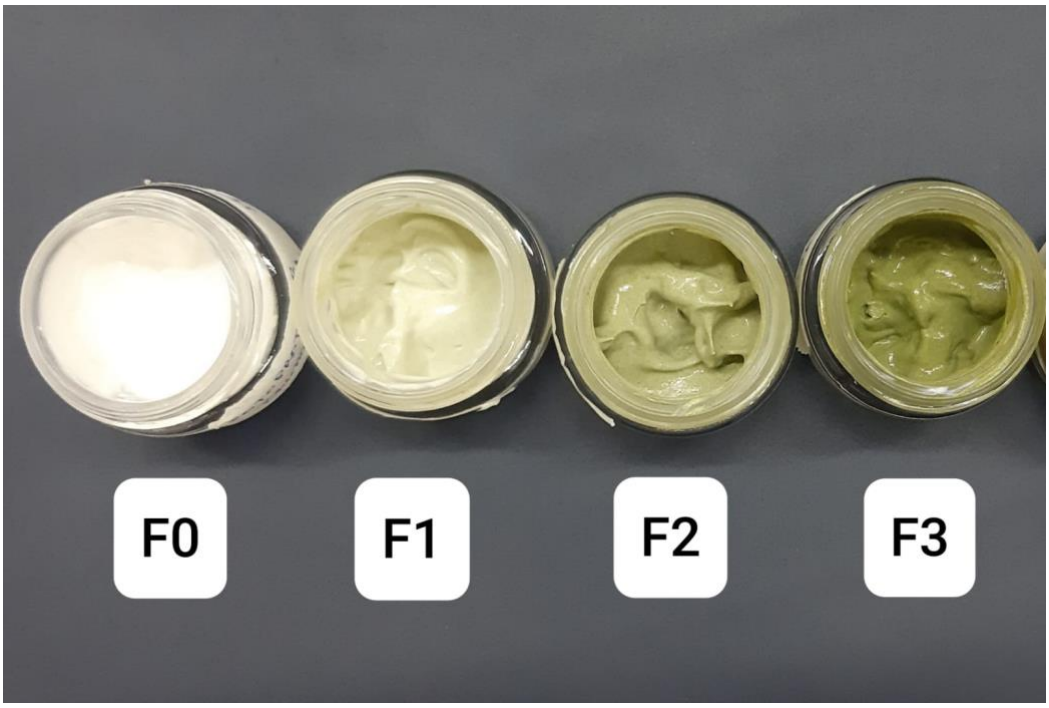
Proteins have been considered as an important product of microalgae and is widely used for human and animal nutrition (CHEW et al., 2017), and literature have been showing another use of proteins extracted from microalgae, such as *C. vulgaris,* especially in fields, like cosmetic industry (URSU et al., 2014; HAMED, 2016; MOURELLE; GÓMEZ; LEGIDO, 2017). Urso and coleagues (2014) showed that proteins extracted from *C. vulgaris* had emulsifying property and could be used as a functional ingredient. Another study pointed out that protein-rich extract from microalgae could repair the signs of early skin aging, exerting a tightening effect and preventing stria formation (SPOLAORE et al., 2006).

C. vulgaris extract can reach 50-60% protein content of the biomass dry weight (VENCKUS; KOSTKEVIČIENĖ; BENDIKIENĖ, 2017) and has shown to develop similar effects in comparison to other protein-rich extracts (anti-aging, thickening and anti-wrinkle effects, for instance) (SKJÅNES; REBOURS; LINDBLAD, 2013; KOLLER; MUHR; BRAUNEGG, 2014; WANG et al., 2015; FONSECA, 2016; ARIEDE et al., 2017).

5.5 Sunscreen Formulations

The oil-in-water emulsified system containing the UV filters was macroscopically compatible with 3N extract. The UV active ingredients of the sunscreen (avobenzone and octyl p-methoxycinnamate) were selected to obtain a photounstable prototype product aiming at visualizing the attributes from the *C. vulgaris* extract into this system (LAUTENSCHLAGER; WULF; PITTELKOW, 2007). All samples were macroscopically evaluated in regard to the organoleptic properties, being considered suitable, and their pH values (6-7) were biocompatible with the skin (VELASCO et al., 2012). The odor of the samples was characteristic, being more intense as the concentration of the extract increased. The green color of the formulations was due to the color of the extract, which also became more intense according to its concentration. Those properties remained unchanged until the end of the studies.

Figure 8: Photograph of photoprotective formulations.



Source: AUTHOR; F0 = formulation without extract; F1 = 1.0% extract; F2 = 5.0% extract; F3 = 10.0% extract.

The extract of *C. vulgaris* was incorporated into the formulation at different concentrations (1.0, 5.0 and 10.0% w/w) to verify an ideal concentration that would provide the highest *in vitro* SPF and increased photostability. The minimum suitable

SPF value was established as, at least, 6 with critical wavelength of, at least, 370 nm, according the Brazilian Legislation (BRASIL, 2012; RUSCINC, 2018).

Table 16: *In vitro* sun protection factor (SPF) and critical wavelength (nm) of the sunscreen samples (n=3).

Samples	*In vitro* SPF	Standard Deviation	Critical wavelength (nm)	Standard Deviation
F0	54.33^{A}	7.37	382.0^{C}	0.00
F1	45.67^{A}	2.08	381.3^{C}	0.57
F2	43.33^{A}	6.11	381.3^{C}	0.57
F3	80.33^{B}	0.57	381.0^{C}	0.00

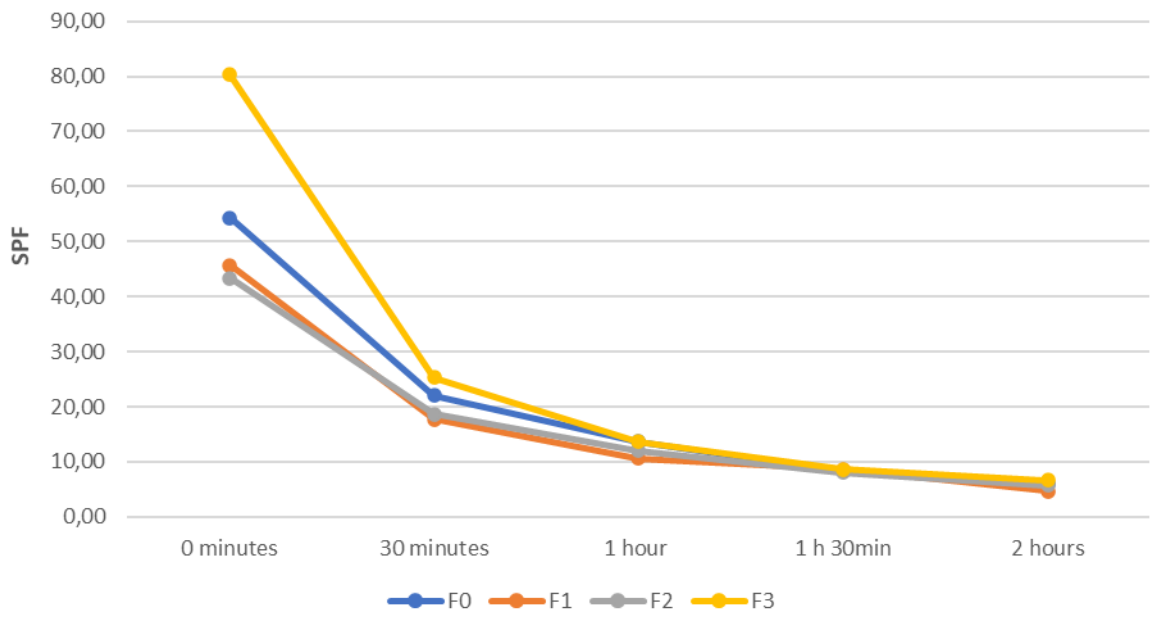
Legend: F0 = formulation without extract; F1 = 1.0% extract; F2 = 5.0% extract; F3 = 10.0% extract. The results that share the same letter are statistically equal (p<0,05).

The extract of *C. vulgaris* was able to promote an increase in the *in vitro* SPF value when at the concentration of 10.0%. For all other concentrations, the extract did not interfere this parameter performance. Regarding the critical wavelength, it was not interfered by the extract and all values were above the minimum established by the Brazilian Legislation. Secondary metabolites could exert some mechanisms to increase the SPF and consequently modify skin-UV interaction, improving the cutaneous protection. The mechanism could involve absorbing UV radiation; inhibiting UV-induced free radical and protecting skin surface lipids; and attenuating inflammatory responses; among other actions (KOSTYUK et al., 2018). According to the specialized literature, extracts of *C. vulgaris* can have several compounds, such as lutein, α-carotene and xanthophylls; β-1,3 glucan and linoleic acid (a compound identified in our investigation), that also have anti-inflammatory properties, and other compounds, which would act like some of those methods providing skin protection against UV-radiation and, consequently, increasing the SPF of the formulation (ANDO et al., 1998; BARHAM et al., 2000; CHA et al., 2010; SKJÅNES; REBOURS; LINDBLAD, 2013; WANG et al., 2015; LI et al., 2016; HYNSTOVA et al., 2017; MUSZYNSKA et al, 2018; PANAHI et al., 2019 MOORE; WAGNER; KOMARNY, 2020).

The photostability of sunscreens is an important issue that is directly correlated to their performance. The association of different UV filters is one of the alternatives to

improve the photostability of novel sunscreens. However, the association of avobenzone and octyl *p*-methoxycinnamate was showed to be photounstable, as expected (LHIAUBET-VALLET et al., 2010). Hence, another alternative would be an addition of photostabilizers and natural antioxidants into the formulations to maintain or enhance the product efficacy (MOROCHO-JÁCOME et al., 2020). F0, F1, F2 and F3 were irradiated for 2 hours since it is recommended to reapply sunscreens every 2 hours (UNITED STATES, 2021). *In vitro* SPF was read every 30 minutes to monitor the photostability and the results are illustrated at **Figure 9**.

Figure 9: Photostability of the sunscreen samples about the *in vitro* sun protection factor (SPF) values.



Legend: F0 = formulation without extract; F1 = 1.0% extract; F2 = 5.0% extract; F3 = 10.0% extract.

Unfortunately, the extract derived from *C. vulgaris* biomass did not work as a photostabilizer at any of the concentrations tested and the active ingredients were degraded in less than 2 hours of artificial irradiation. Our results suggested that the extract was not able to reduce the formation of free radicals' by-products produced by the photodegradation of the avobenzone and it was also not able to balance the photochemical reaction of the degradation of the octyl *p*-methoxycinnamate (ARIEDE et al., 2017). However, the *C. vulgaris* extract should not be discarded as a photostabilising agent, requiring further tests with an extract obtained under new conditions of microalgae culture. As it was observed, the cultivation conditions directly

interfered with the secondary metabolites produced by this microalgae, which are the bioactives that could play important functions in several types of topical formulations.

Thus, there has been more demand for dermocosmetics developed with natural and sustainable products, giving rise to the opportunity and need for new searches for natural, sustainable and renewable sources of biocompounds and bioactive molecules for the development of formulations that can be considered *eco-friendly* products (MCINTOSH et al, 2018; SHOW et al., 2017; CHRAPUSTA et al., 2017).

6. CONCLUSION

Considering the experimental conditions adopted in this research work, we conclude the following:

- the best growing condition for *C. vulgaris* cultivation was in the 3N concentration of nitrogen (8.82 mM), particularly regarding the yield and cultivation time to obtain the biomass. This process generated cell productivity of 3978.67 m L^{-1} d^{-1}; growth rate of 0.71 $\mu_{máx}$ (d^{-1}); 23.50 and 19.31% of lipid content and protein content, respectively.
- The extract prepared from the biomass cultivated in the 3N condition had 43.17% of lipid content and 19.78% of protein content.
- A sunscreen formulation was prepared as an O/W emulsion, containing as UV filters the octyl *p*-methoxycinnamate and avobenzone. The extract of *C. vulgaris* was incorporated at 0.0, 1.0, 5.0 and 10.0%.
- The sunscreen sample with the best performance was the one with 10.0% of the extract of *C. vulgaris*, considering the *in vitro* SPF equal to 80.33 + 0.57.

REFERENCES

A TOLEDO-CERVANTES, M MORALES, E NOVELO, S. R. Carbon dioxide fixation and lipid storage by Scenedesmus obtusiusculus. *Bioresource technology*, v. 130, p. 652–658, 2013.

ACIOLI. Cultivo da microalga Chlorella spp. em pequena escala visando ao aumento da produção de biomassa e óleos para biodiesel, 2014.

ADAMAKIS et al. Cultivation, characterization, and properties of *Chlorella vulgaris* microalgae with different lipid contents and effect on fast pyrolysis oil composition. *Environmental Science and Pollution Research*, v. 25, n. 23, p. 23018–23032, 2018.

AGÊNCIA nacional de vigilância sanitária. ANVISA. Guia de estabilidade de produtos cosméticos. Brasilia: Anvisa, v. 1, 45 p, 2004. (Séries temáticas da Anvisa. Qualidade em cosméticos, 1).

AHLUWALIA et al. Cultivo de Chlorella sp. em fotobiorreatores enriquecidos com CO_2 e avaliação da biomassa para produção de biocombustíveis. IOSR Journal of Economics and Finance, v. 3, n. 1, p. 56, 2016. Disponível em: <https://www.bertelsmann-stiftung.de/fileadmin/files/BSt/Publikationen/GrauePublikationen/MT_Globalization_Report_2018.pdf%0Ahttp://eprints.lse.ac.uk/43447/1/India_globalisation%2C society and inequalities%28lsero%29.pdf%0Ahttps://www.quora.com/What-is-the>.

ANDERSEN. Algal culturing techniques. [s.l: s.n.]

ANDO; RYU; HASHIMOTO et al. Linoleic acid and alpha-linolenic acid lightens ultraviolet-induced hyperpigmentation of the skin. *Arch. Dermatol*. Res. 290, 375–381, 1998. doi: 10.1007/s004030050320

ANDRADE. Produção De Microalgas E Caracterização De Sua Composição Protêica E Lipídica Via Espectrometria De Massas. Tese de Doutorado, p. 141, 2014.

ANDRADE; MENDES; NASCIMENTO. Avaliação da intensidade luminosa nos cultivos em *shaker* da *Chlorella vulgaris*. n. July, 2013.

AOAC, A. (ed.). AOAC Association of Official Analytical Chemists. 14. ed. [s.l: s.n.]

ARAÚJO. Avaliação do crescimento e composição de micro-organismos fotossintetizantes para uso como matéria-prima em fotoprotetor. 2015b. Universidade de São Paulo, 2015.

ARAÚJO. Desenvolvimento de formulações cosméticas contendo óleos vegetais para a proteção e reparação capilar. UNIVERSIDADE DE SÃO PAULO FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO, 2015.

ARIEDE et al. Cosmetic attributes of algae - A review Algal Research, 2017.

AVADHANI et al. Skin delivery of epigallocatechin-3-gallate (EGCG) and hyaluronic acid loaded nano-transfersomes for antioxidant and anti-aging effects in UV radiation induced skin damage loaded nano-transfersomes for antioxidant and anti-aging effects, v. 7544, 2017.

BABY. Avaliação in vitro da permeabilidade cutânea da rutina em emulsões cosméticas. Universidade de São Paulo, 2007.

BACOVA et al. The effects of 5-azacytidine and cadmium on global 5-methylcytosine content and secondary metabolites in the freshwater microalgae Chlamydomonas reinhardtii and Scenedesmus quadricauda. *Journal of Phycology*, v. 55, n. 2, p. 329–342, 2019.

BAIEE; SALMAN. Effect of phosphorus concentration and light intensity on protein content of microalga *Chlorella vulgaris*. *Mesopotamia Environmental Journal*, v. 2, n. 2, p. 75–86, 2016.

BARHAM; EDENS; FONTEH et al. Addition of eicosapentaenoic acid to gamma-linolenic acid- supplemented diets prevents serum arachidonic acid accumulation in humans. *J. Nutr*. 130, 1925–1931, 2000. doi: 10.1093/jn/130.8.1925

BASHAN. *Chlorella Sorokiniana* (formerly *C. vulgaris*) UTEX 2714, non-thermotolerant microalga useful for biotechnological application and as a reference strain. *J. Appl. Phycol*, 2015.

BHALAMURUGAN; VALERIE; MARK. Valuable bioproducts obtained from microalgal biomass and their commercial applications: A review. *Environmental Engineering Research*, v. 23, n. 3, p. 229–241, 2018.

BOROWITZKA. Commercial production ofmicroalgae: pond, tanks, tubes and fermenters. *Journal of Biotechnology*, v. 70, n. 1–3, p. 313–321, 1999.

BRASIL. Guia para Avaliação de Segurança de Produtos Cosméticos. [s.l: s.n.]. Disponível em: <http://portal.anvisa.gov.br/documents/106351/107910/Guia+para+Avaliação+de+Segurança+de+Produtos+Cosméticos/ab0c660d-3a8c-4698-853a-096501c1dc7c>. Acesso em: 29 out. 2019.

BRENNAN; OWENDE. Biofuels from microalgae — A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, v. 14, n. 2, p. 557–577, 2010. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S1364032109002408>.

BROINIZI et al. Avaliação da atividade antioxidante dos compostos fenólicos naturalmente presentes em subprodutos do pseudofruto de caju (Anacardium occidentale L.). *Ciencia e Tecnologia de Alimentos*, v. 27, n. 4, p. 902–908, 2007.

BUONO; LANGELLOTTI; MARTELLO et al. Biological activities of dermatological interest by the water extract of the microalga Botryococcus braunii. *Arch Dermatol Res*, v. 304, p. 755–764, 2012.

C. VAN DEN HOEK; D.G. MANN. Algae: an introduction to phycology. [s.l: s.n.]

CARVALHO; FRANCISCO; ALMEIDA et al. Cultivation of Arthrospira (Spirulina) platensis by fed-batch addition of ammonium chloride at exponentially-increasing feeding rate. *Journal of Phycology*, v. 40, p. 589–597, 2004.

CARVALHO; MATSUDO; BEZERRA et al. Microalgae bioreactors. In: R. BAJPAI, A. PROKOP, & M. Z. (Ed.). *Algal biorefineries*. [s.l: s.n.]p. 83–126.

CEZARE-GOMES et al. Potential of Microalgae Carotenoids for Industrial Application. *Applied Biochemistry and Biotechnology*, v. 188, n. 3, p. 602–634, 2019.

CHA et al. Effect of pressurized liquids on extraction of antioxidants from *Chlorella vulgaris*. *Journal of Agricultural and Food Chemistry*, v. 58, n. 8, p. 4756–4761, 2010.

CHEW et al. Microalgae biorefinery: High value products perspectives. *Bioresource Technology*, v. 229, p. 53–62, 2017. Disponível em: <http://dx.doi.org/10.1016/j.biortech.2017.01.006>.

CHINNASAMY; RAMAKRISHNAN; BHATNAGAR et al. Biomass production potential of a wastewater alga *Chlorella vulgaris* arc 1 under elevated levels of CO_2 and temperature. *International Journal of Molecular Sciences*, p. 518–532, 2009.

CHISTI. Biodiesel from microalgae. Biotechnology Advances, v. 25, n. 3, p. 294–306, 2007. Disponível em: <http://dx.doi.org/10.1016/j.biotechadv.2007.02.001>.

CHRAPUSTA et al. Mycosporine-Like Amino Acids: Potential health and beauty ingredients. *Marine Drugs*, v. 15, n. 10, p. 1–29, 2017.

COSTA. Constituintes químicos, fenóis totais e atividade antioxidante de Sterculia striata St. Hil. et Naudin Chemical constituents, total phenolics and antioxidant activity

of Sterculia striata St. Hil. et Naudin. v. 40, n. 1, p. 207–212, 2010. Disponível em: <http://www.scielo.br/pdf/aa/v40n1/v40n1a26.pdf>.

DARIO. Desenvolvimento e avaliação de eficácia de nanoemulsão catiônica bioativa na proteção capilar aos danos foto-oxidativos. 2016. Faculdade de Ciências Farmacêuticas, 2016.

DARIO et al. Efficacy of Punica granatum L. hydroalcoholic extract on properties of dyed hair exposed to UVA radiation. *Journal of Photochemistry and Photobiology B: Biology*, v. 120, p. 142–147, 2013. Disponível em: <http://dx.doi.org/10.1016/j.jphotobiol.2012.12.011>.

DARIO et al. Synergistic photoprotective activity of nanocarrier containing oil of Acrocomia aculeata (Jacq.) Lodd. Ex. Martius—Arecaceae. Industrial Crops and Products, 2018.

DE JAGER; COCKRELL; DU PLESSIS. Ultraviolet Light Induced Generation of Reactive Oxygen Species. *Advances in Experimental Medicine and Biology*, v. 996, p. 15–23, 2017.

DELGODA; MURRAY. Evolutionary perspectives on the role of plant secondary metabolites. In: Pharmacognosy. *Elsevier*. [s.l: s.n.]p. 93–100.

DERNER. Microalgas, produtos e aplicações. p. 1959–1967, 2006.

DIAS; ALMEIDA; PICHLER. The Shampoo pH can Affect the Hair: Myth or Reality? Int *J Trichology*, v. 6, n. 3, p. 95–99, 2014.

DIFFEY et al. In vitro assessment of the broad-spectrum ultraviolet protection of suscreen products. *Journal American Academy Dermatology*, v. 43, n. 6, p. 1024–1035, 2000.

DURAN; KUMA; SANDHU. A review on microalgae strains, cultivation, harvesting, biodiesel conversion and engine implementation. *Biofuels*, v. 7269, p. 1–12, 2018. Disponível em: <https://doi.org/10.1080/17597269.2018.1457314>.

DVORETSKY et al. Enhanced lipid extraction from microalgae *Chlorella vulgaris* biomass: Experiments, modelling, optimization. *Chemical Engineering Transactions*, v. 49, p. 175–180, 2016.

ECCLESTON. Functions of mixed emulsifiers and emulsifying waxes in dermatological lotions and creams. Colloids Surfaces A: Physicochemical and Engineering Aspects, v. 123–124, p. 169–182, 1997.

EVANS; MARSH; WICKETT. The structural implications of water hardness metal uptake by human hair. *International Journal of Cosmetic Science*, v. 33, n. 5, p. 477–482, 2011.

EVANS. Evaluating hair conditioning with instrumental combing. *Cosmetics and Toiletries*, v. 126, n. 8, 2011.

FERNÁNDEZ-LINARES et al. Assessment of *Chlorella vulgaris* and indigenous microalgae biomass with treated wastewater as growth culture medium. *Bioresource Technology*, v. 244, p. 400–406, 2017. Disponível em: <http://dx.doi.org/10.1016/j.biortech.2017.07.141>.

FONSECA. Aplicação de Algas na Indústria Alimentar e Farmacêutica. 2016.

FREGONESI et al. Brazilian oils and butters: The effect of different fatty acid chain composition on human hair physiochemical properties. *Journal of Cosmetic Science*, v. 60, n. 2, p. 273–280, 2009.

FREITAS. Estudo da solubilidade de sais inorgânicos em misturas de solventes. 2016. Universidade de Tiradentes, 2016.

GOLDSTEIN. Recuperação do DNA e fotoenvelhecimento. *Cosmetcs & toiletries*, v. 20, n. 1, p. 48–51, 2008.

GOMES; DAMAZIO. Cosmetologia: descomplicando os princípios ativos. 3. ed. [s.l: s.n.]

GOSHIYAMA. Avaliação das propriedades das fibras capilares tratadas com alisante ácido com diferentes valores de pH, 2019. Disponível em: <http://www.teses.usp.br/teses/disponiveis/9/9139/tde-07052019-154247/pt-br.php%0Ahttp://fi-admin.bvsalud.org/document/view/87855>.

GRIFFITHS; HARRISON. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J Appl Phycol*. 21:493–507, 2009.

HAMED. The Evolution and Versatility of Microalgal Biotechnology: A Review. *Comprehensive Reviews in Food Science and Food Safety*, v. 15, n. 6, p. 1104–1123, 2016.

HAMEDI; MAHDAVI; GHESHLAGHI. Improved lipid and biomass productivities in *Chlorella vulgaris* by differing the inoculation medium from the production medium. *Biofuel Research Journal*, v. 3, n. 2, p. 410–416, 2016.

HARTMAN; LAGO. Rapid preparation of fatty acid methyl esters from lipids. *Lab Prat*, v. 22, p. 475–476, 1973.

HIRATA; SATO; SANTOS. Radicals livres e o envelhecimento cutâneo. *Acta Farmaceutica Bonaerense*, v. 23, n. 3, p. 418–424, 2004.

HYNSTOVA et al. Separation, identification and quantification of carotenoids and chlorophylls in dietary supplements containing *Chlorella vulgaris* and *Spirulina platensis* using high performance thin layer chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, 2017. Disponível em: <https://doi.org/10.1016/j.jpba.2017.09.018>.

IS Palmitic Acid Bad for the Environment and Your Skin? Disponível em: <https://thedermreview.com/palmitic-acid/>. Acesso em: 15 ago. 2021.

JÁCOME. Estudo do reaproveitamento de meio no cultivo de Arthrospira (Spirulina) platensis. p. 1–143, 2014.

JIANG; KALODIMOS. NMR Studies of Large Proteins. *Journal of Molecular Biology*, v. 429, n. 17, p. 2667–2676, 2017. Disponível em: <http://dx.doi.org/10.1016/j.jmb.2017.07.007>.

JOSHI; KUMARI; UPASANI. Applications of algae in cosmetics: an overview. *International Journal of Innovative Research in Science*, *Engineering and Technology*, v. 7, n. March, p. 1269–1278, 2018.

KHAN; SHIN; KIM. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, v. 17, n. 1, p. 1–21, 2018. Disponível em: <https://doi.org/10.1186/s12934-018-0879-x>.

KHAN; GAWAS; RATHOD. Virendra K. Enzyme-catalysed production of n-butyl palmitate using ultrasoundassisted esterification of palmitic acid in a solvent-free system. *Bioprocess and Biosystems Engineering*, 2018.

KLASSON et al. Bioreactor design for sirjthesis gas fermentations. [s.l: s.n.].

KOCKLER et al. Photostability of sunscreens. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, v. 13, n. 1, p. 91–110, 2012. Disponível em: <http://dx.doi.org/10.1016/j.jphotochemrev.2011.12.001>.

KOLLER; MUHR; BRAUNEGG. Microalgae as versatile cellular factories for valued products. *Algal Research*, v. 6, n. PA, p. 52–63, out. 2014. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S2211926414000782>.

KORTE et al. Distribution and Localization of Hydrophobic and Ionic Chemical Groups at the Surface of Bleached Human Hair Fibers. *Langmuir*, v. 30, n. 41, p. 12124−12129, 2014.

KOSTYUK et al. Natural Substances for Prevention of Skin Photoaging: Screening Systems in the Development of Sunscreen and Rejuvenation Cosmetics. *Rejuvenation Research*, v. 21, n. 2, p. 91–101, 2018. Disponível em: <https://www.liebertpub.com/doi/abs/10.1089/rej.2017.1931>. Acesso em: 22 ago. 2021.

KUMAR et al. UV-B radiation stress causes alterations in whole cell protein profile and expression of certain genes in the rice phyllospheric bacterium Enterobacter cloacae. *Frontiers in Microbiology*, v. 7, n. SEP, p. 1–14, 2016.

CLESCERI; GREENBERG. Standard methods for the examination of water and wastewater. *American Public Health Association*, p. 1484, 1989.

LAM; LEE. Potential of using organic fertilizer to cultivate *Chlorella vulgaris* for biodiesel production. *Applied Energy*, v. 94, p. 303–308, 2012. Disponível em: <http://dx.doi.org/10.1016/j.apenergy.2012.01.075>.

LAUTENSCHLAGER; WULF; PITTELKOW. Photoprotection. *Lancet*, v. 370, n. 9586, p. 528–537, 2007.

LEITE; MAIA CAMPOS. Photoprotective Effects of a Multifunctional Hair Care Formulation Containing Botanical Extracts, Vitamins, and UV Filters. *Photochemistry and Photobiology*, v. 94, n. 5, p. 1010–1016, 2018.

LHIAUBET-VALLET et al. Filter-filter interactions. Photostabilization, triplet quenching and reactivity with singlet oxygen. *Photochemical & Photobiological Sciences*, v. 9, p. 552–558, 2010.

LI et al. Effects of sodium bicarbonate on cell growth, lipid accumulation, and morphology of *Chlorella vulgaris*. *Microbial Cell Factories*, v. 17, n. 1, p. 1–10, 2018. Disponível em: <https://doi.org/10.1186/s12934-018-0953-4>.

LI et al. Morphology, growth, biochemical composition and photosynthetic performance of *Chlorella vulgaris* (*Trebouxiophyceae*) under low and high nitrogen supplies. *Algal*, v. 16, p. 481–491, 2016. Disponível em: <http://dx.doi.org/10.1016/j.algal.2016.04.008>.

LIAO et al. Simultaneous enhancement of *Chlorella vulgaris* growth and lipid accumulation through the synergy effect between light and nitrate in a planar

waveguide flat-plate photobioreactor. *Bioresource Technology*, v. 243, p. 528–538, 2017. Disponível em: <http://dx.doi.org/10.1016/j.biortech.2017.06.091>.

LINOLEIC Acid. Disponível em: <https://thedermreview.com/linoleic-acid/>. Acesso em: 15 ago. 2021.

LIU; CHEN. Biology and Industrial Applications of Chlorella: Advances and Prospects. n. December, 2014.

LORDAN; ROSS; STANTON. Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. *Marine Drugs*, 2011.

LOURENÇO. Cultivo de microalgas marinhas: princípios e aplicações. 2006. Disponível em: <http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=AGB.xis&method=post&formato=2&cantidad=1&expresion=mfn=242602>. Acesso em: 28 nov. 2019.

LÚCIO. Cultivo de microalgas *Chlorella vulgaris* com efluente doméstico como meio de cultura alternativo. Trabalho de Conclusão de Curso do Curso de Engenharia Sanitária e Ambiental da UFCS, p. 71, 2013.

LV et al. Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions. *Bioresource Technology*, v. 101, n. 17, p. 6797–6804, 2010.

MAMBRO; FONSECA. Mudanças por radicais livres e ação tópica de antioxidantes. *Cosmetcs & toiletries*, v. 19, n. 5, p. 74–6, 2007.

MARINO. Análises dos parâmetros de cultivo da microalga *Chlorella vulgaris*. 2018. Universidade de São Paulo, 2018.

MARKOU; VANDAMME; MUYLAERT. Microalgal and cyanobacterial cultivation: The supply of nutrients. *Water Research*, v. 65, p. 186–202, 2014. Disponível em: <http://dx.doi.org/10.1016/j.watres.2014.07.025>.

MARTINS; CAETANO; MATA. Microalgae for biodiesel production and other applications: A review, v. 14, p. 217–232, 2010.

MCINTOSH et al. Alkenones as a promising green alternative for waxes in cosmetics and personal care products. *Cosmetics*, v. 5, n. 2, 2018.

MIRANDA; SATO. Antioxidant activity of the microalga *Chlorella vulgaris* cultered on special conditions. *Boll Chim Farm*, v. 3, n. 140, p. 165–8, 2001.

MOBIN; CHOWDHURY; ALAM. Commercially important bioproducts from microalgae and their current applications-A review. *Energy Procedia*, v. 160, n. 2018, p. 752–760, 2019. Disponível em: <http://dx.doi.org/10.1016/j.egypro.2019.02.183>.

MONTAGNER; COSTA. Bases biomoleculares do fotoenvelhecimento. *Anais Brasileiros de Dermatologia*, v. 84, n. 3, p. 263–269, 2009.

MONTE. Desenvolvimento e avaliação da estabilidade física de condicionador capilar contendo óleo de pequi (Caryocar brasiliense Camb.), 2013.

MONTOYA et al. Production of *Chlorella vulgaris* as a source of essential fatty acids in a tubular photobioreactor continuously fed with air enriched with CO_2 at different concentrations, 2014.

MORAES. Síntese e avaliação da segurança in vitro da rutina e do succinato de rutina visando sua incorporação em formulações fotoprotetoras eficazes associados a filtros químicos e físico, 2012.

MOROCHO JÁCOME et al. Kinetic and thermodynamic investigation of *Arthrospira* (Spirulina) platensis fed-batch cultivation in a tubular photobioreactor using urea as nitrogen source. *Journal of Chemical Technology and Biotechnology*, v. 87, n. 11, p. 1574–1583, 2012.

MOROCHO-JÁCOME; FREIRE; OLIVEIRA et al. In Vivo SPF from Multifunctional Sunscreen Systems Developed with Natural Compounds — A Review. *Journal of Cosmetic Dermatology*, 20, 729–737, 2020. doi:10.1111/jocd.13609.

MOURELLE; GÓMEZ; LEGIDO. The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. *Cosmetics*, v. 4, n. 4, 2017.

MUSZYŃSKA et al. Bioaccessibility of phenolic compounds, lutein, and bioelements of preparations containing *Chlorella vulgaris* in artificial digestive juices. *Journal of Applied Phycology*, v. 30, n. 3, p. 1629–1640, 2018.

PANAHI et al. Impact of Cultivation Condition and Media Content on *Chlorella vulgaris* Composition. *Advanced Pharmaceutical Bulletin*, v. 9, n. 2, p. 182–194, 2019. Disponível em: <http://dx.doi.org/10.15171/jcvtr.2015.24>.

PEREIRA. Produção de biomassa e de lipídeos por cultivo misto de microalgas suplementado com CO_2. Universidade Federal Do Paraná, 2013.

PERES et al. Quality by design (QbD), Process Analytical Technology (PAT), and design of experimente applied to the development of mulfuncional sunscreens. *Drug Development and Industrial Pharmacy*, v. 43, n. 2, p. 246–256, 2017.

POHNDORF; CAMARA; LARROSA et al. Production of lipids from microalgae *Spirulina sp*.: Influence of drying, cell disruption and extraction methods. *Biomass and Bioenerg*y, v. 93, p. 25–32, 2016.

PRIYADARSHANI; RATH. Commercial and industrial applications of micro algae – A review. v. 3, n. 4, p. 89–100, 2012.

RASTOGI; INCHAROENSAKDI. UV radiation-induced accumulation of photoprotective compounds in the green alga *Tetraspora sp*. CU2551. *Plant Physiology and Biochemistry*, v. 70, p. 7–13, 2013. Disponível em: <http://dx.doi.org/10.1016/j.plaphy.2013.04.021>.

RAVEN; EVERT; EICHHORN. Biologia Vegetal. In: Biologia Vegetal. 7. ed. Rio de Janeiro: Editora Guanabara Koogan, p. 906, 2007.

REBENFELD; WEIGMANN; DANSIZER. Temperature Dependence of the Mechanical Properties of Human Hair in Relation to Structure. undefined, 1966.

REBOLLOSO-FUENTES et al. Biomass nutrient profiles of the microalga Nannochloropsis. *Journal of Agricultural and Food Chemistry*, v. 49, n. 6, p. 2966–2972, 2001.

ROBBINS. Chemical and Physical Behavior of Human Hair. In: Springer-Verlaq. Third Edit ed. [s.l: s.n.]

ROBBINS; REICH. Prediction of hair assembly characteristics from single-fiber properties. Part II. The relationship of fiber curvature, friction, stiffness, and diameter to combing behavior. *Journal of the Society of Cosmetic Chemists*, v. 37, n. 3, p. 141–158, 1986.

RODRIGUES-RACT; G. L. A. Lipídios modificados obtidos a partir de gordura do leite, óleo de girassol e ésteres de fitosteróis para aplicação em spreads. *Quimica Nova*, v. 31, p. 1960–1965, 2008.

RUSCINC. Fotoprotetores bioativos contendo extrato de mirtilo (*Vaccinium myrtillus L*.): Caracterização físico-química e funcional. 2018. Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, 2018.

SAFI et al. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review, 2014.

SANTOS. Seleção de cepas oleaginosas da microalga *Chlorella spp*. e otimização do seu cultivo em meio sintético e vinhaça visando à produção sustentável de biodiesel. Universidade De São Paulo Escola De Engenharia De São Carlos, 2013.

SCARSELLA et al. Achievements and Perspectives in Hetero- and Mixotrophic Culturing of Microalgae. *Chemical Engineering Transactions*, v. 17, p. 1065–1070, 2009.

SCHOELLER. Hydrologie Dynamique Et Chimique, Recherche, Exploitation Et Évaluation Des Ressources. Paris: Masson Et Cie, p. 642, 1962.

SERRA-MAIA et al. Influence of temperature on *Chlorella vulgaris* growth and mortality rates in a photobioreactor. *Algal Research*, v. 18, p. 352–359, 2016.

SHARMA; SCHUHMANN. High lipid induction in microalgae for biodiesel production. *Energies*, v. 5, p. 1532–53, 2012.

SHOW et al. A holistic approach to managing microalgae for biofuel applications. *International Journal of Molecular Sciences*, v. 18, n. 1, 2017.

SILVA. Avaliação do crescimento de micro-organismos fotossintetizantes em fotobiorreator tubular empregando nutrientes provenientes da vinhaça tratada, 2020.

SILVA; LEITE; MAIA CAMPOS. Development of hair care formulations containing Spirulina platensis and Ascophyllum nodosum extracts. *International Journal of Phytocosmetics and Natural Ingredients*, v. 6, n. 1, p. 13–13, 2019.

SINGH; SHARMA. Development of suitable photobioreactor for algae production—a review. *Renewable and Sustainable Energy Reviews*, v. 16, n. 4, p. 2347–2353, 2012.

SKJÅNES; REBOURS; LINDBLAD. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Critical Reviews in Biotechnology*, v. 33, n. 2, p. 172–215, 2013.

SOLOMON; BERG; MARTIN. Biology. 9. ed. [s.l.] Fort Worth: Saunders College Publishing, 2011.

SOROKIN. The Effects of Light Intensity on the Growth Rates of Green Algae. *Plant Physiology*, v. 2, n. 33, p. 109–113, 1958.

SPOLAORE et al. Commercial applications of microalgae. Journal of Bioscience and *Bioengineering*, v. 101, n. 2, p. 87–96, 2006.

SPRINGSTEEN et al. In vitro measurement of sun protection factor of sunscreens by diffuse transmittance. *Anal Chim Acta*, v. 380, p. 155–164, 1999.

STAGES. Effects of Marine Toxins on the Reproduction and Early Stages Development of Aquatic Organisms. p. 59–79, 2010.

STATES, U. Food and Drug Administration. Department of Health and Human Services. 21 CFR Parts 201 and 310. Labeling and effectiveness testing; sunscreens drug products for over-the-counter human use. Federal Register, v. 76, n. 117, p. 35620–35665, 2011.

STATES, U. Food and Drug Administration. Department of Health and Human Services. Sunscreen: How to Help Protect Your Skin from the Sun. Available in: https://www.fda.gov/drugs/understanding-over-counter-medicines/sunscreen-how-help-protect-your-skin-sun

STEINER. Antioxidantes no tratamento do envelhecimento cutâneo. Disponível em: <https://www.cosmeticsonline.com.br/noticias/detalhes-colunas1/714/antioxidantes+no+tratamento+do+envelhecimento+cutâneo>. Acesso em: 22 jan. 2020.

UGWU; AOYAGI; UCHIYAMA. Photobioreactors for mass cultivation of algae. *Bioresource Technology*, v. 99, n. 10, p. 4021–4028, jul. 2008. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0960852407001368>.

URSU et al. Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella vulgaris*. *Bioresource Technology*, v. 157, p. 134–139, 2014. Disponível em: <http://dx.doi.org/10.1016/j.biortech.2014.01.071>.

VELASCO et al. Influences of bioactive substances on the physicochemical and functional stability of sunscreen emulsions. *Biomedical and Biopharmaceutical Research*, v. 1, n. 9, p. 119–130, 2012.

VELASCO et al. Associação da rutina com p-metoxicinamato de octila e benzofenona-3: Avaliação in vitro da eficácia fotoprotetora por espectrofotometria de refletância. *Latin American Journal of Pharmacy*, v. 27, n. 1, p. 23–27, 2008.

VENCKUS; KOSTKEVIČIENĖ; BENDIKIENĖ. Green algae *Chlorella vulgaris* cultivation in municipal wastewater and biomass composition. *Journal of Environmental Engineering and Landscape Management*, v. 25, n. 1, p. 56–63, 2017.

VIOLA; VIOLA. Virgin olive oil as a fundamental nutritional component and skin protector. *Clin. Dermatol*, v. 27, p. 159–165, 2009.

VONSHAK. *Spirulina platensis* (Arthrospira) physiology, cell-biology and biotechnology. London: *Taylor & Francis*, p. 233, 1997.

WANG et al. Exploring the potential of using algae in cosmetics. *Bioresource Technology*, v. 184, p. 355–362, 2015. Disponível em: <http://dx.doi.org/10.1016/j.biortech.2014.12.001>.

WIDJAJA; CHIEN; JU. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *Journal of the Taiwan Institute of Chemical Engineers*, v. 40, p. 13–20, 2009.