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Microalga *Scenedesmus obliquus*: extraction of bioactive compounds and antioxidant activity[1]

Microalga *Scenedesmus obliquus*: extração de compostos bioativos e atividade antioxidante

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ABSTRACT - Microalgae have been researched as a sustainable source of bioactive compounds for the food and pharmaceutical industries. The objectives of this work were the extraction and the characterization of fatty acids of Scenedesmus obliquus and to separate, quantify, and evaluate the antioxidant capacity of carotenoids and phenolic compounds of this microalga. The microalga cells were disrupted by using ultrasound. The centesimal composition of lyophilized cells was determined. Fatty acids of the oil were converted into methyl esters and analyzed by gas chromatography (flame ionization detector) to obtain the fatty acid profile. Acetone, petroleum ether, and hexane were the solvents applied to extract the carotenoids and ethanol was used to separate the phenolic compounds. The antioxidant activity of the extracts was determined by the DPPH method. The amount of polyunsaturated fatty acids corresponded to 47.31% of the total for unsaturated, with the predominance of linolenic acid (16.74%) and approximately 9.70% of omega-6. The content of extracted carotenoids was higher by using hexane. Total phenolics concentration of 1.12 g 100 g^{-1} microalga was found. High percentages of oxidation inhibition were observed for the extracts of carotenoids (>88%) and phenolic compounds (>72%). Therefore, *Scenedesmus obliquus* presents itself as an alternative, natural, and sustainable source of bioactive compounds.

Key words: Linolenic acid. Carotenoids. Phenolic compounds. Polyunsaturated fatty acid.

RESUMO - As microalgas têm sido pesquisadas como fonte sustentável de compostos bioativos para as indústrias alimentícia e farmacêutica. Os objetivos deste trabalho foram extrair, caracterizar ácidos graxos de *Scenedesmus obliquus* e separar, quantificar e avaliar a capacidade antioxidante de carotenóides e compostos fenólicos desta microalga. As células de microalga foram rompidas usando ultra-som. Determinou-se a composição centesimal das células liofilizadas. Os ácidos graxos do óleo foram convertidos em ésteres metílicos e analisados por cromatografia gasosa (detector de ionização de chama) para obtenção do perfil de ácidos graxos. Acetona, éter de petróleo e hexano foram os solventes aplicados para extrair os carotenóides e o etanol foi utilizado para separar os compostos fenólicos. A atividade antioxidante dos extratos foi determinada pelo método DPPH. A quantidade de ácidos graxos poliinsaturados correspondeu a 47,31% do total para insaturados, com predomínio de ácido linolênico (16,74%) e aproximadamente 9,70% de ômega-6. O teor de carotenóides extraídos foi maior usando hexano. Concentração fenólica total de 1,12 g·100 g^{-1} microalga foi encontrada. Altos percentuais de inibição da oxidação foram observados para os extratos de carotenóides (> 88%) e compostos fenólicos (> 72%). Portanto, *Scenedesmus obliquus* apresenta-se como uma fonte alternativa, natural e sustentável de compostos bioativos.

Palavras-chave: Ácido linolênico. Carotenóides. Compostos fenólicos. Ácido graxo poliinsaturado.

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INTRODUCTION

Microalgae have been researched as an attractive source of bio-compounds for the biofuel, cosmetics, food, and pharmaceutical industries (SASSI *et al*., 2019; SATHASIVAM *et al*., 2019), such as lipids, proteins, carbohydrates, carotenoids, phenolics compounds, minerals, and vitamins. Besides the nutritional value, the food industry interest resides in the microalgae capability to be an alternative material, non-climate-dependent that grow on non-arable and non-productive land and do not compete with food production sites (CHAN *et al*., 2013; WAGHMARE *et al*., 2016). These microorganisms, as compared with other traditional plant sources, exhibit greater efficiency in the conversion of solar energy to biomass, which results in high growth rates (10 to 50 times faster than plants) (CHEN *et al*., 2016; WAGHMARE *et al*., 2016).

The use of microalgae-derived products has grown exponentially in recent decades, and microalgae-derived food and nutraceutical products have enormous potential to slow the rate of malnutrition in developing nations. At the global level, the need for the development of clean, sustainable, and organic technologies to obtain food products, such as nutrients and natural bioactive compounds, demands a continuous search for species and/or varieties capable of synthesizing large amounts of specific compounds. The abundance of proteins and other essential nutrients in microalgae can be an alternative for the high production of healthy and functional foods (KRISHNA KOYANDE *et al*., 2019; MAADANE *et al*., 2015).

The genus *Scenedesmus* spp., and species *Scenedesmus obliquus* has been recently researched because containing various valuable bio-compounds and it is a very robust species, resistant to different pHs and climatic variations (SILVA *et al.*, 2020). It is possible to be used in large scale and several places of the world, furthermore, exhibits a high growth rate (presenting great potential for exploitation) and is rich in lutein - a carotenoid of high biological value (CHNG; CHAN; LEE, 2015; PŘIBYL *et al*., 2015). Some authors extracted carotenoids from *Scenedesmus* spp. and stated that the ease of cultivation and robustness of some strains of *Scenedesmus* makes them more suitable for sustainable large-scale production, being prone to the production of carotenoids and other compounds (PŘIBYL *et al*., 2015).

Research for the discovery of new pigments, their quantification, and verification of properties, covers both the medical and industrial area, due to their biological actions. In particular, the food sector is one of the most attracted by the challenge of searching natural and functional, in order to replace synthetic components to formulate food products (MAADANE *et al*., 2015; SATHASIVAM *et al*., 2019).

There is a wide range of compounds that could potentially be used as antioxidant agents, such as pigments including carotenoids, phenolic compounds, sulfated polysaccharides and long-chain polyunsaturated fatty acids (HAJIMAHMOODI *et al*., 2010; KIM *et al*., 2014; SINGH *et al*., 2015). There is a growing interest in finding new, powerful, and safe antioxidants from natural sources, to minimize oxidative injury to living cells and prevent oxidation in food, pharmaceutical or cosmetic products (HAJIMAHMOODI *et al*., 2010; MAADANE *et al*., 2015). Microalgae can provide solutions for these questions. Therefore, the objectives of this work were the (*i*) extraction and characterization of fatty acids of *S. obliquus* and (*ii*) extraction, quantification, and evaluation of the antioxidant capacity of carotenoids and phenolic compounds of the studied microalga.

MATERIAL AND METHODS

The microalga *Scenedesmus obliquus* was cultivated in a raceway tank (capacity of 4.000 L of cultivation; d = 0.5 $g \cdot L^{-1}$), with sunlight incidence and in semi-discontinuous mode, in a medium rich in potassium chloride (173.9 $mg \cdot L^{-1}$) and Urea (180.0 $mg \cdot L^{-1}$) at the Biofuels Laboratory, Federal University of Viçosa, Viçosa, BR. The microalga growth curve was determined by optical density using absorbance of 650 nm, and the biomass was collected by flocculation in the stationary growth phase (12 days). Cells were washed with distilled water and concentrated using a centrifuge (3400·g, 5 min, Thermo Scientific, Heraeus multifuge X3R, USA) until the solid content reached approximately 10 to 15% (w / w).

In order to release the biological compounds from the microalga cells, cell disruption is required. The rupture was tested with a tip ultrasound (Sonics, VCX 750, USA) under the following conditions: frequency = 20 kHz; amplitudes = 60; 75; or 90%; times = 1; 3; or 5 min; under ice bath to avoid the samples to be overheated. No longer times were tested to prevent overheating of the samples and consequent degradation of the compounds of interest.

For the quantification of ruptured and unruptured cells, cell counting was performed after staining with erythrosine B (1 mg mL^{-1}) using a light microscope, following the methodology described by Gminski *et al*. (2011). In this assay, membranes from ruptured cells are permeable to the dye, while the unruptured cells remain uncolored. Besides, erythrosine B penetrates cells that have suffered critical damage to their plasma membranes. The breaking capacity is expressed as the percentage of broken cells compared to the total number of cells. From the results, the best disruption was chosen for further extraction of the compounds of interest. Upon choosing the best cell

disruption (20 kHz frequency, 90% amplitude, 5 min), the biomass was frozen at - 40 °C, freeze-dried, and stored in sterilized containers. The chemical composition of the dry microalgal biomass was characterized in terms of its content of moisture (AOAC 925.09, 2005), ashes (AOAC 923.03, 2005), lipids (AOAC 920.85, 2005), and protein (AOAC 920.87, 2005). For the protein estimation, a conversion factor of N = 5.89 was used *(*AFIFY *et al.*, 2018). The carbohydrate content was obtained by difference (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 2005).

The microalgal oil was converted to fatty acid methyl esters (FAMEs) to obtain the fatty acid profile of *Scenedesmus obliquus*, according to Ichihara and Fukubayashi (ICHIHARA; FUKUBAYASHI, 2010). Samples of the microalga oil were diluted in chloroform: methanol solution (2:1) and the lipids were transesterified with the addition of 8 M HCl solution in methanol, followed by incubation at 100 °C for 1 h. The FAMEs were extracted using hexane, which was collected from the upper phase after centrifugation. The supernatant (solvent phase) was injected into a gas chromatograph equipped with a Flame Ionization Detector (GC-FID) (Shimadzu, GC-2010, Japan) and a capillary column of 100 m x 0.25 mm (SP-2560, Sigma-Aldrich, USA). The analysis was performed by direct injection of 1 µL of the sample. Helium gas was used as the dragging gas and maintained at a constant flow rate of 363 kPa. The FAMEs were separated using a linear heating ramp from 60 °C to 330 °C, at a heating rate of 20 °C min^{-1}, and high linear velocity for better peak resolution. Peak identification was confirmed by comparison with the standard FAME mix (SupelCo 37 FAME mix, Sigma-Aldrich, USA).

The extraction of carotenoids was based on the methodology proposed by Howe and Tanumihardjo (2006). Carotenoids extraction was carried out using two organic solvents (petroleum ether or hexane) in order to evaluate the extraction yield. The extraction was started by adding pure acetone in the proportion of 6 mL for each 100 mg of microalga. After 2 hours, the sample was centrifuged for 10 minutes at 7100·g. The procedure was repeated with acetone for 15 min, washing until a clarified extract was obtained. From the extract obtained with acetone, extraction with the organic solvent in the ratio of 2:3 (organic solvent: acetone) was started. Deionized water was added, and the aqueous phase separated from the organic phase.

The organic phase, containing the carotenoids and interferents, such as chlorophyll and lipids, was saponified with a solution of 10% w/v KOH in ethanol (SOARES *et al*., 2016). After 12 h, the solution was washed with deionized water until complete separation in two phases: the greenish aqueous phase, and the yellow organic phase, in which the carotenoids would be contained. Absorbances were measured in a UV-Vis spectrophotometer (Cary 50), 350 nm at 700 nm, and, thus, the amount of carotenoids was quantified according to Equation 1.

$$Ca(mg.100g^{-1}) = \frac{AbsxVx10^{4}xD}{Axm} \quad \text{Equation 1}$$

in which, Ca is carotenoids, Abs is the absorbance of the sample at the wavelength corresponding to carotenoids; 'V' is the volume of the sample; 'D' is the dilution factor; 'A' is the extinction coefficient, and 'm' is the initial mass of the microalga.

Phenolic compounds extraction was performed based on the methodology proposed by Singleton and Rossi (SINGLETON; ROSSI, 1965), with some modifications. Ethanol was utilized as the extraction solvent, in which the mixture was left under stirring for 3 h. Subsequently, a filtration step was carried out, followed by 2 washes with ethanol, obtaining a solution containing the compounds of interest.

In order to quantify the amount of phenolic compounds, 0.4 mL of extract was mixed with 2.1 mL of deionized water, 2.0 mL of 2.0% sodium carbonate (Na_2CO_3) and 0.5 mL of Folin-Ciocalteau reagent, adjusting the final volume to 10 mL. The analytical curve of gallic acid with 10 points, was prepared by using 2.4 mL of deionized water, maintaining the remaining proportions of the reagents. Then the blends were allowed to stand for 60 min at room temperature so that the absorbances were measured on a UV-Vis spectrophotometer (Cary 50) at 750 nm using deionized water as blank.

The total concentration of phenolic compounds in the extract was determined from linear regression with the analytical curve of gallic acid (AGE), varying the concentration between 0 and 1000 mg mL^{-1} of gallic acid, with the results expressed in mg of AGE·mL^{-1} of extract and mg of phenolic compounds·100 g^{-1} of microalga. A standard curve of gallic acid (AGE) was made to find the concentration of the total phenolics.

The method used to evaluate the antioxidant activity is based on the reduction of 2,2-diphenyl-1-picrylhydrazyl, DPPH, by the action of an antioxidant or a radical species, to form diphenyl-picrylhydrazine, which presents a yellowish color. The results were obtained by monitoring the decrease in the absorbance values.

The percent inhibition of DPPH was obtained by modifying the method of Brand-Williams, Cuvelier and Berset (1995). Briefly, a solution of 60 µmol·L^{-1} DPPH in methanol was prepared. Then, aliquots of 2.9 mL were withdrawn from the resulting solution and mixed with 100 µL of the phenolic compounds extract (CF) (411.69 µg AGE·mL^{-1} extract), and methanol as control. The same procedure was performed for carotenoids extracts (195.79 µg·mL^{-1} in hexane – CHX, and 184.71 µg·mL^{-1} in petroleum

ether – CEP, respectively). After stirring, the mixture was left in the dark at room temperature for 90 min.

Then, the absorbance of the samples was read, every 30 min of reaction, in a spectrophotometer at 517 nm, in order to obtain the percentage of inhibition, expressed by Equation 2.

$$AA(\%) = \frac{(A_{sample} - A_{blank})x100}{A_{control}} \quad \text{Equation 2}$$

in which, $A_{control}$ is the absorbance of the DPPH solution with methanol as the control, A_{sample} is the absorbance of the DPPH solution with the test sample and A_{blank} is the absorbance of the sample without the DPPH solution.

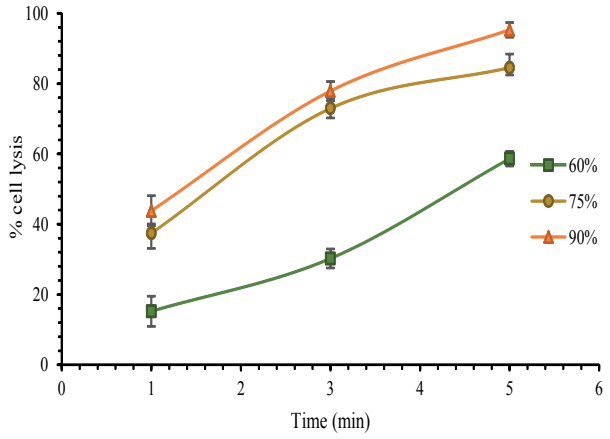
RESULT AND DISCUSSION

The ability of cells to rupture, using ultrasound as a mechanical disruption method, can be seen in figure 1. The ratio of ruptured cell augmentation was directly proportional to the increase in equipment amplitude and time. Therefore, the capacity of cellular rupture and consequent extravasation of the cellular material is directly related to the amplitude of the equipment and time of treatment undergone.

With the maximum amplitude (90%) and longer time (5 min) tested a breakability of the cells of approximately 95% was observed, and this treatment was chosen for later application and extraction of compounds.

Singh *et al*. (2015), verified the extraction of carotenoids with and without cell disruption and concluded that acetone is unable to extract all intracellular carotenoids without effective cell disruption. From the moment, the cell wall was disrupted by ultrasound, the astaxanthin yield was exponential, and this was directly related to the percentage of ruptured cells.

Figure 1 - Percentage of ruptured cells of microalga *Scenedesmus obliquus* using ultrasound, with an amplitude of 60, 75, or 90%, at times of 1; 3 or 5 min



The results of the centesimal composition of *S. obliquus* microalgal biomass are presented in Table 1. As can be seen, the microalga presents high amounts of compounds that are considered of great interest in the food industry, such as high protein, lipid, and carbohydrate contents. Protein values of 40.42 and 40.69% were found by Silva *et al*. (2020), Afify *et al*. (2018), respectively, for microalga *Scenedesmus obliquus*. Silva *et al*. (2020), found values of 05.57% for lipids, 28.0% for carbohydrates, and 15.64% for ash. The values of the centesimal composition of microalgae vary within the same species according to the type of cultivation used, collection time, and stress factors, such for example, as absence or excess of nutrients (AFIFY *et al*., 2018;; ROCHA *et al*., 2019; SILVA *et al*., 2020; SOARES *et al*., 2018).

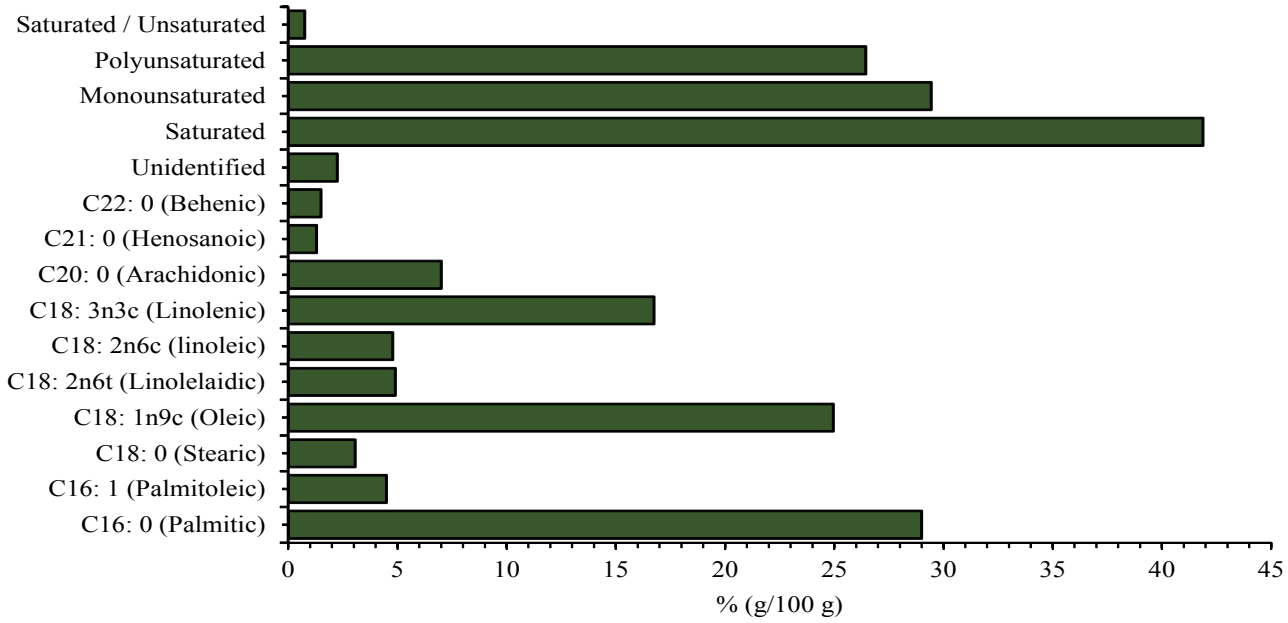
The fatty acid profile of the microalga *Scenedesmus obliquus*, presented in Figure 2, indicates the predominance of unsaturated and polyunsaturated fatty acids.

Regarding the saturated fatty acid methyl ester (FAME), palmitic acid (C16: 0) accounts for 29% of total fatty acids. The fatty acid profile of the microalga *Scenedesmus obliquus*, presented in Figure 2, indicates the predominance of unsaturated and polyunsaturated fatty acids. Regarding the saturated fatty acid methyl ester (FAME), palmitic acid (C16: 0) accounts for 29% of total fatty acids. On the other hand, for unsaturated fatty acids, the amount of polyunsaturated fatty acids (PUFA's) corresponds to 47.31% of the total, with predominance of omega-3 (n-3 PUFA), linolenic acid (16.74%), and approximately 9.70% of omega-6, with the presence of linoleic acid (n-6 PUFA) and linolelaidic acid. This high amount of linolenic acid (n-3), an essential fatty acid of the omega-3 family present in microalga *S. obliquus*, suggests the use of this species as a source of such fatty acid, which can be extracted and used to supplement food and increase consumption for the population. In addition, *S. obliquus* also has linoleic acid (n-6), which is an essential fatty acid of the omega-6 family, in a significant amount (4.79%).

Table 1 - Centesimal composition of microalga *Scenedesmus obliquus*

Components	(% g.100 g^{-1})
Moisture	8.03
Protein (factor = 5.89)	37.15
Lipids	10.29
Ashes	20.24
Carbohydrates	21.93

Figure 2 - Fatty acid composition of microalga *Scenedesmus obliquus* oil



The results found in the present study from the fatty acid profile of microalga *S. Obliquus*, are in agreement with results found by other authors (ROCHA *et al*., 2019; SILVA *et al*., 2020; WILTSHIRE *et al*., 2000). Silva *et al*. (2020), found significant amounts of C18:3n3, C18:2n6, and C18:1n9 in oil extracted from the microalgae *S. obliquus*, stating that this microalga has the potential to be sources of these compounds. Rocha *et al*. (2019), found high amounts of linolenic (> 15%), oleic (> 14%), and linoleic acids (> 10%) for the different types of crops tested by these authors and demonstrate, in their work, that the type of cultivation significantly affects the fatty acid profile of the microalgae *S. obliquus* oil.

The benefits of omega-3 polyunsaturated fatty acids consumption for health are already well known, and their influence on brain development and prevention of cardiovascular diseases has been proven in different epidemiological and clinical studies (CALDER, 2014; GHEYSEN *et al*., 2018). Besides, consumption of n-3 PUFA is lower than that of n-6 PUFA, so the incentive for the consumption of n-3 PUFA is high, and there is a growing interest in enriching food products with n-3 PUFA. It should be noted that the ingestion of such products is still far below that recommended, in several countries (RYCKEBOSCH *et al*., 2012; SIOEN *et al*., 2010). Several authors report some species of microalgae as a sustainable alternative source of polyunsaturated fatty acids of the omega 3 family. The use of those microalgae species could be a way to increase the consumption of n-3 PUFA, given the reduction of the world stocks of fish and fish oils (GHEYSEN *et al*., 2018; RYCKEBOSCH *et al*., 2012; SATHASIVAM *et al*., 2019; SILVA *et al*., 2020). The omega-3 lipid accumulation in the microalgae *Dunaliella salina* and *Chlorella vulgaris* was induced and stimulated by the use of saline stress (RISMANI; SHARIATI, 2017).

The levels of total carotenoids extracted from *S. obliquus* were 1.61 ± 0.18 and 1.03 ± 0.03 g·100 g^{-1} microalga, respectively for hexane and petroleum ether, with hexane being the most efficient solvent for this extraction compared to petroleum ether.

Most carotenoids have therapeutic value, being effective agents for preventing a variety of human diseases, including anti-cancerous and anti-inflammatory activities, due to its strong antioxidant effect which is used to protect against oxidative stress. The natural form of these compounds has a stronger effect and can be easily absorbed by the body when compared to the synthetic form. Therefore, the high consumption of natural carotenoids and the research of new sources and ways to increase the production of these bioactive compounds has been widely encouraged (CHEN *et al*., 2016). Furthermore, several authors reported the high accumulation of lutein as the major carotenoid of the microalgae of the genus *Scenedesmus* spp. (CHAN *et al*., 2013; PŘIBYL *et al*., 2015).

According to Pribyl *et al*. (2015), on their study with the microalgae Scenedesmus sp. in a 7-day culture in the laboratory, these microalgae presented a content of 0.68% carotenoids, while in a 14-day culture with saline stress, it reached 2.08% of carotenoids, indicating that the

culture time and the induced stress influenced the amount of accumulated carotenoids. Besides, these authors reported that thermotolerance is a common feature in Scenedesmus spp., which makes this microalgae genus more interesting for future large-scale production of biomass rich in carotenoids.

Several studies have been reported about the influence of stresses on microalgae for carotenoid accumulation. Some studies have shown that the production of β-carotene can be increased by intervention in the growing conditions. For instance, high salinity, reduced nutrient content, high light incidence, and extreme temperature are extrinsic parameters that could be controlled in order to increase the carotenoid contents in *Scenedesmus* spp. (KRISHNA KOYANDE *et al*., 2019; RAJA *et al*., 2007). In addition, an increase in lutein and astaxanthin has also been reported with induced stress in the culture (CHAN *et al*., 2013; HIGUERA-CIAPARA; FÉLIX-VALENZUELA; GOYCOOLEA, 2006).

In some species of microalgae, the synthesis of lipids and pigments occurs in a coordinated manner. Thus, in adverse conditions, lipids are produced in response to stress and accumulate in cytoplasmic oily bodies. In these places, some carotenoids are also deposited and can exert antioxidant activity in protecting unsaturated lipids against peroxidation (KIM *et al*., 2014). In this way, the accumulation of these compounds of interest can be induced even without affecting its quality.

The microalga *S. obliquus* presented high amount of carotenoids and high extraction capacity of such bioactive compounds by using organic solvent and thus considered as a source of natural carotenoids. However, this amount can still be improved through the induction of stress in microalga cultivation.

The results obtained for the extraction of phenolic compounds was 411.69 µg AGE·mL^{-1} in the extract. The microalga *S. obliquus* presented 1.12 g of total phenolic compounds in 100 g of microalga. Similar results were found by Silva *et al*. (2020). The microalga *Arthrospira platensis*, mutants were induced by an electron beam, and the total phenolics increased from 9.7 mg L^{-1} to 17.0 mg L^{-1} and consequently increased antioxidant activity (KIM *et al*., 2014).

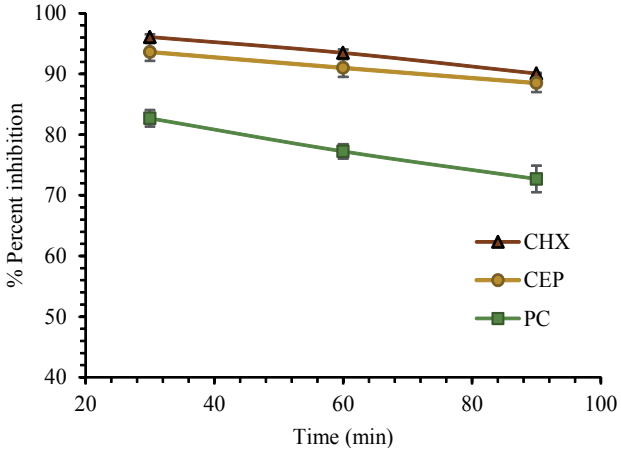
The microalgae react in different ways in the defenses against free radicals and oxidations, including reactive enzymes in the elimination of oxygen and antioxidant activity. Antioxidant agents, such as phenolic compounds, acted as free radical oxidation terminators and were recognized for their physiological, biological and medicinal activity, receiving increasing attention in the areas of health, biology, and food, especially in the search for natural antioxidants that can prevent degenerative diseases (KIM *et al*., 2014; ROJAS; BUITRAGO, 2019).

Studies evaluating the in vivo activity of phenolic compounds have found several beneficial actions in these extracts, including anti-prostate cancer agents, plasma antioxidant, with a beneficial to oxidative damage, and activity antioxidants for the prevention of degenerative diseases (HAJIMAHMOODI *et al*., 2010; KAZUI *et al*., 2018).

The results of the antioxidant activity of carotenoid extracts and phenolic compounds of the microalga *Scenedesmus obliquus* were obtained using the DPPH method (Figure 3).

Carotenoid extracts and phenolic compounds showed a high percentage of inhibition of oxidation >88% and >72%, respectively) at all times analyzed (30, 60, 90 min) (Figure 3). The higher the percentage of inhibition, the higher the DPPH consumption by the sample, and the higher the antioxidant activity (MAADANE *et al*., 2015). In this way, it is noticeable that the carotenoids and phenolic compounds extracted from the microalga *Scenedesmus obliquus* presented antioxidant activity with up to 95% inhibition even when using low concentrations. Silva *et al*. (2020), found a percentage of inhibition of DPPH oxidation of 95.53% for carotenoid extracts and 96.09% for phenolic compounds extracted from the microalga *S. obliquus*. Other species reported in the literature also showed antioxidant potential, such as the study by Maadane *et al*. (2015), in which they found high antioxidant potential in several strains of microalgae, with greater DPPH inhibition capacity (> 80%) Dunalliela sp. Tetraselmis sp. and Nannochloropsis gaditana. These microalgae had a high content of total polyphenols,

Figure 3 - Evaluation of the antioxidant activity of the carotenoids extracts (195.79 µg·mL^{-1} in hexane - CHX, and 184.71 µg·mL^{-1} petroleum ether – CEP) and phenolic compounds (411.69 µg AGE·mL^{-1} extract) of the microalga *Scenedesmus obliquus*



carotenoids and PUFA, indicating that these strains may be a potential new source of natural antioxidants.

The experiment was conducted by measuring the absorbance of the samples every 30 min., during 90 min. The decrease in the percentage of inhibition was observed over time. This reduction in antioxidant activity is associated with the oxidation process of carotenoids during the reaction with the free radicals (FR) generated by DPPH. The antioxidant activity of phenolic compounds is mostly due to their oxi-reduction properties, which may play a role in the absorption and neutralization of free radicals (MAADANE *et al*., 2015).

The microalga *S. obliquus* presented a high amount of carotenoids, phenolic compounds, and antioxidant activity even in low concentrations, in relation to other species reported in the literature (HAJIMAHMOODI *et al*., 2010; MAADANE *et al*., 2015; RISMANI; SHARIATI, 2017) demonstrating a potential use in the food industry. According to Devi *et al*. (2009), the food industry is seeking the use of natural antioxidants, isolated from plants and seaweed, to replace synthetic food additives, since these non-natural additives may have harmful effects on health. Also, the oxidative stress that is involved in the development of various degenerative diseases attracted the interest of researchers in investigating the antioxidant activity of natural products. The consumption of phenolic compounds can help the human body to reduce the damage oxidative diseases related to aging and diseases such as arteriosclerosis, ulcer, diabetes, and cancer (DEVI *et al*., 2009; KAZUI *et al*., 2018).

In a study carried out by Silva *et al*. (2020), it was verified the consumption of microalga *S. obliquus* by Wistar rats and observed that the amounts of bioactive compacts of this microalgae, including carotenoids, phenolic compounds, essential fatty acids, etc., significantly affected the content of triglycerides (70%), the atherogenic index (80%) and the concentration of serum glucose (42%), in the blood of the animals, even using a balanced diet. In addition, these authors reported that *S. obliquus* may represent a promising sustainable source of functional and nutraceutical foods for possible prevention and treatment of diabetes and dyslipidemia.

CONCLUSIONS

1. The microalga *S. obliquus* showed a high concentration of polyunsaturated fatty acids including alpha-linolenic acid, from the omega-3 family. In addition, significant amounts of carotenoids and phenolic compounds were extracted from *S. obliquus*, and showed antioxidant activity with a percentage of inhibition of up to 95%, even using low concentrations;
2. Thus, it is concluded that the microalga *Scenedesmus obliquus* is an alternative, natural and sustainable source of biologically active compounds, which can potentially be used in supplementation or as an additive in the food and pharmaceutical industries.

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