Increase in antioxidant activity of *Nannochloropsis* sp. through stress cultivation conditions

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INTRODUCTION

Nowadays, there is a great demand on the use of microalgae, or their derivatives, as novel foods or food ingredientes. Microalgal supplements provide an optimal solution to balance the diet of people who do not consume fish, promoting a healthy diet and reducing metabolic and aging pathologies with an environmentally and economically sustainable approach (1).

The genus *Nannochloropsis* sp. has a high content of antioxidants, specific vitamins, PUFAs (in particular EPA) and is suitable for intensive culture thus being a good candidate for human consumption (1).

This research work was done under the scope of the project Nº 33784: EXTRATOTECA – High Value Added Microalgae Extracts.

AIMS

Comparison of antioxidant activity and total phenolic content of a strain of *Nannochloropsis* sp. cultivated under stress and unstressed conditions subject to extraction by High Pressure (HPE). The strain was cultivated by A4F – Algae for future and extracted by HPE by the Chemistry Department of the University of Aveiro.

MATERIALS AND METHODS

A strain of *Nannochloropsis* sp. was cultivated in A4F – Algae for Future - facilities, Lisbon, Portugal in normal and stress induced conditions. Stress induced conditions intended to increase the production of compounds of interest and are under the confidentiality of the company. The biomass was concentrated and sent as frozen paste. High pressure extration (HPE) methodology (with processing conditions of 500 MPa during 15 min at room temperature) was applied to samples diluted in tap water (approximately 1:2) of the frozen paste, to promote cell disruption and improve the release of compounds of interest from the cells. After processing by HPE, the samples were stabilized in frozen form at -20ºC and also submitted to freeze-drying process for further chemical analysis.This task was done at the Chemistry Department of the University of Aveiro. There were control batches on all steps of the experiment, for both normal and stress induced conditions, in a total of 10 samples. Briefly the samples were as follows for both culture conditions: integral biomass frozen paste (IB), extract (control) from the integral biomass frozen paste (EC-FP), extract (control) from the sample diluted in tap water but without HPE treatment followed by liofilization (EC-L), extract from the sample that underwent HPE followed by liofilization (HPE-L) and extract from the sample that underwent HPE followed by freezing (HPE-FP).

Preparation of microalgae extracts:

10 g of microalgae powder or frozen paste, according to the condition of the sample, were added to 100 mL of absolute ethanol. The extraction flask was kept on a platform shaker at 180 rpm for 24h at room temperature and the supernatant was recovered. The extraction was repeated three times and all three supernatants were combined. The solvent was removed by rotary evaporation at room temperature. The concentrated extracts were weighed and the yield expressed as (w/w) of sample. At this preliminary stage, only one extract per sample was performed, so the yield value is not the average of triplicate measurements, but a single one.

Methodology for DPPH – free radical scavenging assay, ferrous-ion chelating ability (FICA) and total phenolic content was determined according to Corrêa *et al* (2022) (2) in triplicate samples. The mean and standard deviation were calculated.

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PRELIMINARY RESULTS AND DISCUSSION

For comparison reasons, to standardize the results, assay data was multiplied by the extract yield, dry weight percentage and dilution factor for HPE, thus presenting the results per unit of initally weighed dry biomass.

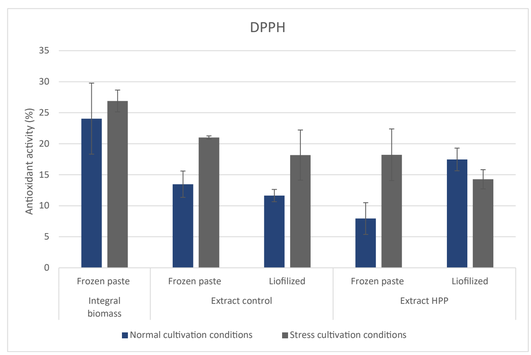

Fig. 1 – Antioxidant activity (DPPH) of *Nannochloropsis* sp. under stressed and unstressed conditions of cultivation.

From the graphic in Fig. 1, it is visible that in four out of five paired samples, the stress cultivation conditions yielded higher values. The exception is the extract HPE liofilized which can be due to the higher extract yield on the unstressed conditions of cultivation.

In FICA assay (Fig. 2) the difference between stress and normal cultivation conditions is not so significant as in DPPH assay. The reason must be elucidated with further analysis of the extract composition.

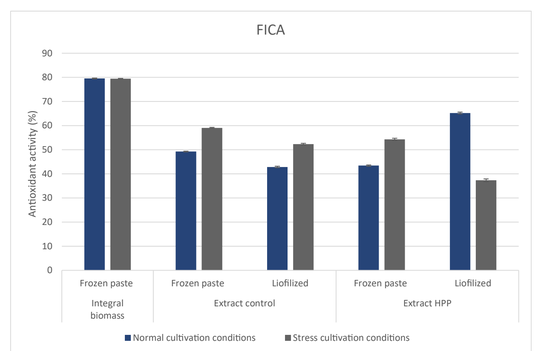

Fig. 2 – Antioxidant activity (FICA) of *Nannochloropsis* sp. under stressed and unstressed conditions of cultivation.

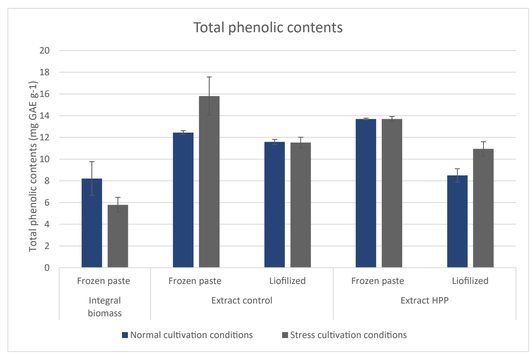

Fig. 3 – Total phenolic contents of *Nannochloropsis* sp. under stressed and unstressed conditions of cultivation.

Contrary to DPPH, and as shown in Fig. 3, total phenolic content was higher for all the extracts than for the integral biomass, indicating that maybe there are other compounds that contribute to radical scavenging activity, more readily available in the extracts than in the integral biomass.

CONCLUSIONS AND PERSPECTIVES

There was a visible increase in the antioxidant activity (DPPH) in stress cultivation conditions in comparison to normal cultivation conditions.

The antioxidant activity may be ascribed to compounds other than total phenolic since their quantification does not follow the graphic tendency of the antioxidant activity (DPPH).

For both antioxidant assays, it seems that the integral biomass has higher values than any of the extracts. However, in this experiment, it was used the same antioxidant quantification methodology for both the integral biomass and extracts. Some authors have different methodologies, therefore it is needed further research for confirmation.

Water extracts were also performed and should be soon analysed for comparison with the ethanolic extracts not only for antioxidant activity but for other parameters.

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