Optimization of green extraction methods for the effective recovery of bioactive compounds from *Nannochloropsis oceanica* microalgae

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1. Introduction

Advanced high-pressure techniques such as supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) are being widely employed to recover bioactive compounds from different sources. Furthermore, these techniques can be considered as environmental friendly as they allow the use of GRAS (generally recognized as safe) solvents such as CO_2, ethanol or water[1].

Microalgae are a promising and renewable feedstock since they are rich in high-value bioactive compounds that are currently used in many industries, such as food, cosmetic, biodiesel or pharmaceutical industries[2]. An interesting microalga with a high content of bioactive compounds is *Nannochloropsis oceanica.* This green microalga has a thick cellulosic wall that protects all its components, including polyunsaturated fatty acids (PUFAs) and terpenoids, mainly carotenoids (such as violaxanthin and vaucheraxanthin, which are highly appreciated and valuable for commercial applications).

A relevant challenge, considering the bioactives that can be extracted from this microalga, is to design a suitable method able not only to efficiently extract the target compounds but also to comply with the green chemistry principles and sustainability issues. Thus, the aim of this work is to optimize the recovery of bioactives from *N. oceanica* using a procedure based on the use of green compressed fluids. Moreover, since this microalga has a strong cell wall, the effect of different pretreatments and high pressures for cell wall disruption were studied, in order to improve the extraction efficiency. All extracts were chemically characterized using chromatographic methods.

2. Results and discussion

The strategy has been selected considering the compounds of interest that can be found in *N. oceanica*, the rigidity of its cell wall and the use green pressurized liquids to extract these compounds.

In this way, a first screening of compounds was performed using a conventional ethanol extraction. Extracts were analyzed by HPLC-DAD-MS/MS, showing that *N. oceanica* is rich in carotenoids such as violaxanthin and vaucheraxanthin, among others, and also in chlorophyll a and derivatives. These results are in agreement with those described in the literature[3].

In order to select the optimum pretreatment for better breakage of the cell wall, a conventional carotenoid extraction was performed after each procedure. Results indicated that the highest content of carotenoids was extracted after the pretreatment based on freeze-thaw (FT) cycles, so it was selected as pretreatment before the pressurized extraction.

For the optimization of the carotenoid recovery, an experimental design using PLE was proposed. Pressure and extraction time were kept constant (100 bar and 20 min) whereas extraction temperature from 40 to 150 ^{o}C and number of cycles from 1 to 3 cycles were the studied variables. Extraction yield and total carotenoids content were the response variables considered. After all experimental analyses, the optimum conditions

proposed by the statistical model were 57 ºC and 3 cycles. As expected, experimental results for these conditions were similar to those predicted by the model.

Alternatively, a one-step procedure including pretreatment and extraction was studied. Here, the initial biomass is subjected to ultra-high pressure (up to 6000 bar) extraction that should be able to break the cell wall, whereas extraction using ethanol as solvent takes place simultaneously. Considering the results obtained in the previous optimization and the working conditions allowed by the equipment, extraction temperature and cycles parameters were set as 50 ºC and 3 cycles, respectively. Moreover, three different pressures were studied: 1000, 3000 and 6000 bar.

A preliminary test, in which only one extraction cycle was applied, was performed in order to verify that *N. oceanica* cell wall was being effectively damaged by high pressure. As it can be seen in **Figure 1**, high pressure positively affects to more intense disruption of *N. oceanica* cell walls. Next, 3-cycle experiments were carried out. As expected, results showed an important increase in both extraction yield and carotenoid content, indicating that this one-step procedure using ultra-high pressure could be a useful and quicker method to extract bioactive compounds directly from microalgae biomass and, thus, avoiding all pretreatment steps.

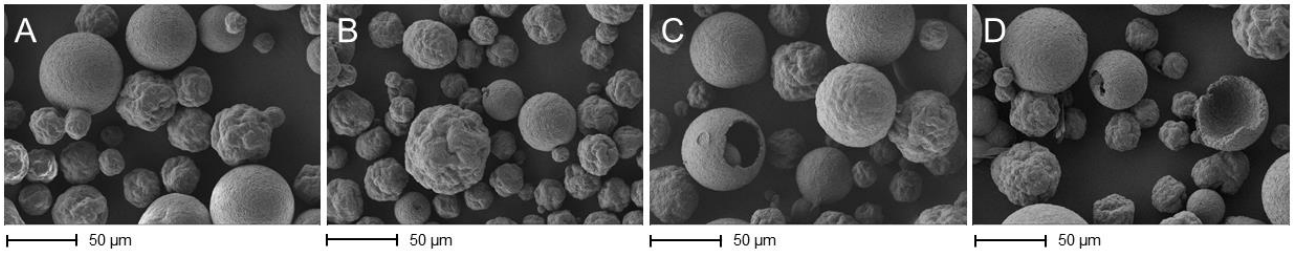

Figure 1. SEM images of *N. oceanica* before and after high pressure. A: not treated; B: 1000 bar; C: 3000 bar; D: 6000 bar.

3. Conclusions

In the present work, an optimization process for the recovery of bioactives from the microalga *N. oceanica*, based on the use of green compressed fluids, is proposed. As main carotenoids in *N. oceanica* are relatively polar, ethanol was used as the pressurized solvent, and a high carotenoid content was achieved using parameters proposed by a 3-level factorial model. The pigment profile of *N. oceanica* showed violaxanthin and vaucheraxanthin as main pigments. Further studies using different solvents or pressurized techniques such as SFE or GXL should be carried out using the remaining residue in order to extract other bioactive compounds in *N. oceanica*, following a biorefinery approach. Finally, ultra-high-pressure processing revealed as a useful tool for cell wall disruption, facilitating the extraction of bioactives directly from the biomass.

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