# Green compressed fluid technologies for the extraction of bioactive compounds from *Porphyridium cruentum* in a biorefinery approach

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## 1. Introduction

Microalgae are promising microorganisms that can play a key role in bio-based economy, since they may serve as a continuous and reliable source of safe natural products[1]. *Porphyridium cruentum* is a red microalga rich in bioactive compounds, such as proteins, polysaccharides, PUFAs and pigments. This microalga is able to accumulate great amounts of zeaxanthin and β-carotene, with beneficial physiological functions, such as anti-cancer, anti-diabetic, and aged-related macular degeneration[2]. However, in order to improve the sustainability and economic feasibility of the biomass production process, it is necessary to obtain other high value compounds from the same biomass, following a biorefinery approach. Indeed, *P. cruentum* can accumulate up to 2 % (w/w) of phyocoerythrin, which is a protein commonly used as a fluorescence-based indicator in biomedical investigations and also as a natural colorant in the food and cosmetic industries. Furthermore, it may have therapeutical value since some researchers demonstrated its immunomodulatory and anti-cancer activities[3].

In the present study, two new biorefinery approaches for the extraction of bioactive compounds from *P. cruentum* microalgae have been developed using pressurized green solvents with different techniques, such as pressurized liquid extraction (PLE) and high pressure processing (HPP), in order to achieve high added-value extracts. The extractions were carried out in two sequential stages: (1) pressurized pure water at 25 ºC, (2) pressurized pure ethanol at high temperature. The residual biomass after each extraction was used as raw material for the following extraction. The first step was carried out in order to obtain phycoerythrin-enriched extracts, while the second step was performed with the main objective of extracting carotenoids. All extracts were chemically characterized using spectrophotometric and chromatographic methods.

## 2. Results and discussion

The strategy has been selected considering the extraction of phycoerythrin in the first step, to avoid its thermal degradation [4] and with the main aim of recovering the amount of carotenoids in the second step. For this purpose, carotenoids extraction was independently optimized and, later on, integrated in a complete biorefiney process.

For the optimization of the carotenoids extraction, pressurized ethanol extractions (100 bar) were carried out at five different temperatures (from 50 ºC to 150 ºC) for 20 min. These results were compared with the values obtained from a conventional extraction with ethanol (24 hours, 500 rpm, atmospheric pressure, 20 ºC). All extraction yields varied between 3.12 and 11.36 %, increasing with temperature due to an improvement in the mass transfer from the sample to the extraction solvent, as a result of an increase in the vapor pressure of the compounds and a decrease in the viscosity of the solvent. According to carotenoids content, all the pressurized extractions were significantly higher than the conventional extraction. Results indicate that the highest content of carotenoids was obtained at 125 ºC, so it was selected as the extraction temperature for PLE. However, no significant differences between 70-150 ºC were found.

Then, the extracts from the biorefinery process using PLE (100 bar, 20 min) were obtained. In the first step, aqueous extracts were obtained (at 25 ºC and 20 min), with high amounts of phycoerythrin (up to 25 mg/g

extract); second step considered optimum conditions selected previously (ethanol, 125 ºC, 20 min), achieving high carotenoids content (with no significant differences compared to the optimization step). HPLC-MS characterization confirmed zeaxanthin and β-carotene as the major carotenoid compounds.

Alternatively, a second biorefinery process was evaluated using ultra-high pressure (up to 6000 bar). Taking into account the results obtained in the previous optimization and the working conditions allowed by the equipment, extraction temperatures were set at 25 ºC (for water) and 70 ºC (for ethanol). Furthermore, three different pressures were studied: 1000, 3000 and 6000 bar. For comparison purposes time was fixed at 20 min.

Extraction yields and phycoerythrin content in the first step were above the 7% and 30 mg/g extract, respectively, higher than the values obtained using PLE. The higher the pressure, the higher the phycoerythrin content; nevertheless, results indicates that at the highest pressure (6000 bar), not only breakage of the cell wall was observed but also a degradation of the protein itself. Moreover, the protein purity values obtained at 1000-3000 bar are higher than 3, which are also higher than the obtained using PLE. Recovery of carotenoids in the second HPP step using ethanol as extracting solvent provide a new process alternative that should be studied in depth in order to select the best biorefinery approach.

## 3. Conclusions

In the present work, two biorefinery approaches were described for the first time to extract bioactives compounds from microalga *P. cruentum* using GRAS – generally recognized as safe – solvents and pressurized technologies. Extractions were performed in two sequential steps using (1) water and (2) ethanol, considering the residue of the first extraction step as the raw material for the second extraction. Step 1 provides the extract enriched in phycoerythrin, while step 2 provides the extract enriched in carotenoids, mainly zeaxanthin and β-carotene. These biorefinery approaches carried out using technologies considered as green, such as PLE and HPP could be considered sustainable processes.

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