17th European Meeting on SUPERCRITICAL FLUIDS

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

Mónica Bueno[a], Rocío Gallego[a], Angelica M. Chourio[b], Marina Martínez[a], Rémi Pradelles[c], Elena Ibáñez[a], Marleny D. A. Saldaña[b], Miguel Herrero[a]*

[a]*Laboratory of Foodomics, Institute of Food Science Research (CIAL, CSIC), Nicolas Cabrera 9, Madrid, 28049, Spain*
[b]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton AB, T6G 2P5, Canada*
[c]*Microphyt, 713 Route de Mudaison, Baillargues, 34630, France*

monica.bueno@csic.es

Introduction

Porphyridium cruentum is a red microalga rich in bioactive compounds, such as proteins, polysaccharides, PUFAs and pigments, including phycoerythrin, with immunomodulatory and anti-cancer activities [1] and zeaxanthin and β-carotene, with beneficial physiological functions, such as anti-cancer, anti-diabetic, and aged-related macular degeneration [2].
In order to improve the sustainability and economic feasibility of the biomass production process, it is necessary to obtain these high value compounds following a **biorefinery approach**, in which the residue of each extraction step is used as raw material for the next step.

Objective

The main goal is the recovery of bioactive compounds of interest from *P. cruentum*, specifically phycoerythrin in the fist step to avoid thermal degradation [3] and carotenoids zeaxanthin and β-carotene in the second step, using pressurized green solvents with different techniques, such as pressurized liquid extraction (PLE) and ultra-high pressure extraction (UHPE):

STEP 1: pure water, 20 min, 25 °C → phycoerythrin-enriched extracts

STEP 2: pure ethanol or ethyl acetate, 20 min, high temperature → carotenoids-enriched extracts

Materials and methods

Extraction methodologies

Solid-liquid extraction

Conventional extraction for carotenoids: 0.2 g dry algae, 20 mL acetone, 20 °C, 24 h

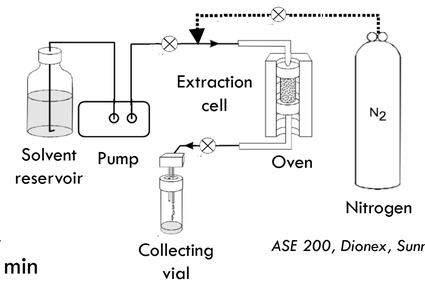
Conventional extraction for phyocoerythrin: 0.5 g dry algae, 50 mL Milli-Q water, 20 °C, 1 h

PLE

10 MPa

1) Water, 25 °C, 20 min

2) Ethanol, 50, 75, 100, 125,150 °C, 20 min



ASE 200, Dionex, Sunnyvale, CA, USA

UHPE

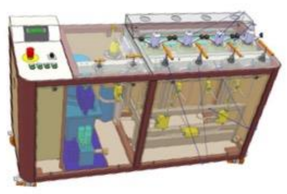
100 MPa, 300 MPa, 600 MPa

1) Water, 25 °C, 20 min

2) Etanol (EtOH) or ethyl actete (EtOAc), **70 °C***, 20 min

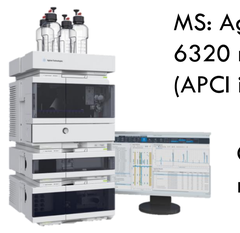
*Maximum temperature allowed by the equipment for these solvents

Apparatus U111 Unipress, Warszawa, Poland



Characterization

HPLC-MS/MS



MS: Agilent ion trap 6320 mass spectrometer (APCI interface).

Column: YMC-C30 reversed phase

Agilent 1100 series liquid chromatograph, Santa Clara, CA, USA

UV

Phycoerythrin: 280, 565, 620, 650 nm [4]

Total carotenoids content: 470 nm [5]

Absorbance microplate reader

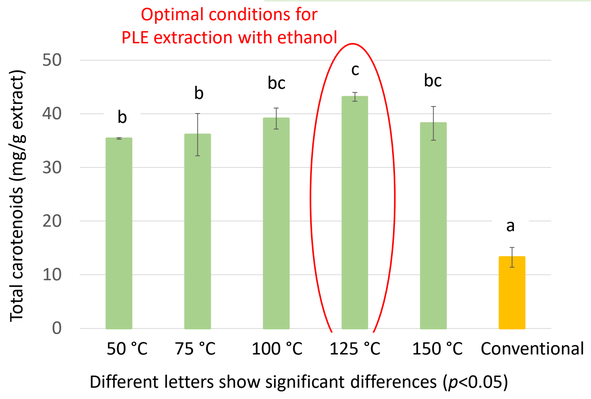
SEM

Carbon coating

Zeiss Sigma 300 VP-FESEM

Results

Optimization of carotenoids extraction by PLE



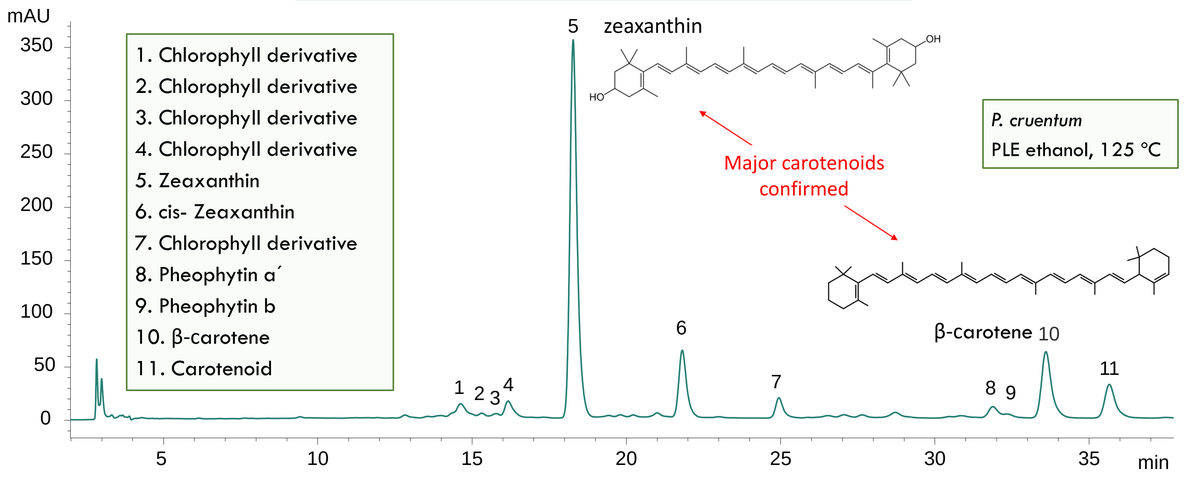
Different letters show significant differences (p<0.05)

In terms of total carotenoids, all pressurized extractions produced significantly richer extracts than the conventional extraction.

Extraction yields varied between 3.12% and 11.36%, increasing with temperature due to an improvement in the mass transfer from sample to the extraction solvent.

For the biorefinery process, 125 °C was selected as the optimal temperature for the second step.

Chemical characterization - HPLC



1) Biorefinery first step – Phycoerythrin extraction

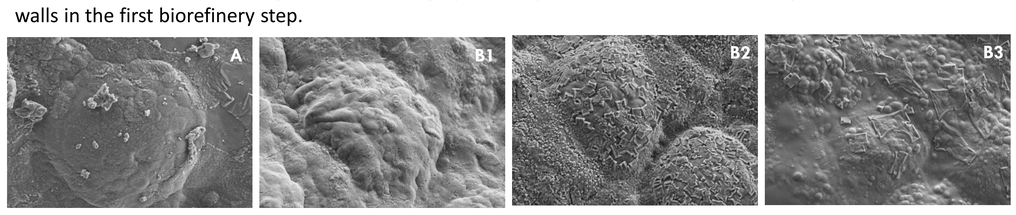
	Extraction yield (%)	B-Phycoerythrin (mg/g extract)	Purity (A_{545}/A_{280})
Conventional	36.14 ± 0.18^{e}	93.07 ± 2.68^{c}	1.71 ± 0.05^{c}
PLE 10 MPa	3.03 ± 0.19^{a}	13.21 ± 0.77^{a}	0.61 ± 0.03^{a}
UHPE 100 MPa	12.19 ± 1.71^{c}	86.97 ± 7.88^{c}	2.16 ± 0.11^{d}
UHPE 300 MPa	7.43 ± 0.46^{b}	144.43 ± 5.00^{d}	2.37 ± 0.09^{e}
UHPE 600 MPa	16.06 ± 3.42^{d}	33.51 ± 3.76^{b}	0.96 ± 0.05^{b}

Highest phycoerythrin amount and purity

Different letters show significant differences (p<0.05)

A pressure of 600 MPa is not only enough to break the cell wall, it also seems to denature the protein itself.

As it can be seen in SEM images (A, B1-B3), high pressure produces more intense disruption of *P. cruentum* cell walls in the first biorefinery step.



1 µm SEM images of *P. cruentum* before the extraction process (A), residue after the first step of HPE with water (25 °C) at 100 MPa (B1), 300 MPa (B2) and 600 MPa (B3).

2) Biorefinery second step – Carotenoids extraction

	Extraction yield (%)	Total carotenoids (mg/g extract)	Zeaxanthin (mg/g extract)	β-carotene (mg/g extract)
Conventional	7.67 ± 0.49^{c}	13.25 ± 1.86^{a}	7.49 ± 0.07^{ab}	
Optimum PLE	9.00 ± 0.67^{d}	43.15 ± 0.84^{e}	19.11 ± 4.33^{c}	6.48 ± 0.02^{b}
UHPE-EtOH 100 MPa	4.00 ± 0.17^{b}	24.46 ± 1.79^{c}	6.27 ± 1.05^{ab}	4.52 ± 0.65^{a}
UHPE-EtOH 300 MPa	3.44 ± 0.16^{b}	22.40 ± 1.53^{bc}	4.29 ± 0.71^{a}	4.07 ± 0.07^{a}
UHPE-EtOH 600 MPa	3.73 ± 0.68^{b}	21.31 ± 2.03^{b}	6.08 ± 0.08^{ab}	3.62 ± 0.47^{a}
UHPE-EtOAc 100 MPa	1.00 ± 0.04^{a}	59.81 ± 0.08^{f}	23.96 ± 0.51^{cd}	9.69 ± 0.51^{c}
UHPE-EtOAc 300 MPa	0.99 ± 0.18^{a}	65.05 ± 0.01^{g}	26.75 ± 5.63^{d}	11.89 ± 0.34^{d}
UHPE-EtOAc 600 MPa	1.37 ± 0.19^{a}	30.24 ± 0.09^{d}	10.97 ± 0.01^{b}	3.92 ± 0.34^{a}

Different letters show significant differences (p<0.05)

In terms of total carotenoids, all pressurized extractions produced significantly richer extracts than the conventional extraction.

More selective extraction of carotenoids is observed in the second step using UHPE at 100-300 MPa with ethyl acetate compared to PLE optimal conditions

Conclusions

- Two **biorefinery approaches** using **PLE** and **UHPE** were described for the first time to extract bioactive compounds from ***P. cruentum*** microalga using **GRAS** – generally recognized as safe – solvents.
- **Step 1** (water) provides extracts enriched in **phycoerythrin,** while **step 2** (ethanol or ethyl acetate) provides extracts enriched in **carotenoids,** mainly zeaxanthin and β-carotene.
- An important increase in the extraction selectivity of both phycoerythrin and carotenoids, indicates that the biorefinery approach using **UHPE at 300 MPa** with (1) **water at 25 °C** and (2) **ethyl acetate 70 °C** could be a useful and quicker method to extract bioactive compounds directly from *P. cruentum* biomass.

Acknowledgements

Authors thank projects ABACUS (Algae for a Biomass Applied to the production of added value compounds, grant agreement No 745668, funded by the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme), AGL2017-89417-R (MINECO, Spain), ILINK+1096 (CSIC, Spain) and Natural Sciences and Engineering Research Council of Canada (NSERC, #05356-2014) for financial support. M.B. acknowledges MINECO for a "Juan de La Cierva-Formación" postdoctoral grant FJCI-2016-30902.

References

1. R. Gallego, L. Montero, A. Cifuentes, E. Ibáñez, M. Herrero, *J. Anal. Test*. **2018**, 2, 109-123.
2. R. Bermejo, F.G. Acién, M.J. Ibáñez, J.M. Fernández, E. Molina, J.M. Alvarez-Pez, *J. Chrom. B* **2003**, 790, 317-325.
3. M. Martínez-Alonso, R. Gallego, E. Ibáñez, A. Cifuentes, M. Herrero, *XI FLUCOMP Meeting* **2018**, O17, 33.
4. R. Bermejo Román, J. M. Alvárez-Pez, F.G. Acién Fernández, E.J. Molina Grima, *Biotechnol*. **2002**, 93, 73–85.
5. B. Gilbert-López, J.A. Mendiola, J. Fontecha, L.A.M. van den Broek, L. Sijtsma, A. Cifuentes, M. Herrero, E. Ibáñez, *Green Chem*. **2015**, 17, 4599–4609.