„Development and Optimization of a Novel and Sustainable Process for the Extraction of Bio-based Phycobiliproteins from the Red Algae Palmaria palmata“
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1. Topic and objective

Phycobiliproteins (PBPs) are fluorescent proteins present in red macroalgae and cyanobacteria. These pigments constitute one of the most important groups of marine proteins and are used in several industries such as the cosmetic, food and pharma due to their multiple bioactivities such as antioxidant, antibacterial, antitumor, etc.[1,2]. Therefore, the aim of this work is the investigation of a new approach for the selective extraction and separation of phycoerythrin (PE), the most abundant PBP in red macroalgae, from *Palmaria palmata*. With this aim, a combined process based on enzyme-assisted extraction (EAE) for cell disruption and deep eutectic solvents (alone and in combination with aqueous two-phase system) as purification step has been performed. EAE is a promising approach for extracting macroalgae compounds with high selectivity, low energy requirements, and gentle condition[3]. Deep eutectic solvents (DES) are biodegradable, non-toxic, easy to prepare and low cost solvents systems than can be used in the extraction and separation of bioactive compounds from natural resources[4].

2. Work plan

An overview of the work plan of this project is shown in Figure 1.

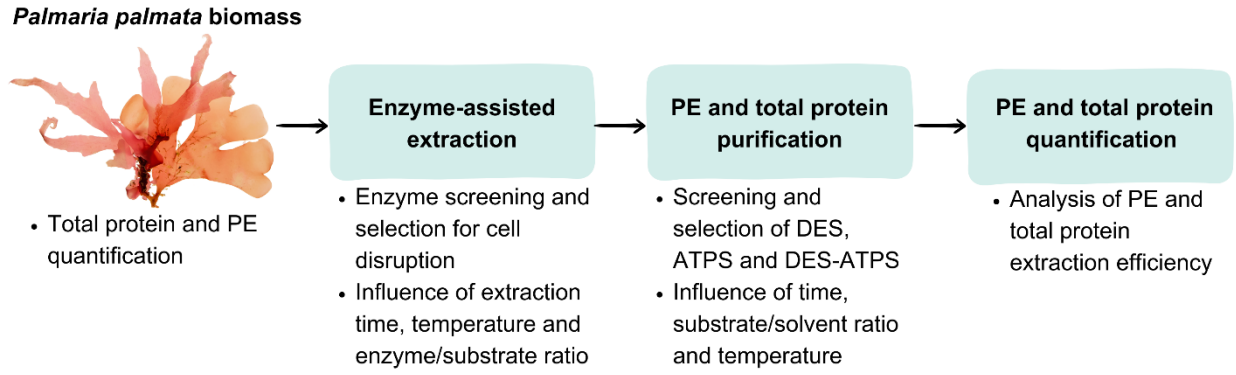

Figure 1. Overview of the steps performed in this project (PE = phycoerythrin, DES = deep eutectic solvents, ATPS = aqueous two-phase system).

Palmaria palmata biomass was first characterized in terms of the total protein and PE content. Enzyme-assisted extraction was used as cell disruption method and based on *P. palmata* cell wall composition, xylanase and Celluclast 1.5L were selected. For both enzymes, the influence of temperature (30 – 50 °C xylanase and 40 – 60 °C Celluclast 1.5L), extraction time (3 – 7 h) and enzyme/substrate ratio (1 – 5 % v/w) was studied on the recovery of total proteins and PE

by a statistical experimental design. For purification, a screening of single deep eutectic solvents (choline chloride – glycerol, choline chloride – butanediol and betaine – propylene glycol) and combined with aqueous two phase system (betaine – glycerol, betaine – urea – water, betaine – glucose – water, choline chloride – glycerol and choline chloride – urea) was performed. Single ATPS based on polyethylene glycol (PEG) was also investigated. After screening, the effect of temperature, extraction time and substrate/solvent ratio on the recovery of PE and total proteins was investigated. Finally, a first attempt was made to scale up the DES system (choline chloride – glycerol) to a final volume of 0.4 L.

3. Results

The *Palmaria palmata* biomass used in this project contained 15.12 % dw of total proteins, with 14.1% dw of this fraction consisting of PE. The total protein content of the used biomass is in the middle range of what has been reported for *Palmaria* species in literature (8-35 % dw)[5]. Regarding enzyme-assisted extraction, results showed that xylanase is more effective than Celluclast 1.5L in the extraction of PE and total proteins, probably explained by the higher content of xylan than cellulose in *P. palmata* cell wall. Among the three studied parameters (temperature, extraction time and enzyme/substrate ratio), extraction time showed the highest influence on the recovery of total proteins when using xylanase, resulting in an increase from 12.2 to 13.6 % (dw) when increasing the extraction time from 3 to 7 h. Nevertheless, a significant effect of these parameters was not observed on PE recovery and purity, achieving a maximum yield of 6 % dw at 30 °C, 3 h and 3 % enzyme/substrate ratio. Celluclast 1.5L showed a statistically significant impact on the extraction efficiency of total proteins and PE, allowing a maximum experimental total protein recovery of 13.2 % dw (50 °C, 3 h and 5 % enzyme/substrate ratio) and 0.77 % of PE (40 °C, 5 h and 5 % enzyme/substrate ratio).

For both enzymes, the obtained models predicted that shorter extraction times and low temperatures are favorable for PE extraction. This behavior might be due to potential PE instability and loss of photoactivity at higher temperatures and extraction times as shown in Figure 2.

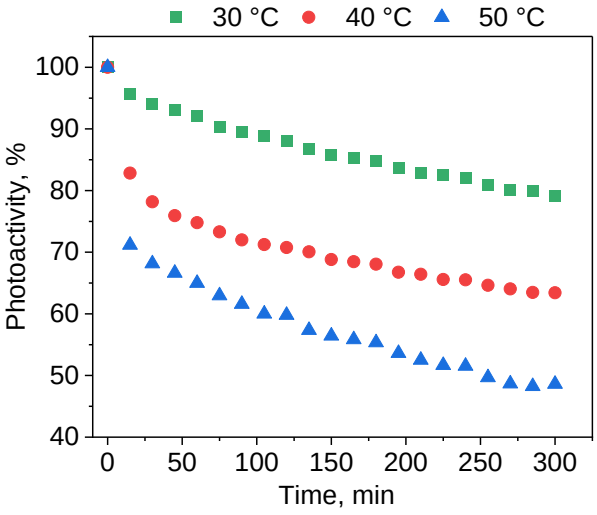

Figure 2. Temperature and time stability of PE at 30, 40 and 50 °C over 5 h.

After screening, ATPS with Polyethylene Glycol (PEG) proved to be the most effective method with 31.88, 45.17 and 45.74 % of PE recovery when using PEG 400, 6000 and 1500 molecular weight, respectively. This was followed by the DES-ATPS betaine – glycerol (10.98 %) and betaine - urea – water (2.42 %). The use of choline chloride – glycerol DES showed the lowest PE recovery (0.14 – 1.68 %) but the highest total protein recovery, with a maximum of 38 % dw. In this case, the ratio between substrate and solvent (w/v) was the parameter with the highest influence on the total protein extraction, leading to a decrease in protein recovery with increased substrate/solvent. This might be due to mass transfer limitations due to the high DES viscosity. The high viscosity of the choline chloride – glycerol DES was a challenge during the scale-up due to the difficulty to stir the system properly and allow the release and separation of PE, which led to a decreased recovery of PE in comparison to the screening experiments that were performed with a lower volume (0.01 L vs. 0.4 L).

The highest total antioxidant capacity (TAC), 102.6 µM Trolox Equivalents, was obtained on the hydrolysate after EAE with xylanase (30 °C, 3 h and 3 % enzyme/substrate ratio) and ultrafiltration. A significant decrease of 92.7 and 36.8 % was registered on the TAC after DES (choline chloride – glycerol) and ATPS (PEG 1500) treatment, respectively.

4. Conclusion

In this project, enzyme assisted extraction (EAE) and deep eutectic solvents (alone and in combination with ATPS) have been used to extract and separate phycoerythrin and total proteins from the red macroalgae *Palmaria palmata*. Regarding EAE, xylanase proved to be more effective than Celluclast 1.5L in terms of PE and total proteins recovery. The use of enzyme combinations could further increase extraction yields. Regarding the separation step, PEG-based ATPS was the most effective system followed by DES-ATPS and DES. Due to the low stability of phycobiliproteins and loss of photoactivity, low temperatures and shorter extraction times might be favorable for the extraction and separation process. Further experiments about the influence of other process parameters (pH, reaction media, etc.) on PE stability and bioactivity would provide useful information to increase the extraction efficiency of the used methods.

5. Presentation of the project in a DECHEMA event

Poster

Malvis Romero A., Wolter C. and Liese A. Enzyme-Assisted Extraction and Purification of Phycobiliproteins from the Red Macroalgae *Palmaria palmata*. Himmelfahrtstagung on Bioprocess Engineering 2023, 15 - 17 May 2023, Weimar

6. Literature

1. Saluri, M. et al. *Algal Res.* 37, 115–123 (2019).
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3. Dobrinčić, A. et al. *Mar. Drugs*, 18, 168, (2020).
4. Dai, Y. et al. *Anal. Chim. Acta* 766, 61–68 (2013).
5. Bjarnadóttir, M. et al. *J. Appl. Phycol.* 30, 2061-2070 (2018).