

## OPTIMIZATION OF R-PHYCOERYTHRIN EXTRACTION PROCESS FROM *Solieria filiformis*

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### ABSTRACT

R-Phycoerythrin (R-PE) is a high-value biomolecule found in red algae (Rhodophyta), which acts as a photosynthetic accessory pigment in red algae (Rhodophyta) and has gained importance in many biotechnological applications. *Solieria filiformis*, a species of red algae, has garnered attention due to its high content of phycobiliproteins, particularly R-PE. This work proposes to evaluate the effect of the grinding time, proportion algae:buffer and extraction time on R-phycoerythrin yield from *Solieria filiformis* and to determine optimized conditions of R-phycoerythrin mechanical extraction through a Central Composite Design (CCD). The solid-liquid extraction of R-PE was performed using 25 mM potassium phosphate buffer solution (pH 6.5) with different combinations of grinding time (2, 4, 6 min), proportion (1:2, 1:3, 1:4 (w/v)) and extraction time (2, 4, 6 h), as determined by CCD. This study confirmed the influence of grinding time and extraction time on the R-PE extracted by homogenization of *S. filiformis*. The optimized conditions predicted by the model equation were 6 minutes of grinding time, proportion algae : buffer 1:2 (m/v) and 4h15 of extraction time.

**Keywords:** Phycobiliprotein. Yield. Seaweed. Surface response methodology.

## 1 INTRODUCTION

R-Phycoerythrin (R-PE) is a high-value biomolecule found in red algae (Rhodophyta)<sup>1</sup>, which acts as a photosynthetic accessory pigment in red algae (Rhodophyta). This protein has gained importance in many biotechnological applications in food science, immunodiagnostic, therapy, cosmetics, protein and cell labelling, and analytical processes<sup>2</sup>.

*Solieria filiformis*, a species of red algae, has garnered attention due to its high content of phycobiliproteins, particularly R-PE. Despite its potential, the extraction process from *S. filiformis* poses several challenges, including the optimization of various parameters to maximize yield.

Considering that these biomolecules are located inside the cells, specifically attached on the stromatic side of the thylakoid membrane<sup>3</sup>, to extract them it's important to expose the pigment by breaking the cell wall, therefore grinding time becomes an interesting parameter to optimize extraction yield. Knowing that R-PE is a photosensitive red protein<sup>4</sup>, it is important to optimize extraction time to prevent exposure and degradation during the process, thus enhancing R-PE yield. Determining an optimal proportion allows reproducibility and the possibility of scaling the process.

This work proposes to evaluate the effect of the grinding time, proportion algae : buffer and extraction time on R-phycoerythrin yield from *Solieria filiformis* and to determine optimized conditions of R-phycoerythrin mechanical extraction.

## 2 MATERIAL & METHODS

The red macroalgae *Solieria filiformis* (Solieriaceae, Rhodophyta) was cultivated and furnished by Flecheiras and Guajiru Algae Producers Association (APAFG) - Trairi, Ceará State, Brazil. This species is registered under code A41C95F in the SisGen (National Genetic Heritage Management System). The macroalgae were harvested and were properly washed in potable water, separated from epiphytes, washed with distilled water, and stored at -20 °C until use.

The solid-liquid extraction of R-PE was performed following the adapted procedures previously described in literature<sup>5</sup>. The wet seaweed was ground in an electric mill in 25 mM potassium phosphate buffer solution (pH 6.5) and homogenated under mechanical agitation (model 713DS, Fisatom) at 250 rpm. The grinding time (min), proportion algae:buffer (m/v) and the extraction time (h) were determined by the experimental design. After this period, each homogenate was filtered through nylon fabric, the solid content was reserved, and the liquid phase was centrifuged at 17,000 × g for 30 min at 4 °C. The collected red supernatant, called crude extract of *S. filiformis* (CE-Sf).

The effects of grinding time ( $X_1$ ), proportion algae : buffer ( $X_2$ ), and extraction time ( $X_3$ ) on the R-PE yield ( $Y_1$ ) were evaluated. A Face-Centered Composite Design (FCCD), with 17 assays (14 factorial points and 3 replicates in the central point) was performed. Each factor in the design was studied at three different levels (-1, 0 and +1), as shown in Table 1.

**Table 1** Coded and actual values of the independent variables used in the experimental designs for the optimization of R-phycoerythrin extraction from *Solieria filiformis*

| Independent variables | Units | Coded and actual levels |     |     |
|-----------------------|-------|-------------------------|-----|-----|
|                       |       | -1                      | 0   | 1   |
| Grinding time         | min   | 2                       | 4   | 6   |
| Proportion            | m/v   | 1:4                     | 1:3 | 1:2 |
| Extraction time       | h     | 2                       | 4   | 6   |

The concentration of R-PE<sup>6</sup> of *S. filiformis* resulting from each extraction was determined in a spectrophotometer (Amersham Biosciences Ultrospec 2100 pro), from Equation 1:

$$R - PE \left( \frac{mg}{mL} \right) = 0,1247 * [(A_{564} - A_{730}) - 0,4583 * (A_{618} - A_{730})] \quad (1)$$

where *A* stands for the absorbance at different wavelengths. Equation 2 shows how the yield was calculated:

$$Y_{R-PE} (mg \cdot g^{-1}) = \frac{R - PE \left( \frac{mg}{mL} \right) \times Volume (mL)}{Seaweed wet weight (g)} \quad (2)$$

The method of analysis of variance (ANOVA) was used to verify the statistical differences between the groups, followed by Tukey's test of comparisons of means at a significance level of 95%. The experimental designs for CCD and their analysis were carried out by using the software STATISTICA (version 10.0, TIBCO Software Inc.). The regression coefficients (linear, quadratic and interaction) involved in the models were investigated by the Pareto analysis of variance at a significance level of 95%.

### 3 RESULTS & DISCUSSION

The results obtained by the FCCD are shown in Table 2, in which R-PE yields varied from 0.53 to 0.75 mg·g<sup>-1</sup> fresh weight.

**Table 2** Experimental matrix with the values (levels) of the dependent and independent variables evaluated in the FCCD for the R-Phycoerythrin extraction from *Solieria filiformis*

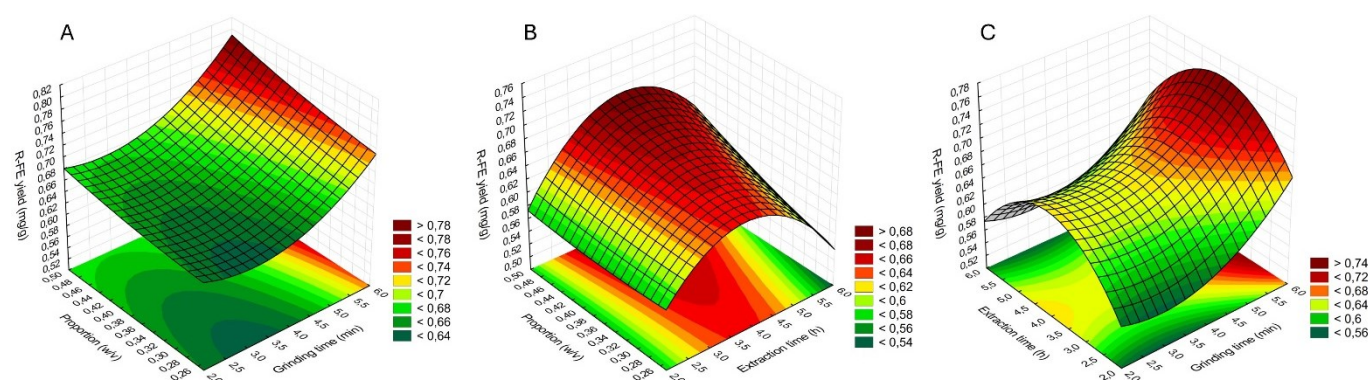
| Experiments | Independent variables |                  |                     | Dependent variable                 |
|-------------|-----------------------|------------------|---------------------|------------------------------------|
|             | Grinding time (min)   | Proportion (w/v) | Extraction time (h) | R-FE Yield (mg·g <sup>-1</sup> fw) |
| 1           | 2,00                  | 0,25             | 2,00                | 0,59                               |
| 2           | 2,00                  | 0,25             | 6,00                | 0,54                               |
| 3           | 2,00                  | 0,50             | 2,00                | 0,53                               |
| 4           | 2,00                  | 0,50             | 6,00                | 0,62                               |
| 5           | 6,00                  | 0,25             | 2,00                | 0,65                               |
| 6           | 6,00                  | 0,25             | 6,00                | 0,65                               |
| 7           | 6,00                  | 0,50             | 2,00                | 0,68                               |
| 8           | 6,00                  | 0,50             | 6,00                | 0,70                               |
| 9           | 2,00                  | 0,33             | 4,00                | 0,68                               |
| 10          | 6,00                  | 0,33             | 4,00                | 0,75                               |
| 11          | 4,00                  | 0,25             | 4,00                | 0,62                               |
| 12          | 4,00                  | 0,50             | 4,00                | 0,74                               |
| 13          | 4,00                  | 0,33             | 2,00                | 0,59                               |
| 14          | 4,00                  | 0,33             | 6,00                | 0,56                               |
| 15 (C)      | 4,00                  | 0,33             | 4,00                | 0,64                               |
| 16 (C)      | 4,00                  | 0,33             | 4,00                | 0,53                               |
| 17 (C)      | 4,00                  | 0,33             | 4,00                | 0,56                               |

Experiment n° 10, correspondent to the highest level of grinding time (6 min) and medium levels of proportion (1:3 (w/v)) and extraction time (4 h), led to the highest R-PE extraction yield. Conversely, for the experiment n° 3, correspondent to the highest level of proportion (1:2 (w/v)) and the lowest level of grinding time (2 min) and extraction time (2 h), the lowest R-PE extraction yield was obtained, with 0.53 mg·g<sup>-1</sup> fw. These results indicate the importance of higher grinding times, given that an increase in 4 min presented a difference of 1.4 times between the experiments. Taking into consideration R-PE has a high market value (US\$ 225/mg), this yield increase becomes relevant.

In this study, the experiment under 4 min of grinding time, proportion 1:3 (w/v) and 6 h of extraction time presented 0.55 mg·g<sup>-1</sup> fw of R-PE yield. The results obtained for the same extraction time were greater than previously reported by within our research group obtained from the same species: 0.14 mg·g<sup>-1</sup> fw of R-PE using the homogeneization method under 6 h of extraction time and proportion 1:3 (w/v)<sup>5</sup>; and 0.21 mg·g<sup>-1</sup> fw using cellulase from *Aspergillus niger* at concentration of 160 U·g<sup>-1</sup> fw, temperature 35 °C by 6 h<sup>7</sup>. Various factors such as seasonality, depth of cultivation ropes at sea, and other cultural conditions can directly influence the results<sup>8,9</sup>.

The influence of each variable on the R-PE extraction yield was determined. The linear variable of grinding time has a significant ( $p < 0.05$ ) positive effect (2.96) on the R-PE extraction yield, whilst the quadratic variable of extraction time has a significant negative effect (-2.91) upon on the evaluated response.

The response surface graphs (Fig. 6a-c) show the linear influence of the independent variables on the R-PE yield, whereas proportion and grinding time reach the highest response in its highest levels, the extraction time presents highest yield close to medium level.



**Figure 1** Response Surface Graphs showing the interactions of the effects between two variables on the R-PE yield. A – The interactive effect of proportion (w/v) and grinding time (min), B - The interactive effect of proportion (w/v) and extraction time (h) and C - The interactive effect of extraction time (h) and grinding time (min).

The  $R^2$  was determined for the dependent variable being 0.75 for  $Y_1$ . The ANOVA and lack of fit (LOF) test indicated a good model fit for extraction of the R-PE from *S. filiformis*. The model equation is shown below in Equation 3 and represent the yield of R-PE extracted.

$$Y_1 = 0,524875223 - 0,0784042844 \times X_1 + 0,011815247 \times X_1^2 - 0,522986643 \times X_2 + 0,314532524 \times X_2^2 + 0,152635583 \times X_3 - 0,0224025344 \times X_3^2 + 0,0300469665 \times X_1 \times X_2 - 0,000930927067 \times X_1 \times X_3 + 0,0864404785 \times X_2 \times X_3 \quad (3)$$

The model equation predicted the optimal conditions as: 6 minutes of grinding time, proportion algae:buffer 1:2 (m/v) and 4h15 of extraction time. Under these conditions, the predicted response was 0.79 and the confidence interval for validation varied between 0.63 and 0.95.

## 4 CONCLUSION

This study confirmed the influence of grinding time and extraction time on the R-PE extracted by homogenization of *S. filiformis*. The determined optimized conditions (6 min, 1:2 (w/v), 4h15min) for the R-PE extraction made it possible to obtain a 1.4 times higher yield. The improved extraction of R-PE could be attributed to an efficient cell wall breakdown, resulting in higher material transfer to the soluble phase, and a diminished extraction time, preserving the pigment. Based on these results, it would now be pertinent to validate the model equation, to characterize and to purify the R-PE, and to make an economic evaluation of this process thereafter.

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