OPTIMIZATION OF BIOACTIVE PEPTIDES EXTRACTION FROM CHLORELLA VULGARIS

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Introduction

Chlorella vulgaris may be a source of several interesting compounds, namely bioactive peptides with anticancer, antioxidant, anti-hypertensive activities. Furthermore, microalgae peptides may also be of great interest due to their functional properties as solubility, emulsifying and foaming properties. Algae peptides may be of great interest as active food or cosmetic ingredients, as preservatives for food or cosmetics, as pharmaceutical or nutraceutical to control or prevent diseases.

To empower peptides action in pharmaceuticals, food or cosmetics, they must be able to resist to adverse external factors. For it, peptide encapsulation can be a possibility.

Objectives

The microalgae cell wall is rich in polysaccharides making it rigid and difficult to digest and, consequently, limiting the extraction of proteins and generation of peptides. Whereby it is important to break cell wall to achieve a more efficient peptide extraction. Therefore, this work aimed to obtain an optimized microalgae extract rich in bioactive peptides, through the combination of acid and enzymatic hydrolysis. For that, *Chlorella vulgaris* was submitted to several extraction conditions, with variable factors including temperature, pH values, enzymes type, enzymes concentration, incubation time, use of salts and acids. To confirm the optimal extraction conditions, a Box-Behnken experimental design was performed using statgraphic centurion software, with three central points and in duplicated.

Methods

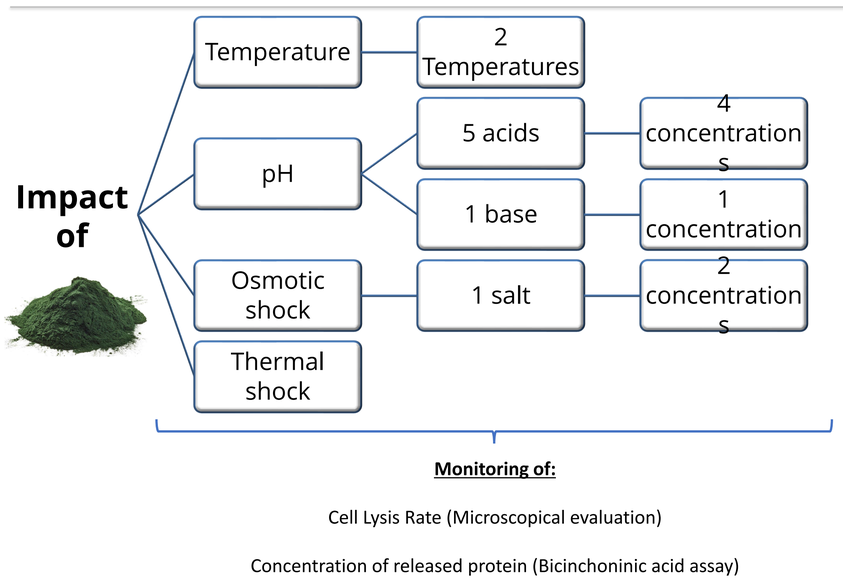

Figure 1: Conditions tested in order to verify ability of disrupting the microalgae cell wall.

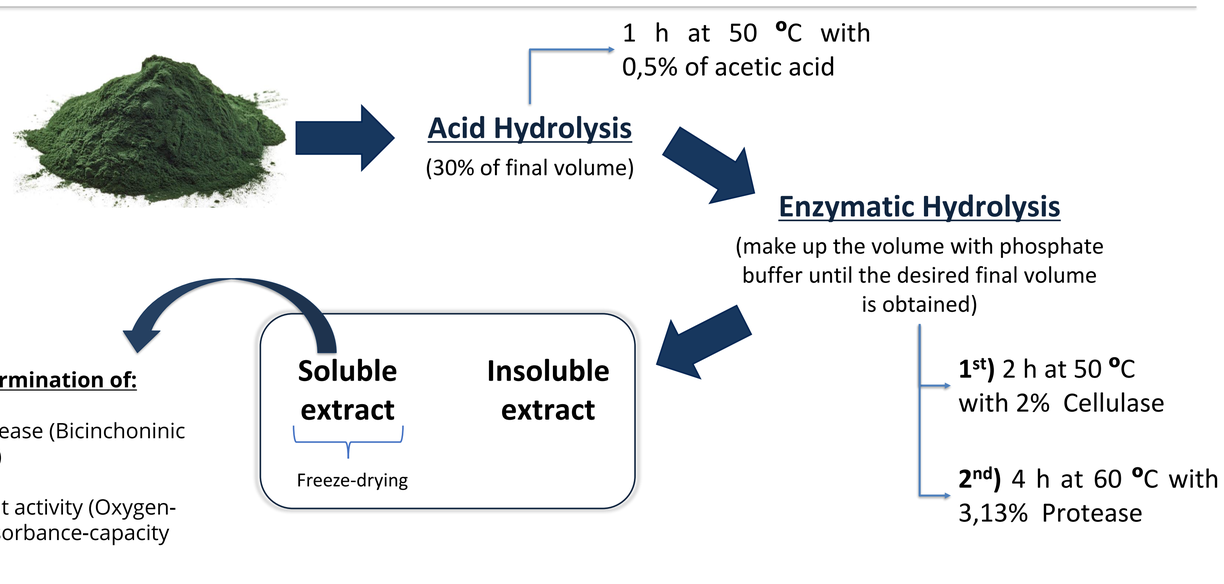

Figure 2: Optimized extraction method from *Chlorella vulgaris.*

Results

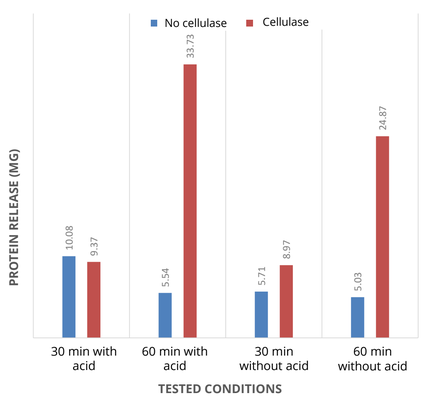

Figure 3: Evaluation of protein release after incubation with and without acid followed by an incubation with 2% cellulase.

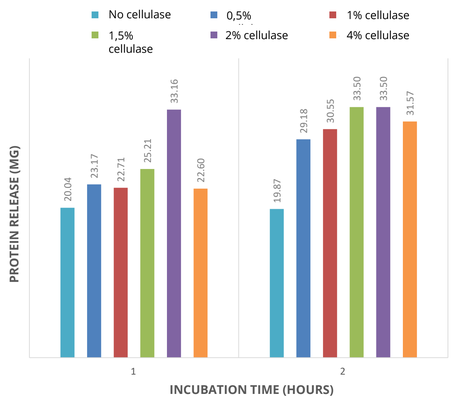

Figure 4: Evaluation of protein release after incubation with acid followed by incubation with several cellulase concentrations.

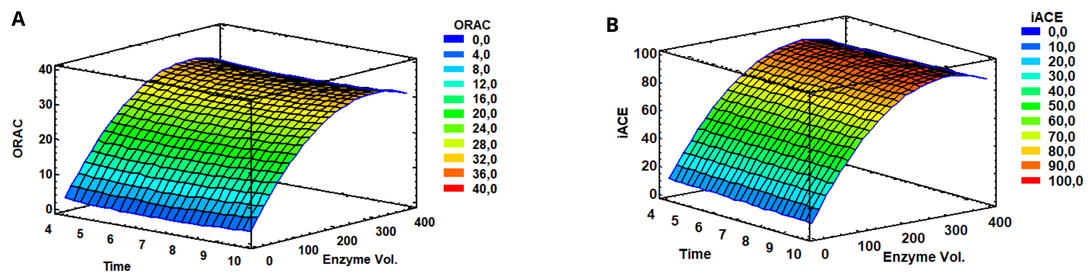

Figure 6: Obtained chart for antioxidant (A) and anti-hypertensive (B) activities in the performed experimental design, showing the best factors combination that allows to achieve a higher bioactive peptides release.

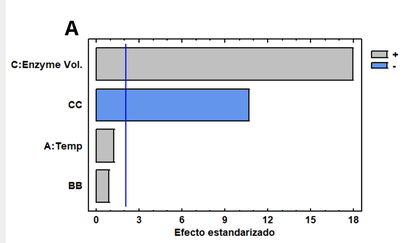

Figure 5: Pareto charts obtained for antioxidant (A) and anti-hypertensive (B) activity in the experimental design, showing the most influent factors.
A-Temperature of incubation with the protease. B-Incubation time with the protease. C- Protease concentration.

Table 1: Nutritional composition of the tested microalgae.

Nutrients	Composition (g/100g)
Proteins	52,2
Lipids	7,9
Carbohydrates	10,9
Fibre	15,5
Mineral matter	11,1
Moist	2,4

Table 2: Optimal conditions obtained in the analysis of experimental design.

Incubation Factors	Optimal conditions
Temperature (ºC)	60
Time with protease (hours)	4
% of Protease	3,13%

Table 3: Expected values in an extraction performed with the optimal conditions described in table 2.

Evaluated characteristics	Expected results
Protein concentration (mg / mL)	6,0
Antioxidant activity (mmol TE/g sample)	69,68
Anti-hypertensive activity (%)	92,75

Conclusions

A combination of an acid and an enzymatic hydrolysis using a cellulase and a protease, appeared to be the best method to achieve protein and peptide extraction. Factorial design allowed an evaluation of the effect of three factors (protease concentration, temperature and hydrolysis time) on protein release and extracts bioactivities. The best extracts showed high antioxidant (69,68 mmol Trolox Equivalent/g sample) and anti-hypertensive (IC50 of 12,75 µg protein/mL) activities. Thus, the factorial design allowed to select the best conditions to extract the peptides with highest antioxidant and anti-hypertensive activity. The obtained peptide extract may be further tested toward the development of functional foods.

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