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Optimized ultrasound-assisted extraction of antioxidants from *Himanthalia elongata* by Response Surface Methodology.

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Abstract: *Himanthalia elongata* is one of the most consumed algae. It is also widely used in different fields such as food, pharmaceutical and nutraceutical industries, for its biological properties such as antioxidant, anti-inflammatory, and antimicrobial, among others. Many of these beneficial properties are due to the presence of antioxidant compounds, responsible for the improvement of product preservation, and their therapeutic effects in the prevention of various diseases. The main objective of this study is to optimize the extraction of antioxidants, especially fucoxanthin, from *H. elongata* by ultrasound-assisted extraction (UAE). To this aim three extraction parameters were assessed using a response surface methodology (RSM): the concentration of ethanol in water, extraction time and ultrasound power. Results are expressed as total phenolic content, total flavonoids and antioxidant capacity determined using three methods: sequestering capacity of the diphenyl-2-picryl-hydrazyl radical (DPPH), Trolox equivalent antioxidant capacity (TEAC) and β-carotene discoloration method (BC), and extraction yield. The best yield of fucoxanthin was obtained at 44.9 min, 418.9 W and 86.8% ethanol. Overall, it can be concluded that UAE is an efficient and green technique that would allow the revalorization of *Himanthalia elongata* by its further use as source of antioxidants with application in food and nutraceutical industries.

Keywords: Macroalgae, ultrasound assisted extraction, *Himanthalia elongata*, bioactive compounds, antioxidant, response surface methology.

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1. Introduction

Marine macroalgae are gaining importance in the human diet due to their nutritional composition and potential health benefits. Many of these beneficial properties are triggered by the presence of antioxidant compounds, responsible for the improvement of product preservation, and their therapeutic effects in the prevention of various diseases [1].

Among all the marketed algae, one of the most consumed is *Himanthalia elongata*, also known as sea spaghetti. However, despite such high consumption rates, only few works have been focused on its chemical characterization. However, it has been described as a potential source of polyunsaturated fatty acid, essential amino acids, minerals (K, Na, Ca and Mg), polyphenolic compounds [2] and pigments. Among pigments, fucoxanthin has

been reported as an important molecule for having additional properties such as its antioxidant, anti-inflammatory, anticancer or neuroprotective effects, among others [3] .

Nowadays, there is a wide variability of extraction techniques available for improving recovery rates. Among them, those considered rapid, simple and green, for using fewer and less toxic organic solvents and energy consumption, are much more preferred [4]. In this group of techniques, it can be included the use of ultrasound-assisted extraction (UAE). This technique consists of produce acoustic cavitation which damage the cell walls of the algae matrix and thereby favors the release of bioactive compounds, which improves the extraction efficiency. In fact, the utilization of UAE has already achieved good results in plant matrices [5,6].

The extraction optimization can be enhanced when coupled to potent statistical tools such as the response surface methodology (RSM). This mathematical model permits to assess different combinations of experimental parameters in relation with the selected response. This mathematical approach results fundamental to determine the best conditions to maximize the recovery of the target molecule.

Currently, food, cosmetic and pharmaceutical industries claim natural ingredients for the development of innovative and healthy products. Therefore, the development of studies regarding the optimization of the extraction and the chemical characterization of bioactive compounds from *H. elongata*, especially polyphenols and pigments with antioxidant activity, is key [7].

The present work is aimed to provide an optimized extraction protocol using ultrasound-assisted extraction (UAE) for antioxidants, mainly fucoxanthin, from *H. elongata*..

2. Material and Methods

2.1. Sample preparation

H. elongata samples were provided by the company Algas Atlánticas Algamar S.L located in Pontevedra, Spain. The algae were collected from the coasts of the province of Pontevedra, they were washed with distilled water, frozen at -80 ºC and later lyophilized. Next, the samples were crushed and grinded to obtain a homogeneous matrix, which was stored at -20 ºC until use.

2.2. Ultrasound assisted extraction (UAE)

The process for obtaining bioactive compounds was carried out by UAE, using the CY-500 (Optic Ivymen Systems). The extraction was carried out using 1.05 g of the lyophilized alga and 35 mL of solvent (solute/solvent ratio of 30 g/L). The variables studied were the processing time (*t*), power (*P*) and solvent (*%Et*), as critical extraction parameters. Specifically, the processing time varied between 15-45 min, the power between 0-100% and the concentration of ethanol from 0-100% v/v. Once the extraction was completed, the samples were centrifuged at 9000 rpm for 15 minutes and filtered to separate the supernatant from algae debris. These extracts were stored in a freezer at - 80°C.

In order to determinate the best conditions of extraction of UAE (t, P, *%Et*), an RMS was applied using circumscribed central composite design (CCCD). This model allows to identify the operating conditions that maximize the responses, in this case total phenolic content, total flavonoids, antioxidant capacity, in terms of sequestering capacity of the diphenyl-2-picryl-hydrazyl radical (DPPH), Trolox equivalent antioxidant capacity (TEAC) and β-carotene discoloration method (BC), and extraction yield of *H. elongata*. The optimization process here described can be solved with 28 experimental points plus some preliminary trials to center the ranges of the variables involved:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2 \quad (1)$$

Where Y is the predicted responses, β_0 is the constant of the model, β_i is the linear coefficient, β_{ii} is the coefficient quadratic, β_{ij} is the coefficient of the interaction and X_i and X_j is the dimensionless coded value of the independent variables.

2.3. Determination of bioactive compounds and antioxidant capacity

In this study, the dry weight (dw) of the extract was determined to calculate the extraction performance (**Eq. 2**.):

$$EY\ (\%) = (P_2\text{-}P_1)/P_0 \times 100 \quad (2)$$

Where, P_0 is the mass of lyophilized algae prior to extraction (mg), P_1 is the mass of the empty crucible (mg), P_2 is the mass of the dry extract in the crucible (mg). Furthermore, an identification and quantification of fucoxanthin was carried out by HPLC.

3. Results and discussions

The process was optimized by response surface methodology (RSM) using a five-level central composite design, combining the independent variables of processing time (*t*, 15-45 min), power (*P*, 0-100%) and solvent (*S*, 0-100 % ethanol, v/v) [8]. Specifically, RSM was performed to optimize different responses associated with polyphenol production and antioxidant activity determination. The results analyzed included total phenolic content, total flavonoids, antioxidant, and extraction yield. The theoretical models were fitted to the experimental data, statistically validated, and used in the prediction and optimization steps. The analysis presented provides important data that allows the comparison between different extraction conditions, in terms of efficiency, and consequent related decision making.

4. Conclusion

H. elongata is an alga species with reported antioxidant activity, which has been attributed to the presence of phenolic compounds and some pigments like fucoxanthin, a compound typical of brown algae. In this study, UAE resulted in a suitable technique to extract fucoxanthin and obtain extracts with antioxidant activity. The best yields of fucoxanthin were obtained at 44.9 min, 418.9 W and 86.8% ethanol. The analysis presented provides important data that allows the comparison between different extraction conditions, in terms of efficiency, and consequent related decision making. Overall, it can be concluded that ultrasounds assisted extraction can be an efficient and green technique to revalorize algae of common uses as *H. elongata* into potent antioxidant phenolic for their further application in food and nutraceutical industries.

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