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Proceeding Paper

Green Extraction of Fucoxanthin with Promising Nutraceutical Applications †

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Abstract: *Sargassum muticum* is an invasive brown macroalga in Galicia (Spain). Thus, exploitation of this biomass for the extraction of bioactive compounds could be an interesting strategy to add value to food supplements and functional foods. Among these compounds, fucoxanthin (Fx) has been gaining attention for its promising biological activities, such as its antioxidant, antimicrobial, anticancer, antihypertensive, anti-inflammatory, anti-diabetic, anti-obesity, neuroprotective, anti-angiogenic and photoprotective properties. Fucoxanthin is the most abundant and characteristic pigment in brown algae, accounting for approximately 10% of the total carotenoids in nature. The aim of this study was to optimize the extraction yield (grams extract per 100 g of macroalgae dried weight, g E/100 g Ma dw) and Fx content (mg Fx/g E) from *Sargassum muticum* using ultrasound-assisted extraction (UAE). For this purpose, a response surface methodology (RSM) study with a five-level circumscribed central composite design (28 independent experiments) was applied to optimize three main UAE variables: ethanol concentration (*S*, 35–100%), time (*t*, 5–55 min) and power (*p*, 100–500 W). A second-order polynomial model was used to fit the experimental data (obtained in triplicate). Based on the model prediction (R^2 = 0.965), the optimal conditions that individually maximized extraction yield were 29.98 ± 1.03 g E/100 g Ma dw at a *t*-value of 45.00 ± 3.35 min, an *S*-value of 37.50 ± 3.06% and a *p*-value of 409.46 ± 10.12 W. Meanwhile, for maximizing the Fx content (R^2 = 0.8199), the response was optimal at 0.93 ± 0.10 mg Fx/g Ma dw at a *t*-value of 45.00 ± 3.35 min, an *S*-value of 84.22 ± 4.59% and a *p*-value of 339.73 ± 9.22 W.

Keywords: natural pigments; macroalgae; innovative extraction technology; optimization study

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1. Introduction

Algae are a very important source of many compounds with biological activity or potential beneficial effects for people's health, such as pigments, polysaccharides (especially fiber), polyphenols and polyunsaturated fatty acids. For this reason, in recent years, algae have been gaining great importance among scientific researchers and the food industry [1]. Regarding pigments, carotenoids—fucoxanthin (Fx) in particular—are responsible for the pigmentation of brown algae. This compound represents 10% of the total carotenoids in

nature. Several biological activities have been attributed to fucoxanthin, such as antioxidant, antimicrobial, anticancer, antihypertensive, anti-inflammatory, anti-diabetic, anti-obesity, neuroprotective, anti-angiogenic and photoprotective properties [2]. Therefore, many efforts have been made to extract fucoxanthin from brown macroalgae through conventional techniques (Soxhlet or maceration). However, these techniques are time-consuming, need a lot of solvent and increase the carbon footprint. In this regard, application of the ultrasound-assisted extraction (UAE) methodology appears to be an environmentally friendly alternative as it requires relatively short extraction times, the use of less solvent and low energy and power consumption [3].

Sargassum muticum is an invasive brown macroalga widely distributed along the Atlantic coast of the Iberian Peninsula [4]. Several attempts have been made to control and eradicate this species, but with little success due to the process being time-consuming and costly. Thus, exploitation of this biomass for the extraction of bioactive compounds could be an interesting strategy to add value to food supplements and functional foods while controlling the growth and expansion of this invasive alga.

In this context, the aim of this study was to optimize the ultrasound-assisted extraction conditions (time, solvent concentration and power) to maximize the extraction yield (grams extract per 100 g of macroalgae dried weight, g E/100 g Ma dw) and Fx content (mg Fx/g E) of *S. muticum*. For this purpose, a response surface methodology (RSM) study with a five-level circumscribed central composite design (28 independent experiments) was applied to optimize the above-mentioned UAE variables: ethanol concentration (*S*, 35–100%), time (*t*, 5–55 min) and power (*p*, 100–500 W).

2. Material and Methods

2.1. Sample Collection

Sargassum muticum was kindly provided by Algas Atlánticas Algamar S.L. (https://algamar.com/, accessed on 2 February 2022) located in Pontevedra, Spain. The algae were collected from the coasts of the province of Pontevedra, and once at the lab, they were washed with distilled water to remove sand and other impurities. Then, samples were freeze-dried and ground until obtaining a homogeneous powder, which was stored at −20 °C until use.

2.2. Ultrasound-Assisted Extraction (UAE)

UAE was carried out using a CY-500 system, Optic Ivymen Systems™, equipped with an ultrasonic probe (COMECTA S.A., Barcelona, Spain) with a power range between 100 and 500 W and a frequency of 20 kHz. Extraction time, solvent concentration and ultrasonic power were the critical parameters to be optimized. For the extraction, 1.050 g of *Sargassum muticum* powder was mixed with 35 mL of ethanol in a Falcon tube, obtaining a solid to liquid ratio of 33.33 mL/g. The obtained suspension was exposed to UAE conditions. The ultrasonic probe was inserted in the tube containing the extraction solution and the sonication was conducted in continuous (0s:0s) mode. Temperature was maintained below 30 °C during the whole extraction procedure, and this was monitored by putting the extraction tube in an ice bath. The critical parameters were evaluated in the following ranges: ethanol concentration (*S*), 35–100%; time (*t*), 5–55 min; and power (*p*), 100–500 W. The range of each of the variables was selected based on the literature and practical considerations previously made by this research group. Once extraction was finished, the obtained mixture was centrifuged at 8400 rpm for 7 min and filtered through a 0.22 µm PFTE filter. For further analysis, the samples were stored at −80 °C.

2.3. Determination of Extraction Yield

Five milliliters of algae extracts were added into 30-milliliter crucibles, previously dried at 104 °C for 1–2 h in a TCF 120 Forced air Oven (Argo Lab). Subsequently, crucibles containing extracts were placed in the oven again for 24 h. After that time, the crucibles

were cooled in a desiccator and weighted. Extraction yield was calculated in terms of dry weight (dw) following Equation (1).

$$EY\ (\%) = \frac{P_2 - P_1}{P_0} \times 100 \quad (1)$$

where P_0 is the mass of freeze-dried algae (mg), P_1 is the dw of the crucible before adding 5 mL of the algae extract (mg) and P_2 is the dw of the crucible after 24 h of drying (mg).

2.4. Chromatographic Analysis of Fucoxanthin

Samples were analyzed using Waters HPLC equipment coupled to a photodiode array detector (chromatograms recorded between 450 and 700 nm; 1.2 nm optical resolution). The HPLC equipment included a Waters 600 Controller and Waters 600 Pump, a Waters 2996 PDA Detector, a Waters 717 plus Autosampler and a Waters In-Line Degasser AF. The analytical separations were performed using a Waters Nova-Pak C18 column (150 × 3.9 nm, WAT 088344) thermostated at 25 °C. Three mobile phases were employed: a solution of 5 mM of ammonium acetate in Milli-Q water (A), a solution of 5 mM of ammonium acetate in methanol (B) and pure ethyl acetate (C). The organic solvents employed to prepare the mobile phases were HPLC-grade. The flow rate was fixed at 0.5 mL/min, and the injection volume was 50 µL.

2.5. Experimental Design, Modeling and Optimization

To obtain the optimal processing conditions that allow for maximizing the extraction yield and the fucoxanthin content from *S. muticum*, the response surface methodology was employed with a five-level circumscribed central composite design (CCCD). The RSM models were fitted by calculating least-squares using a second-order polynomial model from Equation (2):

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

where Y is the dependent variable (extraction yield and fucoxanthin content) to be modeled, X_i and X_j are the independent variables (extraction time, solvent concentration and ultrasonic power), b_0 is the constant coefficient, b_i is the coefficient of linear effect, b_{ij} is the coefficient of interaction effect, b_{ii} is the coefficient of quadratic effect and n is the number of variables.

3. Results and Discussion

The experimental results of the RSM of CCCD for the optimization of *S. muticum* UAE for the five considered independent and response variables are represented in Table 1. Table 1 displays the coded and natural values of the independent variables X_1 (extraction time (t), min), X_2 (power (p), W) and X_3 (solvent (S), % of ethanol, v/v). The statistical parameters of the fitted models were calculated according to the procedure explained by Prieto and Vazquez [5].

Based on the model prediction (R^2 = 0.965), the optimal conditions that individually maximized extraction yield were 29.98 ± 1.03 g E/100 g Ma dw at a t-value of 45.00 ± 3.35 min, an S-value of 37.50 ± 3.06% and a p-value of 409.46 ± 10.12 W. Meanwhile, for maximizing the Fx content (R^2 = 0.8199), the response was optimal at 0.93 ± 0.10 mg Fx/g Ma dw at a t-value of 45.00 ± 3.35 min, an S-value of 84.22 ± 4.59% and a p-value of 339 ± 9.22 W, as shown in Table 2.

Table 1. Experimental parameters of the optimization process.

Run	Independent Variables			Response Variables	
	***t* (min)**	***p* (W)**	***S* (%)**	***EY* g E/100 g Ma dw**	**Fx mg Fx/g Ma dw**
1	15.1 (−1)	181.1 (−1)	48.2 (−1)	27.09	270.9
2	15.1 (−1)	181.1 (−1)	86.9 (1)	14.83	148.3
3	15.1 (−1)	418.9 (1)	48.2 (−1)	38.45	384.5
4	15.1 (−1)	418.9 (1)	86.8 (1)	29.27	292.7
5	44.9 (1)	181.1 (−1)	48.2 (−1)	29.14	291.4
6	44.9 (1)	181.1 (−1)	86.8 (1)	16.52	165.2
7	44.9 (1)	418.9 (1)	48.2 (−1)	38.12	381.2
8	44.9 (1)	418.9 (1)	86.8 (1)	21.64	216.9
9	55 (1.68)	300 (0)	67.5 (0)	31.43	314.3
10	5 (−1.68)	300 (0)	67.5 (0)	35.33	353.3
11	30 (0)	100 (−1.68)	67.5 (0)	18.72	187.2
12	30 (0)	500 (1.68)	67.5 (0)	25.61	256.1
13	30 (0)	300 (0)	35 (−1.68)	40.10	401.0
14	30 (0)	300 (0)	100 (1.68)	6.54	65.4
15	5 (−1.68)	100 (−1.68)	35 (−1.68)	22.91	229.1
16	5 (−1.68)	100 (−1.68)	100 (1.68)	4.78	47.8
17	5 (−1.68)	500 (1.68)	35 (−1.68)	34.01	340.1
18	5 (−1.68)	500 (1.68)	100 (1.68)	4.26	42.6
19	55 (1.68)	100 (−1.68)	35 (−1.68)	27.43	274.3
20	55 (1.68)	100 (−1.68)	100 (1.68)	2.01	20.1
21	55 (1.68	500 (1.68)	35 (−1.68)	57.96	579.6
22	55 (1.68)	500 (1.68)	100 (1.68)	7.48	74.8
23	30 (0)	300 (0)	67.5 (0)	29.98	299.8
24	30 (0)	300 (0)	67.5 (0)	31.73	317.3
25	30 (0)	300 (0)	67.5 (0)	31.41	314.1
26	30 (0)	300 (0)	67.5 (0)	28.04	280.4
27	30 (0)	300 (0)	67.5 (0)	29.79	297.9
28	30 (0)	300 (0)	67.5 (0)	28.56	285.7

Note: *EY*, extraction yield; Fx, fucoxanthin.

Table 2. Optimum extraction values for each variable.

Optimum Values		***S* (%)**	***t* (min)**	***p* (W)**
EY %	29.98 ± 1.03	37.50 ± 3.06	45.00 ± 3.35	409.46 ± 10.12
Fx content (mg Fx/g Ma dw)	29.98 ± 1.03	84.22 ± 4.59	45.00 ± 3.35	339± 9.22

Obtaining carotenoids from plant matrices is a growing market; in fact, it is expected that by 2022, it will reach a market value of USD 120 million. In this way, methods to correctly optimize their extraction from previously wasted materials are the object of much study [6].

As is well known, brown algae are among the main sources of fucoxanthin, and some studies have carried out its extraction from the matrix used using other extraction methods, such as extraction by maceration. With the current method, it is expected to obtain about 0.93 ± 0.10 mg Fx/g Ma dw, which is close to and even better than that obtained by conventional extraction methods [7].

4. Conclusions

The results from this study suggest that the optimization of UAE conditions to maximize the Fx content and extraction yield from *S. muticum* represents a promising approach for the recovery of bioactive compounds from invasive macroalgae, with potential application in nutraceutical and food industry sectors. This will also contribute to sustainable management of the expansion of *S. muticum* and the restoration of the ecosystem in coastal areas.

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References

1. Lee, H.J.; Kim, H.C.; Vitek, L.; Nam, M.C. Algae consumption and risk of type 2 diabetes: Korean National Health and Nutrition Examination Survey in 2005. *J. Nutr. Sci. Vitaminol.* **2010**, *56*, 13–18. [CrossRef] [PubMed]
2. Peng, J.; Yuan, J.P.; Wu, C.F.; Wang, J.H. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. *Mar. Drugs* **2011**, *9*, 1806–1828. [CrossRef] [PubMed]
3. Chemat, F.; Rombaut, N.; Sicaire, A.G.; Meullemiestre, A.; Fabiano-Tixier, A.S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.* **2017**, *34*, 540–560. [CrossRef] [PubMed]
4. Olabarria, C. Ecosystem Functioning Impacts of the Invasive Seaweed Sargassum. *J. Phycol.* **2014**, *116*, 108–116.
5. Prieto, M.A.; Vázquez, J.A. In vitro determination of the lipophilic and hydrophilic antioxidant capacity of uroasted coffee bean extracts and their synergistic and antagonistic effects. *Food Res. Int.* **2014**, *82*, 1183–1196. [CrossRef]
6. Lourenço-lopes, C.; Fraga-corral, M.; Jimenez-lopez, C.; Carpena, M.; Pereira, A.G. Trends in Food Science & Technology Biological action mechanisms of fucoxanthin extracted from algae for application in food and cosmetic industries nuclear Magnetic Resonance. *Trends Food Sci. Technol.* **2021**, *117*, 163–181.
7. Holdt, S.L.; Kraan, S. Bioactive compounds in seaweed: Functional food applications and legislation. *J. Appl. Phycol.* **2011**, *23*, 543–597. [CrossRef]