proceedings

MDPI

Proceeding Paper

Green Extraction of Fucoxanthin with Promising Nutraceutical Applications †

Anxo Carreira-Casais [1], Lucia Cassani [1,2], Anton Soria-López [1], Sepidar Seyyedi Mansour [1], Maria Fraga-Corral [1,3], Rosa Perez-Gregorio [1,4], Jesus Simal-Gandara [1,*] and Miguel A. Prieto [1,3,*]

[1] Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain; anxocc@uvigo.es (A.C.-C.); lucia_cassani@hotmail.com (L.C.); anton.soria@uvigo.es (A.S.-L.); sepidar.seyyedi@uvigo.es (S.S.M.); mfraga@uvigo.es (M.F.C.); mariarosa.perez@uvigo.es (R.P.G.)

[2] Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA, CCT-CONICET), Colón 10850, Mar del Plata (7600), Argentina

[3] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

[4] Associated Laboratory for Green Chemistry of the Network of Chemistry and Technology (LAQV-REQUIMTE) Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Porto, Portugal.

* Correspondence: jsimal@uvigo.es (J.S.-G.); mprieto@uvigo.es (M.A.P.)

† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021.

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 antioxidant, antimicrobial, anticancer, antihypertensive, anti-inflammatory, anti-diabetic, anti-obesity, neuroprotective, anti-angiogenic and photo protective 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 an experimental design of 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 by triplicate). Based on the model prediction (R^2= 0.965), the optimal conditions that individually maximize extraction yield were 29.98 ± 1.03 g E/100 g Ma dw at a *t* value of 45.00 ± 3.35 min, *S* value of 37.50 ± 3.06% and *P* value of 409.46 ± 10.12 W. While for maximizing the Fx content (R^2 = 0.8199) the response was 0.93 ± 0.10 mg Fx/g Ma dw at a *t* value of 45.00 ± 3.35 min, *S* value of 84.22 ± 4.59% and *P* value of 339.73 ± 9.22 W.

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

Citation: Carreira-Casais, A.; Cassani, L.; Soria-López, A.; Seyyedi Mansour, S.; Fraga-Corral, M.; Perez-Gregorio, R.; Simal-Gandara, J.; Prieto, M.A. Green Extraction of Fucoxanthin with Promising Nutraceutical Applications. **2022**, *69*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Torsten Bohn

Published: date: 15 March 2022

1. Introduction

Algae are very important source of many compounds with bioactive 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 food industry [1]. Regarding pigments, carotenoids, in particular fucoxanthin (Fx), are responsible for the pigmentation of brown algae. This compound represents 10% of the total carotenoids in

nature. Several biological activities were ascribed to fucoxanthin, such as antioxidant, antimicrobial, anticancer, antihypertensive, anti-inflammatory, anti-diabetic, anti-obesity, neuroprotective, anti-angiogenic and photo protective properties [2]. Therefore, many efforts have been made for extracting fucoxanthin from brown macroalgae through conventional techniques (Soxhlet or maceration). However, these techniques are time consuming, need a lot of solvent and increase of the carbon footprint. In this regard, the application of -assisted extraction (UAE) methodology appears as an environmentally friendly alternative as it requires relatively short extraction times, less use of 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 with little success because its 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, power) to maximize 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, Optic Ivymen Systems™, equipped with a ultrasonic probe (COMECTA S.A., Barcelona, Spain) with a power range between 100–500 W and a 20 kHz of frequency. 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. 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 literature and practical considerations previously done 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

5 mL of algae extracts were added into 30 mL-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 again in the oven for 24 h. After that time, crucibles were cooled in

the desiccator and weighted. Extraction yield was calculated in terms of dry weight (Dw) following Equation (1).

$$EY\ (\%) = \frac{P2 - P1}{P0} \times 100 \quad (1)$$

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

2.4. Chromatographic Analysis of Fucoxanthin

Samples were analyz °C ed using Waters HPLC equipment coupled to a photo diode-array detector (chromatograms recorded between 450 nm and 700 nm; 1.2 nm optical resolution). HPLC equipment includes a Waters 600 Controller and Waters 600 Pump a Waters 2996), 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) thermo-stated 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). Organic solvents employed to prepare 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, Modelling and Optimization

To obtain the optimal processing conditions that allow maximizing the extraction yield and the fucoxanthin content from *S. muticum*, a 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 the 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 independents 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 *S. muticum* UAE for the five considered response variables are represented in Table 1. Table 1 displays coded and natural values of the independent variables X_1 (extraction time (t), min), X_2 (power (W)) and X_3 (solvent (S), % of ethanol, v/v). Statistical parameters of the fitted models were calculated according to the procedure explained by Prieto and Vazquez [5].

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: extraction yield (*EY*), fucoxanthin.

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

Table 2. Optimun 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 120 million US dollars. In this way, the methods that to a correct optimization of their extraction from previously wasted materials are the object of study [6].

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

4. Conclusions

Results from this study suggested that optimization of UAE conditions to maximize Fx content and the 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 sustainably manage the expansion of *S. muticum* and the restoration of the ecosystem in coastal areas.

Author Contributions:

Funding: The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium. The project SYSTEMIC Knowledge hub on Nutri- tion and Food Security has received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 5).

Acknowledgments: The research leading to these results was supported by MICINN supporting the Ramón y Cajal grant for M.A. Prieto (RYC-2017-22891), the María Zambrano grant for R. Perez-Gregorio (CO34991493-20220101ALE481), and the FPU grants for A. Carreira-Casais (FPU 16/06135) and A. Soria-Lopez (FPU2020/06140); by Xunta de Galicia for supporting the program EXCELENCIA-ED431F 2020/12 the post-doctoral grant of M. Fraga-Corral (ED481B-2019/096) and L. Cassani (ED481B-2021/152), Authors are grateful to Ibero-American Program on Science and Technology (CYTED—AQUA-CIBUS, P317RT0003), to the Bio Based Industries Joint Undertaking (JU) under grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019). The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium. The project SYSTEMIC Knowledge hub on Nutrition and Food Security, has received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 696295).

Conflicts of Interest: The authors declare no conflict of interest.

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.
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.
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.
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.
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.