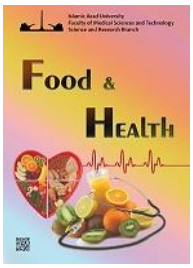

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Optimization of ultrasound-assisted extraction conditions for pigment compounds of the brown algae *Sargassum angustifolium* using response surface methodology

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ABSTRACT

This study aimed to optimize the ultrasound-assisted extraction (UAE) of pigment compounds from the brown alga *Sargassum angustifolium*. UAE offers potential advantages over conventional extraction methods, including reduced solvent consumption, shorter extraction times, and improved yields. To systematically investigate and optimize the extraction process, response surface methodology (RSM) was employed. A central composite design (CCD) was utilized to examine the effects and interactions of four critical process variables: ethanol concentration (ranging from 50% to 100%), extraction time (10 to 30 minutes), solid-liquid ratio (1:5 to 1:15), and ultrasound power (80 to 400 watts). The impact of these variables on four key responses was evaluated: chlorophyll a content, total chlorophyll content, total carotenoid content, and fucoxanthin content. Statistical analysis of the experimental data revealed that optimal conditions for maximizing fucoxanthin and total carotenoid yields were: 75% ethanol concentration, 20 minutes extraction time, 1:5 solid-liquid ratio, and 240 watts ultrasound power. Under these optimized conditions, fucoxanthin yield reached 0.42 mg/g, while total carotenoid yield attained 1.11 mg/g of dry algal biomass. The developed models demonstrated high predictive capability, with experimental results closely aligning with model predictions. This agreement validated the appropriateness and reliability of the RSM approach for optimizing the UAE process. Additionally, the study provided insights into the relative importance and interactions of the investigated process variables. These findings offer a foundation for the efficient and scalable extraction of high-value pigments from *S. angustifolium* using UAE.

1. Introduction

Seaweeds are abundant natural resources of valuable nutritional and bioactive compounds, including pigments such as chlorophylls and carotenoids (1). Generally, based on the pigment composition, seaweeds are categorized into three groups: Chlorophyta (green seaweed), Phaeophyta (brown seaweed), and Rhodophyta (red seaweed). The brown seaweeds contain the highest phytochemicals (e.g., carotenoids) (2). Pigments are responsible for biological activities, such as antiviral, antioxidative, anti-inflammatory, and neuroprotective properties (1, 3). According to World Food Organization statistics, global production of marine algae in 2016 was over 30 million tons (4). Marine brown algae, in particular, those belonging to the genus *Sargassum*, have been studied for their different biological applications such as antioxidants, anti-inflammation, anti-tumor, anti-atherosclerosis, anti-obesity, liver protection against alcohol-induced injury, anti-diabetic, insecticidal activity, antimicrobial activity, and skin whitening activity. Various active constituents, such as sulfated polysaccharides, meroterpinoid, carotenoids, polyphenols, and phlorotannins, are found to be involved in such biological activities (5-9). Different methods of extraction of pigment compounds include immersion methods (10, 11), ultrasound (12), microwave (13), supercritical CO_2 extraction (14), and the use of ionic surfactants (15). Brown algae are brown because of their large amounts of xanthophyll and fucoxanthin pigments (16). Fucoxanthin has been studied clinically for its efficacy against many diseases. It has been shown in vivo to have

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activity against cancer (17, 18), type II diabetes (19), obesity (20), cholesterol (21), inflammatory disorders (22), tumor angiogenesis (23), malaria (24), hypertension (25), and as a β-secretase 1 inhibitor in Alzheimer's disease (26). Studies on the properties of pigments within the food and drug industry show that they combat many diseases, like cancer, and their antioxidant properties prevent food oxidative corruption. Sargassum algae, a rich source of carotenoids and fucoxanthin, are abundant within the Persian Gulf. Therefore, this study was conducted to research the effect of the three variables of ethanol concentration percentage, solid-liquid ratio, and ultrasonic power by minimizing extraction costs and achieving a high-efficiency extraction method.

2. Materials and methods

2.1. Preparation of algae sample

Samples of brown algae *S. angustifolium* were freshly collected from Bushehr Port, Jalali Esqal, Rishahr area (8 km south of Bushehr) in the second half of May 2018. The algae samples were transferred to the laboratory, and after several steps of seawater washing to remove residual epiphytes, sand, and salt, they were rewashed with fresh water. Samples were dried in an oven (Behdad Medical Equipment Manufacturing Company) at 40°C for 24 hours. The dried algae samples were powdered using an electric grinder (Hardstone, Model GCS2700W, UK) and sieved with a mesh size of 900 µm. Particles larger than 900 µm were not used in the experiments. The powdered specimen was stored in a zippered plastic bag in the refrigerator for extraction.

2.2. Extraction by sonication

This method used RSM to design experiments based on the previous method. Extraction using water and ethanol solvents (with 3 concentrations of 50, 75, and 100% ethanol), in 3 extraction times (10, 20, and 30 min), 3 solid-liquid ratios (1:5, 1:10 and 1:15) was performed at three power levels (20, 60 and 100%). The sample and solvent mixture was exposed to ultrasonic waves (ultrasonic homogenizer fapan 400ups) at 400 watts and constant frequencies of 24 kHz according to the ratios and concentrations. (Watman 42). After 30 minutes, the extract was centrifuged for 20 minutes at 9000 rpm. The extracts were kept in dark bottles until evaluation to prevent light degradation (10).

2.3. Pigment analysis (chlorophyll and carotenoids)

0.4 ml of extract was extracted with 5 ml of ethanol and water separately and, after dilution, absorbed by a spectrophotometer (UV/Vis 2100) at wavelengths in the range of 350-800 nm (12).

2.4. Formulas

A_1. Chlorophyll a (mg g^{-1}) = [12.7 (A_{663})-2.69 (A_{645}) V] / (1000 × W) (27)

A_2. Total Chlorophyll (mg g^{-1}) = [20.2 (A_{645}) + 8.02 (A_{663}) V] / (1000 × W) (27)

A_3. Carotenoids (mg g^{-1}) = [7.6 (A_{480}) - 1.49 (A_{510}) V] / (1000 × W) (28, 29)

A_4. Fucoxanthin (mg g^{-1}) = A_{470} −1.239 (A_{631} + A_{581}−0.3 × A_{664}) - 0.0275 × A_{664} / 141 (30)

Where, A=Absorption rate at a particular wavelength, V=The total volume of an extract obtained, W=Sample weight used for extraction. The concentration of pigments was expressed in terms of mg/g and dry matter g/mg pigment.

2.5. Design of experiments and statistical tests

Response surface methodology is a set of statistical and mathematical methods for developing, improving, and optimizing processes that evaluate the relative significance of multiple effective variables even when complex relationships exist (31). Design-Expert software version 11 and central composite design (CCD) with 4 independent variables and 6 replications at the central point were used in an ultrasound method to investigate the process's extraction and optimization conditions. Independent variables, including ethanol concentration (X1), time (X_2), solid-liquid ratio (X_3), and ultrasonic power (X_4) were coded at 3 levels, and the dependent variable (response) was pigment concentration in mg/g (Table 1).

Table 1. Displaying process independent variables and their values in ultrasound

Independent variable	Math symbol	Related level		
Concentration of ethanol to (%) water	X1	50	75	100
Time (hours)	X2	2	4	6
Solid to Liquid Ratio (g/ml)	X3	1:5	1:10	1:15
Wave Intensity (%)	X4	20	60	100

The model used in RSM is generally second-order. The RSM defines a model for each dependent variable that expresses the main effects of the factors of each variable. For the three factors, the polynomial equation is as follows:

$$Y= \beta_0 + \sum_{i=1}^{k} \beta i Xi + \sum_{i=1}^{k} \beta ii\, X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta ij X_i\, X_j + \varepsilon$$

In this function, Y represents the predicted response value of the tested variables, β_0 is a constant number or width of the source, β_i, $\beta_{ii,}$ and β_{ij} are regression model coefficients, and X_i and X_j are the independent variables. $x2_i$, $x2_j$, represent the squared effects of variables, and X_iX_j and X_iX_k represent the effects of interaction between variables. ε is the probability error, and K is the number of independent parameters. In the response surface methodology, these parameters form second-order equations, and the optimal values of each parameter and the level obtained from the second-degree equation are determined on the fitted data. Then, the 3D graphs of the surface response are plotted. ANOVA analysis was used to determine significant differences between the data and the fit of polynomial models with R^2 coefficient, Adj.R^2 coefficient, prediction coefficient (pre.R^2), and standard deviation (Std). The statistical significance of all model components was investigated at probability levels (p) of 0.001, 0.01, and 0.05,

and finally, optimum conditions were determined for each extraction method.

3. Results

A total of 30 experiments were performed randomly to optimize the investigated indices, and the response values in different experimental combinations are shown in Table 2. The amounts of chlorophyll a, total chlorophyll, total carotenoid, and fucoxanthin were respectively between 0.02 to 1.69, 0.06 to 1.85, 0.08 to 1.11, and 0.06 to 0.42 mg/g of dried algae powder obtained. The software provided the predicted models as ANOVAs for each of the answers as follows:

B_1. $Ln(Y_{Chl\ a}) = -4.39419 + 0.037548\ X_1 + 0.068565\ X_2 - 0.178371\ X_3 - 0.002487\ X_4 - 0.000076\ X_1X_2 - 0.001099\ X_1X_3 + 0.000023\ X_1X_4 - 0.000824\ X_2X_3 - 0.000046\ X_2X_4 + 9.53364E\text{-}06\ X_3X_4 + 0.000142\ X_1^2 - 0.000629\ X_2^2 + 0.005990\ X_3^2 + 3.55035E\text{-}06\ X_4^2$

B_2. $\text{Ln}(Y_{\text{Total Chl}}) = -1.98163 + 0.010616\ X_1 + 0.085972\ X_2 - 0.206715\ X_3 - 0.003758\ X_4 - 0.000084\ X_1X_2 - 0.001053\ X_1X_3 + 0.000025\ X_1X_4 - 0.000620\ X_2X_3 - 0.000044\ X_2X_4 + 0.000017\ X_3X_4 + 0.000186\ X_1^2 - 0.001116\ X_2^2 + 0.006899\ X_3^2 + 5.53159E\text{-}06\ X_4^2$

B_3. $1/\sqrt{Y_{\text{Total Cr}}} = +5.09422 - 0.118566\ X_1 - 0.046193\ X_2 + 0.182470\ X_3 + 0.001482\ X_4 + 0.000153\ X_1X_2 + 0.000014\ X_1X_3 - 0.000013\ X_1X_4 - 0.001375\ X_2X_3 + 0.000033\ X_2X_4 - 0.000062\ X_3X_4 + 0.000746\ X_1^2 + 0.000557\ X_2^2 + 0.001367\ X_3^2 - 1.69310E\text{-}06\ X_4^2$

B_4. $\ln(Y_F) = -6.61297 + 0.136537\ X_1 + 0.040823\ X_2 - 0.097696\ X_3 - 0.000688\ X_4 - 0.000103\ X_1X_2 - 0.000516\ X_1X_3 + 0.000017\ X_1X_4 + 0.000463\ X_2X_3 - 0.000036\ X_2X_4 + 0.000089\ X_3X_4 - 0.000805\ X_1^2 - 0.000257\ X_2^2 + 0.001621\ X_3^2 - 9.16004E\text{-}07\ X_4^2$

Table 2. Experimental design of independent variables and responses of chlorophyll a, total chlorophyll, total carotenoid and in ultrasound.

Std.	Run	Factor1 A: Compactness (%)	Factor2 B: Time (min)	Factor3 C: Solid/liquid (g/ml)	Factor4 D: Power (U) watt	Chl. a (a)	Total Chl. (a)	Total Cr. (a)	Fucoxanthin (a)
14	1	100	10	15	400	0.22	0.25	0.12	0.14
5	2	50	10	15	80	0.02	0.06	0.08	0.06
13	3	50	10	15	400	0.02	0.06	0.08	0.07
7	4	50	30	15	80	0.03	0.08	0.11	0.09
26	5	75	20	10	240	0.11	0.19	0.36	0.28
24	6	75	20	10	400	0.13	0.23	0.43	0.30
22	7	75	20	15	240	0.06	0.11	0.18	0.19
12	8	100	30	5	400	1.69	1.85	0.91	0.41
30	9	75	20	10	240	0.14	0.25	0.40	0.27
19	10	75	10	10	240	0.09	0.15	0.28	0.21
25	11	75	20	10	240	0.10	0.16	0.32	0.24
18	12	100	20	10	240	0.51	0.54	0.27	0.22
20	13	75	30	10	240	0.15	0.24	0.46	0.32
2	14	100	10	5	80	1.01	1.10	0.57	0.25
27	15	75	20	10	240	0.11	0.18	0.34	0.24
28	16	75	20	10	240	0.12	0.19	0.34	0.26
4	17	100	30	5	80	1.50	1.66	0.82	0.36
10	18	100	10	5	400	1.49	1.62	0.82	0.34
11	19	50	30	5	400	0.09	0.24	0.45	0.13
16	20	100	30	15	400	0.28	0.30	0.14	0.17
23	21	75	20	10	80	0.14	0.26	0.36	0.23
6	22	100	10	15	80	0.16	0.18	0.09	0.10
9	23	50	10	5	400	0.06	0.18	0.37	0.13
1	24	50	10	5	80	0.06	0.18	0.38	0.13
21	25	75	20	5	240	0.31	0.58	1.11	0.42
15	26	50	30	15	400	0.02	0.07	0.13	0.13
8	27	100	30	15	80	0.26	0.30	0.14	0.17
3	28	50	30	5	80	0.13	0.40	0.75	0.26
17	29	50	20	10	240	0.03	0.10	0.19	0.12
29	30	75	20	10	240	0.12	0.21	0.40	0.30

All data are expressed in mg of pigment/g of dried algae powder.

In the analysis of variance, the quadratic model was used. According to the Table 3 and 4, values of all coefficients of explanation above 95% were obtained, which is significant for these regressions. The adjusted and prediction coefficients of fucoxanthin were above 95% and 90%, respectively. For fucoxanthin, the coefficient of determination and prediction coefficient were above 90% and 70%, respectively. There is a reasonable agreement with the coefficient of explanation. The factors such as percentage of ethanol concentration (X_1), extraction time (X_2), solid-liquid ratio (X_3), interactions of percent ethanol concentration and solid-liquid ratio (X_1X_3), interaction between the percentage of ethanol concentration and power of ultrasound (X_1X_4), interaction of extraction time and power of ultrasound (X_2X_4) and second power of solid-liquid ratio (X_3^2) had the most effect on chlorophyll response and the differences were significant ($p<0.05$). In response to total chlorophyll, extraction time factors (X_2), solid-liquid ratio (X_3), ultrasound power (X_4), interactions of percent ethanol concentration and solid-liquid ratio (X_1X_3), interactions of percent ethanol concentration and ultrasound

Table 3. Results of analysis of variance for independent variables in ultrasound extraction method (Chl a, Total Chl).

Source	Chl. a					Total Chl.					
	Sum of Squares	**df**	**Mean Square**	**F-Value**	**p-value**	**Source**	**Sum of Squares**	**df**	**Mean Square**	**F-Value**	**p-value**
Model	42.60	14	3.04	252.87	< 0.0001*	Model	24.46	14	1.75	109.02	< 0.0001*
A-Compactness	0.0604	1	0.0604	5.02	0.0406	A-Compactness	0.0048	1	0.0048	0.3012	0.5912
B-Time	0.0654	1	0.0654	5.44	0.0340	B-Time	0.1029	1	0.1029	6.42	0.0229
C-Solid/liquid	0.1107	1	0.1107	9.20	0.0084	C-Solid/liquid	0.1487	1	0.1487	9.28	0.0082
D-Power (U)	0.0345	1	0.0345	2.87	0.1111	D-Power (U)	0.0787	1	0.0787	4.91	0.0425
AB	0.0058	1	0.0058	0.4827	0.4978	AB	0.0070	1	0.0070	0.4378	0.5182
AC	0.3017	1	0.3017	25.07	0.0002	AC	0.2774	1	0.2774	17.31	0.0008
AD	0.1342	1	0.1342	11.15	0.0045	AD	0.1545	1	0.1545	9.64	0.0072
BC	0.0271	1	0.0271	2.26	0.1538	BC	0.0154	1	0.0154	0.9594	0.3429
BD	0.0871	1	0.0871	7.24	0.0168	BD	0.0794	1	0.0794	4.96	0.0417
CD	0.0009	1	0.0009	0.0774	0.7847	CD	0.0028	1	0.0028	0.1755	0.6812
A^2	0.0203	1	0.0203	1.68	0.2139	A^2	0.0350	1	0.0350	2.19	0.1600
B^2	0.0102	1	0.0102	0.8508	0.3709	B^2	0.0323	1	0.0323	2.01	0.1762
C^2	0.0581	1	0.0581	4.83	0.0441	C^2	0.0771	1	0.0771	4.81	0.0445
D^2	0.0214	1	0.0214	1.78	0.2022	D^2	0.0520	1	0.0520	3.24	0.0919
Residual	0.1805	15	0.0120	-	-	Residual	0.2404	15	0.0160	-	-
Lack of Fit	0.1109	10	0.0111	0.7973	0.6455ns	Lack of Fit	0.1348	10	0.0135	0.6384	0.7450
Pure Error	0.0696	5	0.0139	-	-	Pure Error	0.1056	5	0.0211	-	-
Cor Total	42.78	29	-	-	-	Cor Total	24.70	29	1.75	109.02	< 0.0001ns
Std.Dev.	0.1097					Std.Dev.	0.1266				
Mean	-1.97					Mean	-1.40				
C.V.%	5.57					C.V.%	9.01				
PRESS	0.8787					PRESS	1.04				
R^2	0.9958					R^2	0.9903				
Adj R-Squared	0.9918					Adj R-Squared	0.9812				
Pred R-Squared	0.9795					Pred R-Squared	0.9578				
Adeq Precision	58.5556					Adeq Precision	39.8402				

*Significant at p< 0.01 ns Not significant at p> 0.05.

Table 4. Results of analysis of variance for independent variables in ultrasound extraction method (Total Cr, Fucoxanthin).

Source	Total Cr					Fucoxanthin				
	Sum of Squares	**df**	**Mean Square**	**F-Value**	**p-value**	**Source**	**Sum of Squares**	**df**	**Mean Square**	**F-Value**	**p-value**
Model	15.95	14	1.14	113.63	< 0.0001*	Model	7.04	14	0.5030	29.26	< 0.0001*
A-Compactness	0.6022	1	0.6022	60.05	< 0.0001	A-Compactness	0.7985	1	0.7985	46.46	< 0.0001
B-Time	0.0297	1	0.0297	2.96	0.1058	B-Time	0.0232	1	0.0232	1.35	0.2635
C-Solid/liquid	0.1159	1	0.1159	11.56	0.0040	C-Solid/liquid	0.0332	1	0.0332	1.93	0.1848
D-Power (U)	0.0123	1	0.0123	1.22	0.2864	D-Power (U)	0.0026	1	0.0026	0.1534	0.7009
AB	0.0233	1	0.0233	2.33	0.1481	AB	0.0107	1	0.0107	0.6214	0.4428
AC	0.0000	1	0.0000	0.0047	0.9464	AC	0.0665	1	0.0665	3.87	0.0679
AD	0.0436	1	0.0436	4.35	0.0546	AD	0.0722	1	0.0722	4.20	0.0583
BC	0.0756	1	0.0756	7.54	0.0150	BC	0.0086	1	0.0086	0.4993	0.4906
BD	0.0434	1	0.0434	4.33	0.0549	BD	0.0523	1	0.0523	3.04	0.1016
CD	0.0394	1	0.0394	3.93	0.0662	CD	0.0816	1	0.0816	4.75	0.0457
A^2	0.5627	1	0.5627	56.12	< 0.0001	A^2	0.6562	1	0.6562	38.18	< 0.0001
B^2	0.0080	1	0.0080	0.8028	0.3844	B^2	0.0017	1	0.0017	0.0999	0.7563
C^2	0.0030	1	0.0030	0.3019	0.5908	C^2	0.0043	1	0.0043	0.2476	0.6260
D^2	0.0049	1	0.0049	0.4854	0.4966	D^2	0.0014	1	0.0014	0.0829	0.7774
Residual	0.1504	15	0.0100	-	-	Residual	0.2578	15	0.0172	-	-
Lack of Fit	0.1155	10	0.0116	1.66	0.3007ns	Lack of Fit	0.2210	10	0.0221	3.00	0.1183ns
Pure Error	0.0349	5	0.0070	-	-	Pure Error	0.0368	5	0.0074	-	-
Cor Total	16.10	29	-	-	-	Cor Total	7.30	29	-	-	-
Std.Dev.	0.1001					Std.Dev.	0.1311				
Mean	1.96					Mean	-1.63				
C.V.%	5.11					C.V.%	8.03				
PRESS	0.8438					PRESS	1.84				
R^2	0.9907					R^2	0.9647				
Adj R-Squared	0.9819					Adj R-Squared	0.9317				
Pred R-Squared	0.9476					Pred R-Squared	0.7481				
Adeq Precision	38.3077					Adeq Precision	20.3147				

*Significant at p< 0.01 ns Not significant at p> 0.05.

wave power (X_1X_4), the interaction of extraction time and ultrasound power (X_2X_4) and second power of solid-liquid ratio (X_3^2), total carotenoid response, ethanol concentration percentage factors (X_1), solid-liquid ratio (X_3), interaction between extraction time and solid-liquid ratio (X_2X_3) and second power percent ethanol concentration (X_1^2) and in response to fucoxanthin, ethanol concentration percentage factors (X_1), solid-liquid ratio interaction, and ultrasound power (X_3X_4) and the second power of percent ethanol concentration (X_1^2) had the highest impact on response rate and significant differences are at ($p<0.05$). The results of Table 3 show that the experiments and the selected models for data analysis and prediction of optimum conditions have followed the correct trend. On the other hand, low standard deviation values are evidence of this and indicate the reproducibility of the models. In addition, the non-fits of the models were not significant (32). Thus, these results suggest that these models have worked well in predicting the optimum conditions for extracting pigment compounds from the brown alga *S. angustifolium*. Ultrasound-based pigment extraction from *S. angustifolium* brown algae was optimized by response surface methodology. In this optimization, the percent of ethanol concentration, extraction time, and solid-liquid ratio were determined based on the study's objectives. The percentages of ethanol concentration in the range of 50 to 100, 10 to 30 minutes, 1:5 to 1:15 solid-liquid ratio, and 80 to 400 watts were measured (Table 5). The three-dimensional graphs of response levels show more clearly the trend of the influence of independent variables on different responses and extraction results. These graphs in 3D show the simultaneous effect of

Table 5. Optimal conditions for extraction of pigment compounds by ultrasound

Responses	Independent variables				Predicted values
	Ethanol: water	Time	Solid-liquid ratio	The power of ultrasound	
Total Chl	99.86	20.50	5.03	399.35	1.90
Total Cr	76.87	12.42	5.04	123.31	1.12
Fucoxanthin	70.99	29.74	5.04	96.86	0.46
Chl a	99.73	27.36	5.10	395.75	1.70

two variables, for example, on the amount of fucoxanthin. The highest point represents the optimal extraction level, and the values for each variable can be deduced from the x and y axes (33). To estimate the effect of each parameter and its interaction with the response, 3D response surface plots were plotted as a function of both variables. In contrast, the other variable was fixed at the central point. The following diagrams show the effect of the initial indices of ethanol concentration, time, solid-liquid ratio, and ultrasound power on chlorophyll a, total chlorophyll, total carotenoid, and fucoxanthin responses. Fig. 1 on chlorophyll shows the simultaneous effect of ethanol concentration percentage and solid-liquid ratio at 20 minutes and a constant power of 240 watts. As shown above, the response rate increases with increasing ethanol concentration and decreasing solid-liquid ratio. Fig. 2, related to chlorophyll a, shows the concomitant effect of ethanol concentration percentage and sonication power at a constant time of 20 minutes and a solid-liquid ratio of 1:10. By increasing these two factors, more chlorophyll a is extracted. Fig. 3, related to chlorophyll, shows the co-effect of ethanol concentration percentage and extraction time at a 1:10 solid-liquid ratio and 240 watts. The response rate increases as the two factors increase. According to the results, the highest chlorophyll content was in 100% ethanol, 30 min duration, 1:5 solid-liquid ratio, and 400 watts. Fig. 4 shows the total effect of ethanol concentration and the solid-liquid ratio at a constant duration of 20 minutes and constant power of ultrasonic waves of 240 watts. The response rate increases with increasing ethanol concentration and decreasing solid-liquid ratio. Fig. 5 on total chlorophyll shows the simultaneous effect of ethanol concentration percentage and sonication power at a constant time of 20 min and a solid-liquid ratio of 1:10. The response rate increases with the increase of these two factors. **Fig. 6** on total chlorophyll shows the concomitant effect of ethanol concentration percentage and extraction time at 1:10 solid-liquid ratio and 240 watts. The response rate increases as the

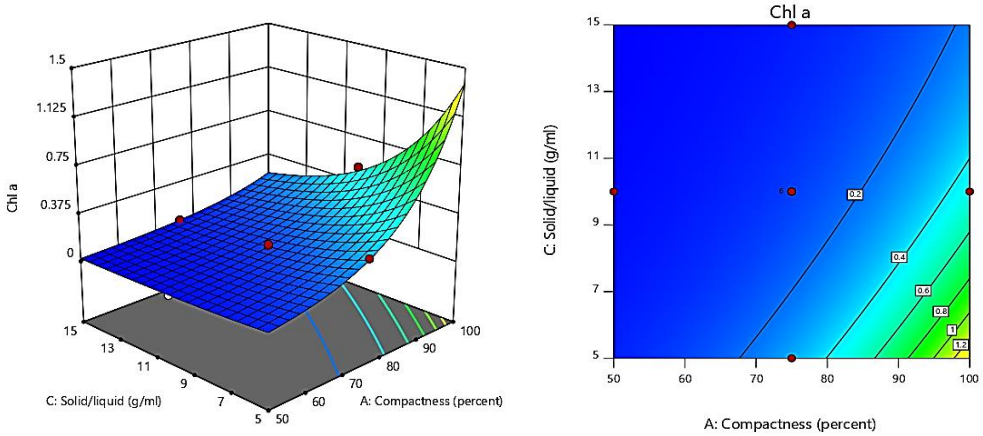

Fig. 1. Effect of concurrent ethanol concentration and solid-liquid ratio (chlorophyll a).

two factors increase. Fig. 7 shows the total chlorophyll content, the solid-liquid ratio's synchronous effect, and the ultrasound's power at a constant time of 20 minutes and a constant concentration of 75% ethanol. Decreasing the solid-liquid ratio and increasing the power of ultrasound increase the total chlorophyll content. According to the results, the highest total chlorophyll content was in 100% ethanol, 30 minutes duration, 1:5 solid-liquid ratio, and 400 watts. Fig. 8 shows the total carotenoid content, the synchronous effect of the extraction time, and the solid-liquid ratio at a constant concentration of 75% ethanol and a constant power of ultrasonic waves of 240 watts.

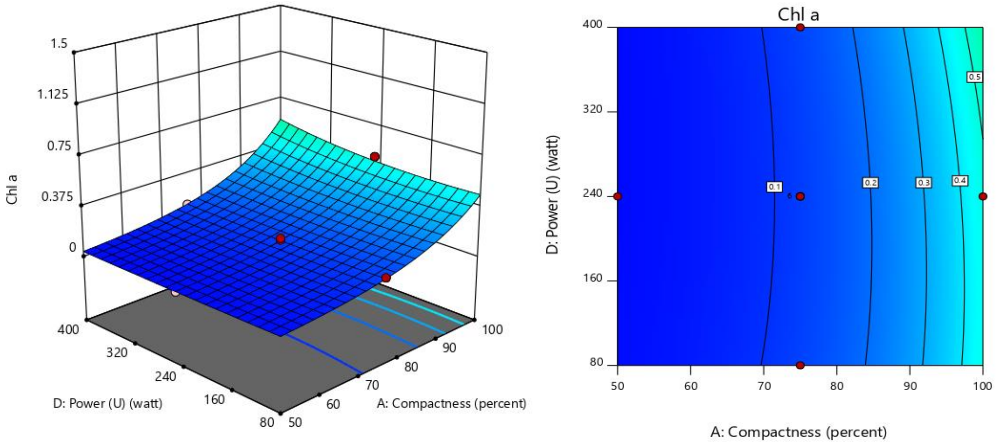

Fig. 2. Simultaneous effect of ethanol concentration percentage and ultrasound power (chlorophyll a).

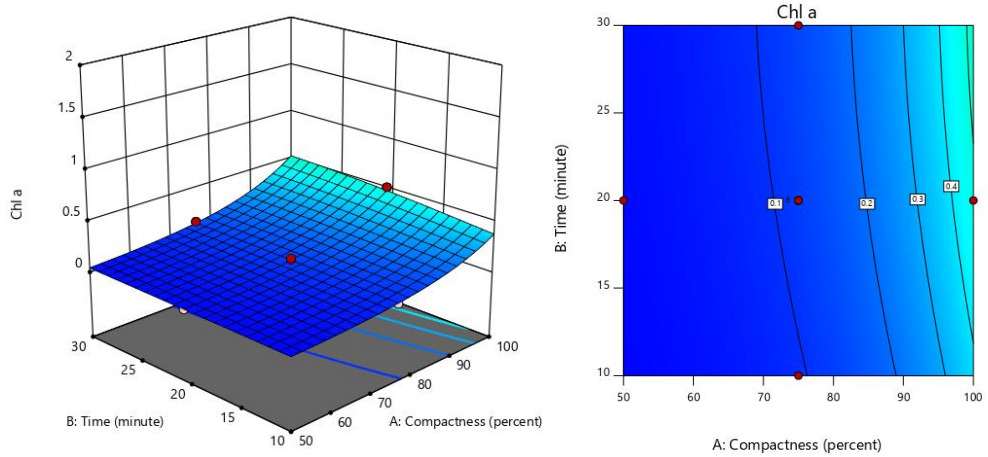

Fig. 3. Simultaneous effect of ethanol concentration percentage and extraction time (chlorophyll a).

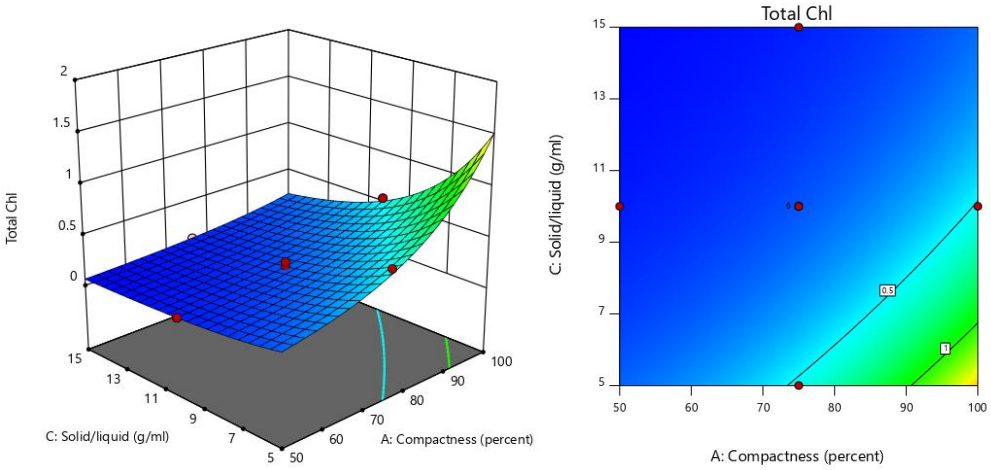

Fig. 4. Simultaneous effect of ethanol concentration and solid-liquid ratio (Total chlorophyll).

Total carotenoid content increases with increasing extraction time and decreasing solid-liquid ratio. Fig. 9 shows the total carotenoid content, the simultaneous effect of the percentage of ethanol concentration and the solid-liquid ratio at a constant time of 20 minutes, and a constant power of ultrasonic waves of 240 watts. Decreasing the solid-liquid ratio and the percentage of ethanol concentration increased the response rate. In the graph above, the highest response was 65 to 95% of ethanol concentration. Fig. 10 shows the total carotenoid content, the synchronous effect of the solid-liquid ratio, and the ultrasound's power at a constant time of 20 minutes and a constant concentration of 75% ethanol. The response rate increases with increasing sonication power and decreasing solid-liquid ratio.

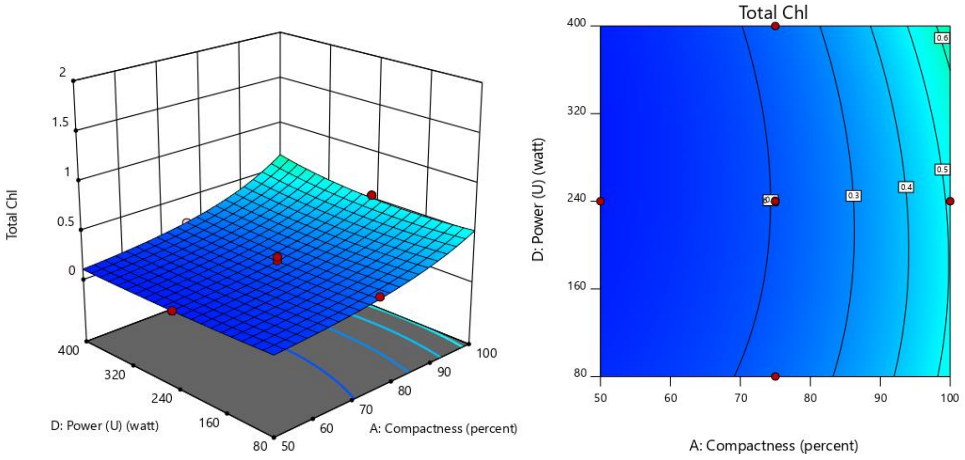

Fig. 5. Synchronous effect of ethanol concentration percentage and ultrasound power (Total chlorophyll).

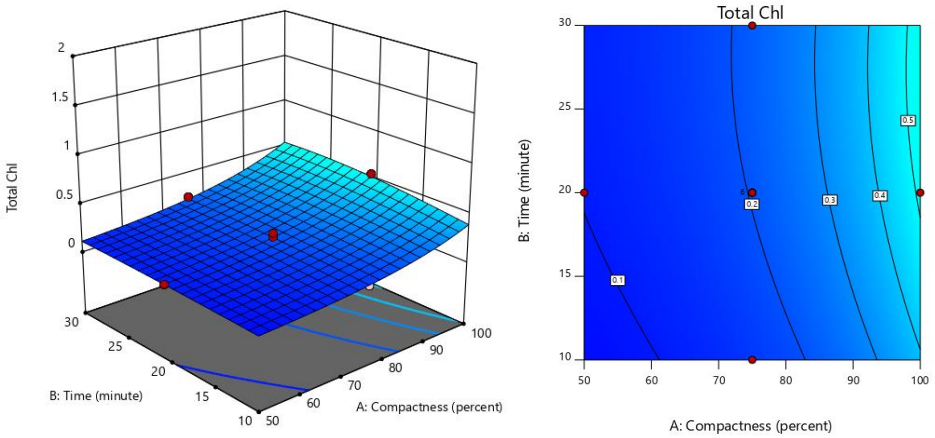

Fig. 6. Effect of concurrent ethanol concentration percentage and extraction time (Total chlorophyll).

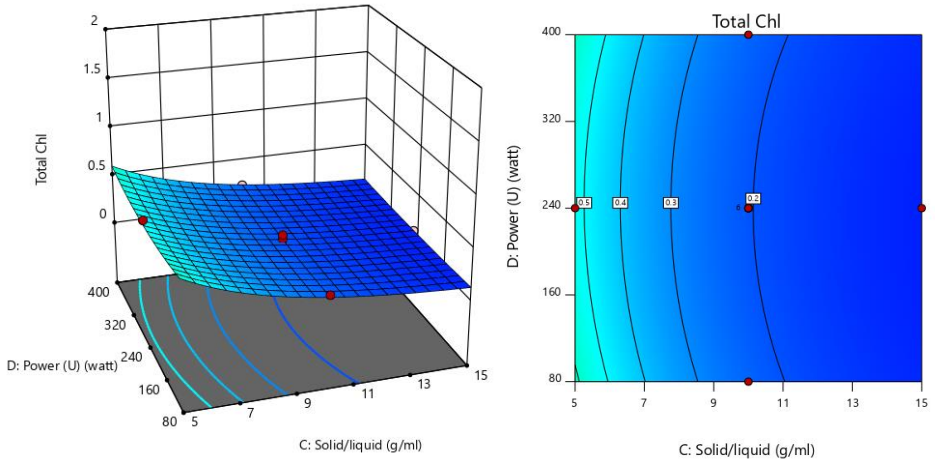

Fig. 7. Simultaneous effect of solid-liquid ratio and ultrasound power (Total chlorophyll).

Fig. 11 shows the total carotenoid content, the simultaneous effect of ethanol concentration percentage, and extraction time on the 1:10 solid-liquid ratio and 240 watts. The response rate increases with the increase of these two factors. According to the graph, the highest response is in the 70 to 90% ethanol concentration range. According to the obtained evidence, the highest total carotenoid content was in 75% ethanol, 20 minutes duration, 1:5 solid-liquid ratio, and 240 watts.

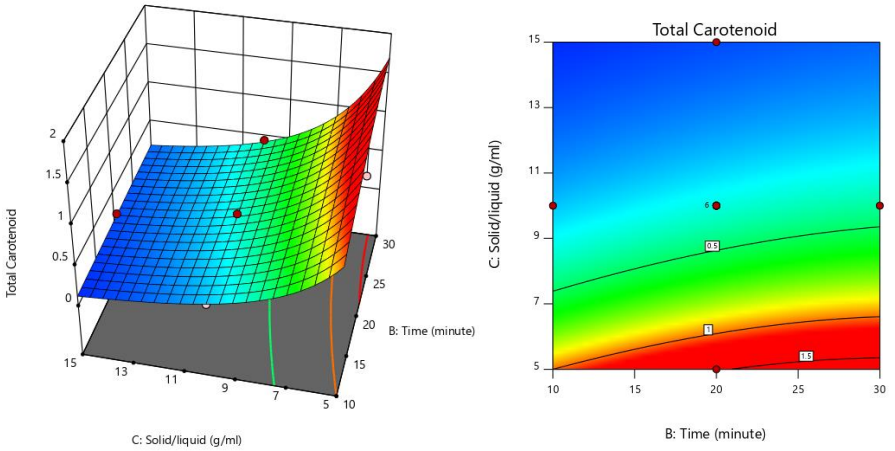

Fig. 8. Simultaneous effect of extraction time and solid-liquid ratio (Total carotenoid)

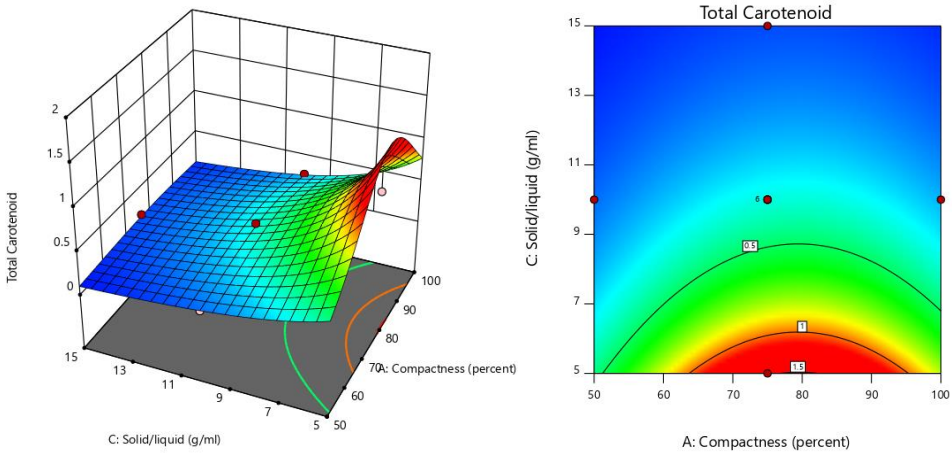

Fig. 9. Simultaneous effect of ethanol concentration percentage and solid-liquid ratio (Total carotenoid)

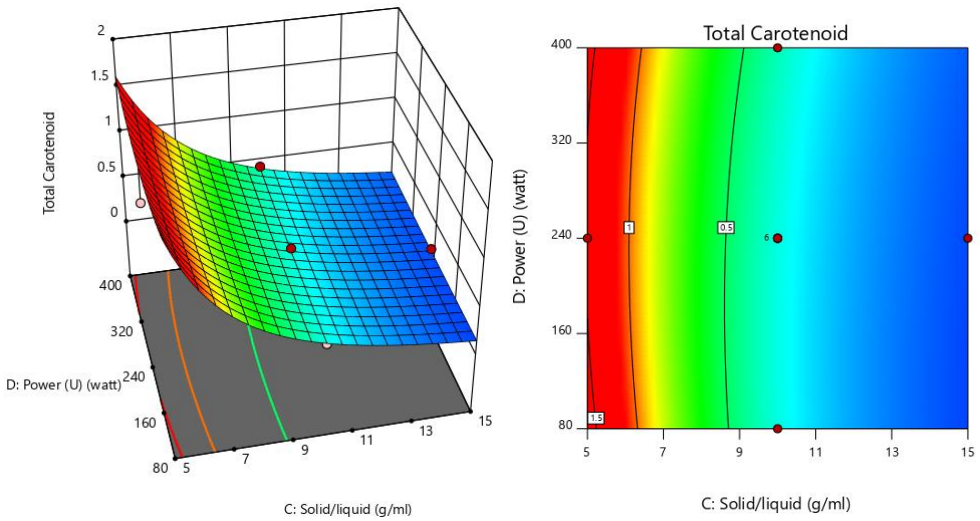

Fig. 10. Effect of Simultaneous Solid-Liquid Ratio and Ultrasound Power (Total Carotenoid).

Fig. 12 shows the amount of fucoxanthin, the synchronous effect of the solid-liquid ratio, and the power of the ultrasound at a constant time of 20 minutes and a constant concentration of 75% ethanol. Decreasing the solid-liquid ratio and increasing the power of the ultrasound increased the response rate.

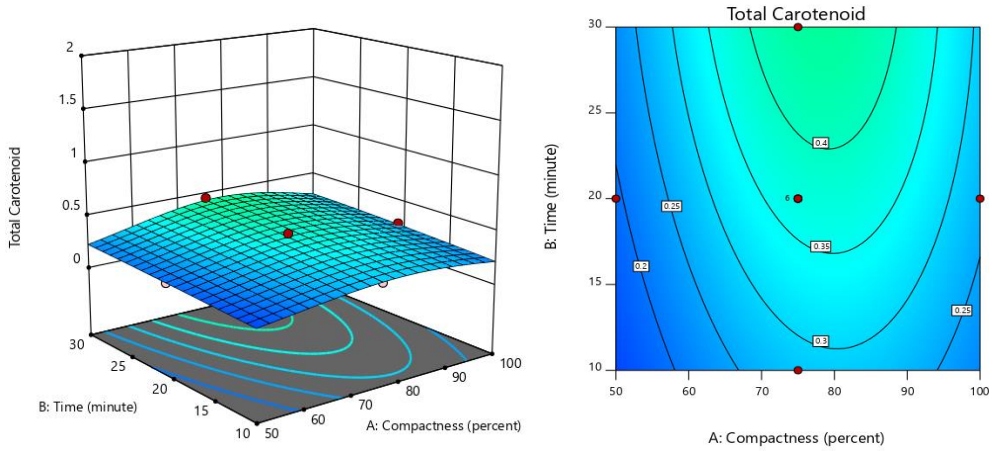

Fig. 11. Effect of simultaneous percentage of ethanol concentration and extraction time (Total carotenoid).

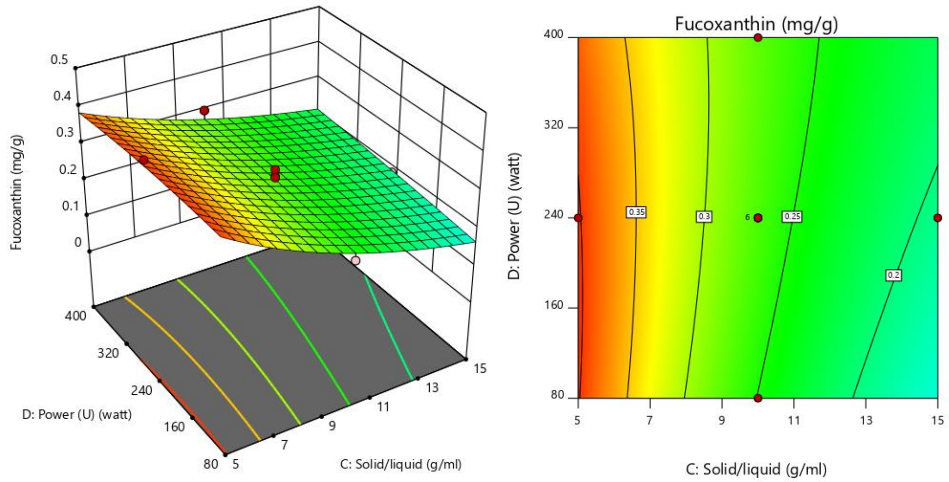

Fig. 12. Effect of simultaneous solid-liquid ratio and ultrasound power (Fucoxanthin).

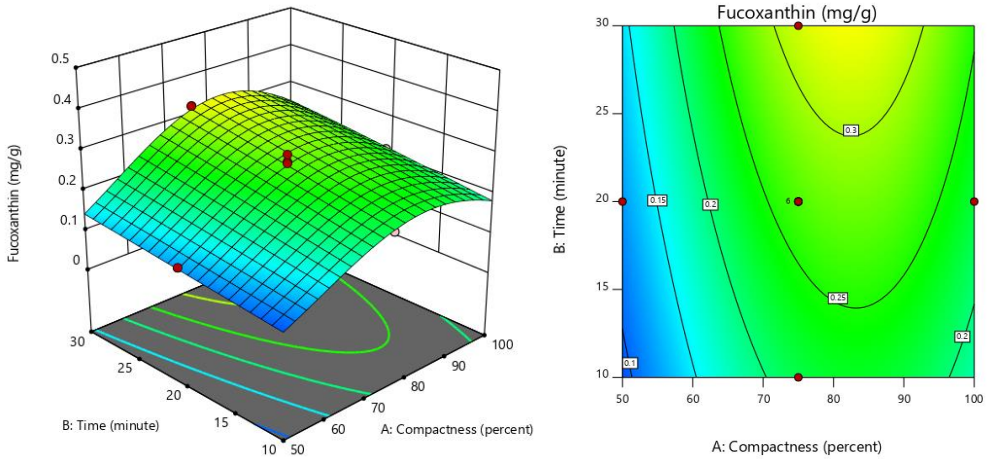

Fig. 13. Simultaneous Effect of Ethanol Concentration Percentage and Extraction Time (Fucoxanthin).

Fig. 13 shows the simultaneous effect on fucoxanthin of the percentage of ethanol concentration and extraction time at 1:10 solid-liquid ratio and 240 watts. The response rate increases with the increase of these two factors. The highest amount of fucoxanthin is in the range of 75 to 95% of ethanol concentration. Fig. 14 shows the effect on fucoxanthin of the simultaneous effect of ethanol concentration and solid-liquid ratio at a constant time of 20 minutes and a constant power of 240 watts. The response rate increases with increasing ethanol concentration and decreasing solid-liquid ratio. The graph shows that the maximum response is in the range of 75 to 95% ethanol. Fig. 15 shows the amount of fucoxanthin, the synchronous effect of the extraction time, and the solid-liquid ratio at 75% constant ethanol concentration and 240 watts. Increasing the extraction time and decreasing the solid-liquid ratio increases the amount of fucoxanthin. The maximum amount of fucoxanthin is at 75% ethanol, 20 minutes duration, 1:5 solid-liquid ratio, and 240 watts.

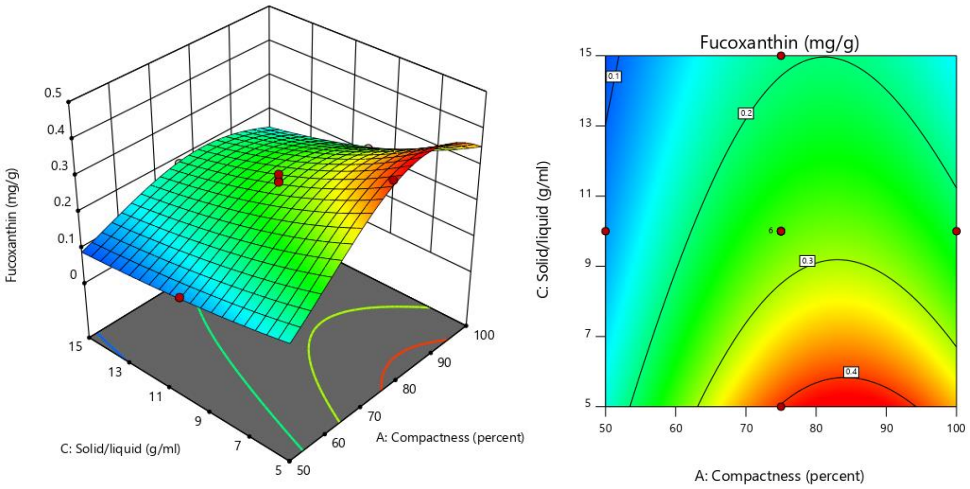

Fig. 14. Effect of Simultaneous Percentage of Ethanol Concentration and Solid-Liquid Ratio (Fucoxanthin).

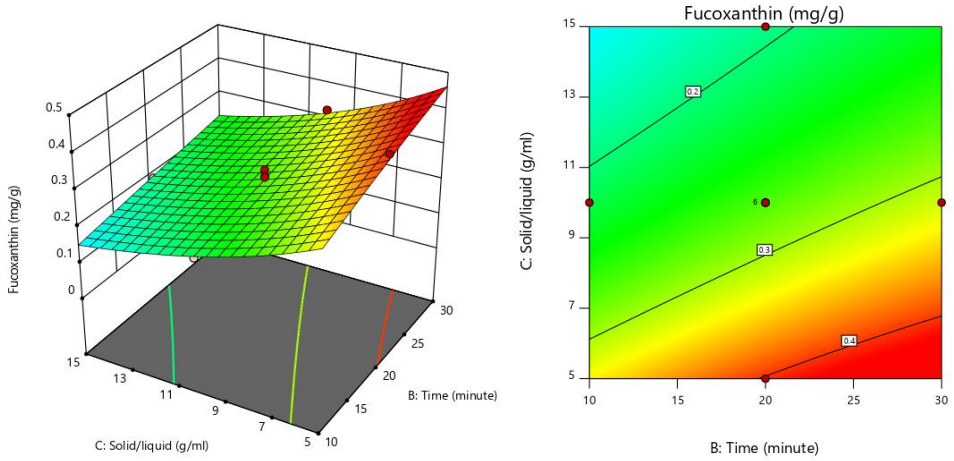

Fig. 15. Simultaneous effect of extraction time and solid-liquid ratio (Fucoxanthin).

4. Discussion

Carotenoid extraction is affected by different species of algae with different structures and compositions (34). Compared to chlorophyll a and c, the fucoxanthin pigment-protein complex is less strongly bound to the thylakoid membrane, as it is an accessory pigment synthesized in response to reduced light availability (35). In the present study, a significant difference was observed regarding the percentage of ethanol concentration in the results. Thus, the highest amount of total chlorophyll and chlorophyll a in ethanol was 100%, and the highest amount of total carotenoids and fucoxanthin was obtained in 75% ethanol. This is because chlorophylls (36) and carotenoids are naturally occurring lipid-soluble pigments produced by plants, algae, phytoplanktons, and some fungi and bacteria (37). Carotenoids are generally hydrophobic molecules and only soluble in organic solvents. The presence of hydroxyl groups at the end of the chains causes carotenoids to be polar molecules and tend to dissolve in various organic solvents (38). It was observed that ethanol was preferred as an excellent solvent for the extraction of fucoxanthin (12). A study on the effect of solvent on the

process of extraction of seaweed pigments showed that in green and brown algae, acetone extracted more carotenoids, and then ethanol had the highest extraction, which is due to the polarity of the solute and the solvent being close. Acetone and ethanol have relatively high polarity (39). In addition to the extraction conditions, different conditions indicated that methanol gave the best result of fucoxanthin extracted (40). However, because ethanol is the best solvent for processing food from a safety viewpoint, it is recommended as an alternative to methanol, although it is slightly more expensive (39). In the ultrasonic extraction method, the power of ultrasound was affected by 3 other factors, so that the maximum amount of chlorophyll and total chlorophyll in 30 minutes, solid-liquid ratio of 1:5, and the power of ultrasonic waves of 400 watts were obtained, while the maximum amount of total carotenoids and fucoxanthin obtained in 20 minutes, the solid-liquid ratio was 1:5. The power of ultrasound was 240 watts. Contrary to the results obtained, in a study on the optimization of fucoxanthin extraction conditions by ultrasound from the alga *Tetrastromatica Padina* and the solid-liquid ratio of 1:10, the power of ultrasound waves was 230 volts. With a frequency of 50 Hz, the concentration of ethanol was considered an important factor, and the results of this study showed that the highest amount of fucoxanthin in ethanol was 80% in 30 minutes (12). This difference is in the present study; increasing the power of ultrasound waves reduced the extraction time of carotenoids. A study on the multistage extraction of seaweed pigments using ultrasound and ultrafiltration performance showed that in the study area, the efficiency of chlorophyll extraction increased with temperature (40 to 60), ultrasound power (100 to 300 Watts), solution pH up to 11 and the solid-liquid ratio to 1:30. However, carotenoid extraction efficiencies peaked at 50. This study demonstrates that the UAE and UF can be employed to enhance the recovery of pigments from seaweed (41). As in this study, the temperature was kept constant at 50±5 °C in the present study. A study on the optimization of fucoxanthin extraction from Irish algae using the response surface method by four factors: time (30 minutes-10 hours), temperature (20-100), solvent pH (5.0-9.0), and percentage of acetone concentration (0-100%) was performed. The results showed that the time factor had no significant effect. In this study, the percentage of acetone had the greatest effect on the performance of fucoxanthin, followed by pH and temperature (35). As in this study, in the present study, the percentage of ethanol concentration had the greatest effect on the extraction performance, but in contrast to these studies in the present study, the time factor affected the extraction of different pigments, and different results were obtained. Due to the presence of ultrasound waves, the extraction time is reduced, which saves time. In this method, the highest levels of fucoxanthin, total carotenoids, total chlorophyll, and chlorophyll a were 0.42, 1.11, 1.85, and 1.69 mg/g, respectively. As can be seen in the results of the present experiment, the maximum amount of pigments was in the solid-liquid ratio of 1:5. This phenomenon is related to the principles of mass transfer, which states that the velocity of diffusion is directly proportional to the concentration gradient, which increases at a lower solid-liquid ratio (42). In this study, the values of all explanatory coefficients above 95% were obtained, which shows the significance of regressions.

5. Conclusion

The results showed that more pigment is extracted with a longer duration of time. In total chlorophyll, total carotenoid, and fucoxanthin, decreasing the solid-liquid ratio increased the response rate, and in chlorophyll a, increasing the solid-liquid ratio increased the response rate. The high fit of the models showed that the second-order polynomial model can be used to optimize the extraction of pigment compounds from brown algae. In addition, it was shown that RSM is an effective method for optimizing extraction conditions. These findings may be used to develop appropriate extraction methods for value-added seaweed products. According to this research, *Sargassum* brown algae is a suitable source for extracting bioactive compounds, and it is necessary to use it as a value-added product in the food and drug industry.

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