Study of Microwave Synergistic Enzyme Method for Extraction from Laminaria Japonica by Response Surface Methodology

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Abstract. Fucoxanthin is a carotenoid which occurs in marine brown algae and exerts multiple beneficial effects on human health, including the anti-diabetic, anti-obesity, anti-cancer, and antioxidant. The aim of this study was to maximise the extraction yield of fucoxanthin from laminaria japonica. By using microwave synergistic enzyme method, the extraction condition, namely the enzyme amount, complex enzyme ratio and microwave temperature were investigated and optimized with Response surface methodology (RSM) combined with Box-Behnken design (BBD). Microwave temperature was found to have the most significant effect on fucoxanthin extraction yield. With the optimized process parameter of extraction in this work, extraction yield of 0.443mg $_{\text{fucoxanthin}}$/g $_{\text{dry algae}}$ was obtained by 0.14g enzyme, complex enzyme ratio (pectinase : cellulase) of 1.12 and microwave temperature at 42.6℃. Therefore, with higher efficiency and low energy consumption system this method can be realized simpler.

1. Introduction

Fucoxanthin(FX) known as a carotenoid derivative found in brown seaweeds. Recently, several researches have demonstrated that FX shows anti-obesity, anti-cancer effects and can be used as free radical scavenger [1]-[3]. Fucoxanthin is estimated to account for more than 10% of the total production of carotenoids in nature, and is responsible for the brown to yellow colour of brown algae [4].

Laminaria japonica, is one species of brown seaweeds and was widely cultured in the northwest Pacific. In many countries, especial in Asian such as China, Korea and Japan, cause the high contents of minerals, vitamins and non-caloric dietary fiber, Laminaria japonica has been consumed as a popular marine vegetable and used as a health food [5].

Suitable operation condition during extraction stage was required due to the thermal sensitivity of fucoxanthin [6]. Extraction of fucoxanthin from laminaria japonica using conventional solvents (methanol, acetone, ethanol) [7] and supercritical CO_2 [8], from Eisenia bicyclis using pressurized liquid extraction [9] and from Hincksia mitchellae using supercritical anti-solvent crystallization[10] have been reported. However, the high operation cost and energy consumption inhibit the industrial production efficiency and popularize.

Microwave can effective break cell wall and has been widely used in environmental, food or pharmacy fields. Enzyme process has been reported to improve the extractions efficient of fucoxanthin from laminaria japonica. Response surface methodology(RSM) is useful for design of the experiments, for estimation the effects and interactions of process variables, and for predict the optimum operational conditions. No reports have been found to extraction fucoxanthin from laminaria japonica by using microwave synergistic enzyme method and optimize the extraction condition with RSM. Therefore, in the present study, RSM based on Box Behnken (BBD) has been used to optimize the microwave synergistic enzyme extractions condition, and determination the effects and interaction of the extraction variables (enzyme amount, complex enzyme ratio, microwave temperature) for extraction yield of fucoxanthin from dry laminaria japonica powder.

2. Materials and methods

2.1. Sample preparation and materials

Fresh Laminaria japonica was used as raw materials in this study and provided from LINHAI Seaweed, Co. Ltd (Xiamen China). Sample was cleaned with deionized water to removed sand, organic debris and epiphytes, the fresh algae was freeze-dried and ground to pieces of about 1mm then stored at -4℃ in darkness until use in order to prevent degradation. Ethanol, pectinase (1000U/g) and cellulase (30000U/g) were purchased from Aladdin biochemical Co. Ltd (Shanghai China). Fucoxanthin standard was from Sigma-Aldrich (Rep. of Ireland).

2.2 Microwave synergistic enzymatic Extraction

Freeze-dried algae (1g) was mixed with pectinase and cellulase, then extracted by using ethanol 95%. A Microwave extractor (APEX YIYAO Shanghai, China) was assisted with enzyme extraction of fucoxanthin. After extraction process, the solution was filtered through a filter (0.45 μm) and the solvent was removed by using a vacuum evaporator. Microwave synergistic enzyme extraction was performed in darkness due to the photo sensitivity of fucoxanthin. The residues were analysis with a UV spectrometer under 449nm. All experiments were conduced in triplicate.

2.3 Extraction yield

The extraction yield was expressed by Eq. (1).

$$X = AV/(A_{1cm}^{1\%} \times 100) \quad (1)$$

where A is the absorption value of sample by 449nm, Abs; $A_{1cm}^{1\%}$ is the theoretical absorption value of sample with concentration of 1g/L in 1cm optical length, here is 1600, Abs; V is the sample volume, ml; X is the extraction yield of fucoxanthin, mg fucoxanthin/g dry laminaria japonica.

2.4 Experimental design

To optimized the extraction process, a response surface method (RSM) was used and with Stat-Ease software (Design-Expert 8.0version) to performed the experimental design and the variances analysis. A three-levels three-factors Box-Behnken design (BBD) was employed and to optimize the extraction conditions. Three variables are presented in Table 1.

Table 1. Factors and levels of the responding surface design

Factor	unit	symbol	Coded level	
			-1	+1
Enzyme amount	g	A	0.07	0.15
Complex enzyme ratio (pectinase : cellulase)	-	B	0.5	2
Microwave temperature	℃	C	40	50

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Each variable and rang was determined by preliminary experiments. An analysis of variance (ANOVA) table was generated to presented the effect and interaction of factors. The significances were judged by calculating the F-value at a probabilites (P-value) of 0.0001, 0.001 and 0.05.

3. Results and Discussion

The UV-spectrometer results from fucoxanthin standard and extracted powder indicated that the extracted powder was fucoxanthin.

3.1 Analysis of Variance (ANOVA)

The designed experiments (17 runs) by using BBD method are shown in Table 2. The extraction yield varies between 0.27 mg/g and 0.45 mg/g, respectively.

Table 2. The experimental results of Box-Benhnken Design

STD	A	B	C	Extraction yield /(mg/g)
1	0.11	1.25	45	0.43
2	0.15	1.25	50	0.31
3	0.15	2.00	45	0.35
4	0.11	1.25	45	0.41
5	0.15	1.25	40	0.43
6	0.11	0.50	50	0.28
7	0.07	0.50	45	0.32
8	0.11	1.25	45	0.43
9	0.15	0.50	45	0.38
10	0.11	1.25	40	0.37
11	0.11	2.00	45	0.42
12	0.11	2.00	50	0.29
13	0.07	1.25	45	0.31
14	0.11	1.25	50	0.45
15	0.07	1.25	50	0.31
16	0.11	2.00	40	0.32
17	0.07	1.25	40	0.33

The analysis of variance (ANOVA) shows in Table 3, the model F-value of 43.03 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could due to noise. P-value is lower than 0.0001, which indicates that the model terms are highly significant. The P-value of factors C, B^2 and C^2 are lower than 0.0001 shows highly significant effect. Values of P great than 0.0001 and less than 0.005 indicate model terms are significant, in this case A, AC and A^2 are significant model terms. Lack of fit is determining how well the model fits the data, in this study, the Lack of fit (P>0.05) is insignificant, the response was sufficiently represented.

Table 3. Anova of regression model

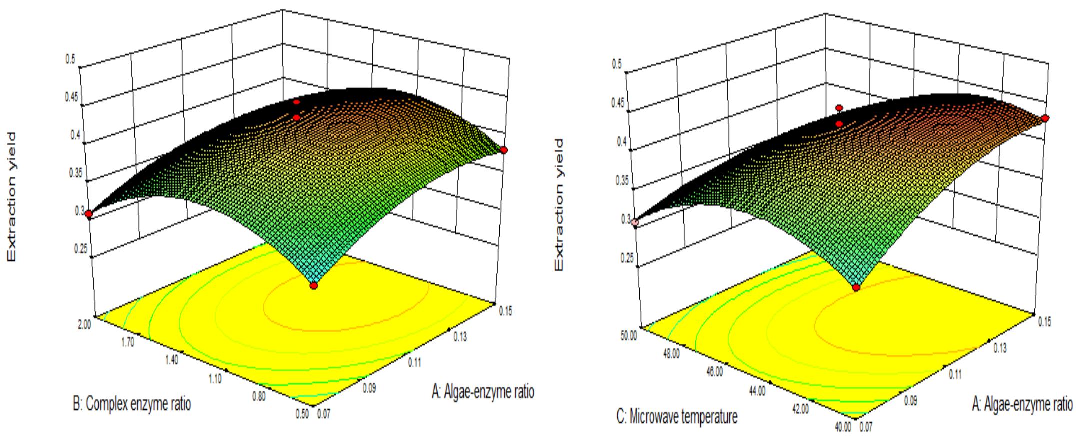
source	Sum of squares	d_f^a	mean square	F	P
Model	0.051	9	5.716E-003	43.03	< 0.0001
A	5.000E-003	1	5.000E-003	37.63	0.0005
B	8.000E-004	1	8.000E-004	6.02	0.0439
C	8.450E-003	1	8.450E-003	63.60	<0.0001
AB	1.000E-004	1	1.000E-004	0.75	0.4144
AC	2.500E-003		2.500E-003	18.82	0.0034
BC	9.000E-004	1	9.000E-004	6.77	0.0353
A^2	3.541E-003	1	3.541E-003	26.65	0.0013
B^2	0.015	1	0.015	110.32	<0.0001
C^2	0.012	1	0.012	92.41	<0.0001
Residual	9.300E-004	7	1.329E-004		
Lack of fit	5.000E-005	3	1.667E-005	0.076	0.9698
Pure erro	8.800E-004	4	2.200E-004		
Cor total	0.052	16			

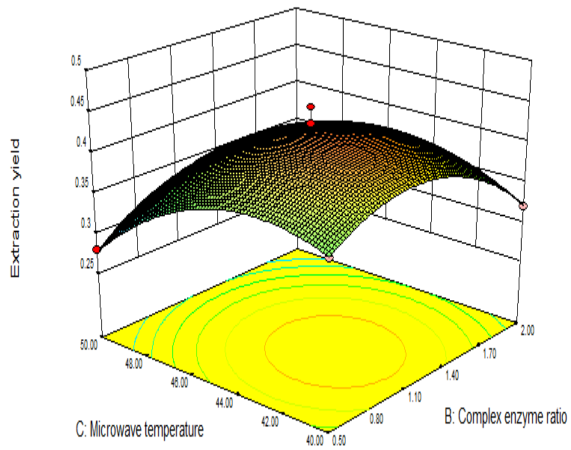
The value of R^2 (0.9822) was close to that of R_a^2 (0.9894). This suggests that the predicated values (response) match well with the observed value, supporting that the regression model is adequate to explain most of the variability for extraction yield of fucoxanthin from Laminaria japonica in the given experimental domain.

3.2 Effect of Extraction Conditions

The three dimensional (3D) surface responses plots were utilized to study the effect and relationship of microwave synergistic enzymatic extraction condition for fucoxanthin from Laminaria japonica. Figure 1(a-c) shows the fitted response surface plots of extraction yield versus extraction variables (enzyme amount, complex enzyme ratio and microwave temperature)

a) b)





c)

Figure.1 Response surface plots

It is observed that the extraction yield is directly correlated with these variables, As shown in Figure 1a and 1b, the plot is not perfectly convex. This indicates that there is less interaction between the two variables. Moreover, the extraction yield increased slightly with the enzyme amount for a given complex enzyme ratio. By enzyme amount at 0.15 g the extraction yield reached a highest value. The increase in extraction yield can be explained by the increase in the amount of enzyme and the effective broken of algae cell wall, thus bringing about an increase in the extraction yield. It is also observed that the extraction yield increase with complex enzyme ratio to obtain a maximum at 1.2 and thereafter decrease with a further increase in complex enzyme ratio for a given enzyme amount. Therefore, a suitable complex enzyme ratio (pectinase: cellulase) about 1.4 is preferable for achieving a higher extraction yield. This trend is consistent to explained as follows: fucoxanthin in laminaria japonica surround mainly by cellulose, semi-cellulose and pectin substance, in this study used enzyme were pectinase (1000U/g) and cellulase (30000U/g), cellulase has higher activity unit than pectinase, with suitable ratio of pectinase and cellulase achieved extraction yield the maximum. Figure 1c presented the effects of complex enzyme ratio and microwave temperature on the extraction yield. As indicated, the extraction yield achieved maximum by microwave temperature at 45℃ for a given complex enzyme ratio, thereafter decrease with increase temperature. This can be attributed to the fact that the fucoxanthin and enzyme were thermal sensitivity. Microwave temperature had an antagonistic effect of extraction yield due to the thermal sensitivity. By microwave temperature at 45℃ the enzymes had a suitable environment, in contrast, the enzymes have less activity by higher temperature.

3.3 Optimized Studies for Maximizing Extraction Yield

The Optimum modification conditions of the extraction yield was carried out by using the Design-Expert software. According to the results above, the optimization equation shows in follow:

$$Y=0.43+0.025A-0.01B-0.033C-5E\text{-}003AB-0.025AC+0.015BC-0.029A^2-0.059B^2-0.054C^2$$

where A is the enzyme amount; B is the complex enzyme ratio and C is the microwave temperature.

Table4. Optimization Results of the Modification variables

Modification variable	Optimum value
Enzyme amount	0.14
Complex enzyme ratio	1.12
Microwave temperature	42.6℃

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The aim of this study was to determine the interaction of extraction variables and maximize the extraction yield with the extraction condition from the model. Enzyme amount, complex enzyme ratio and microwave temperature were selected by applying the regression model. To confirm the model adequacy three additional experiments were carried out under the optimal conditions, the average values of the triplicate experiments 0.443 mg/g (fucoxanthin/dry algae) are close to the predicated results 0.452 mg/g, it indicate that the proposed model can well correlate the microwave assisted enzymatic extraction variables to the extraction yield.

4. Conclusion

In this work, the extraction condition for fucoxanthin from laminaria japonica with microwave synergistic enzyme method were investigated and optimized to improve the extraction yield by using response surface methodology. According to the BBD analysis results, the effect and interaction of the extraction variables including enzyme amount, complex enzyme ratio and microwave temperature on the extraction yield of fucoxanthin was investigated. The term for microwave temperature shows the most significant effect to the extraction yield of fucoxanthin. With the optimized condition (enzyme amount 0.14 g, complex enzyme ratio 1.12 and microwave temperature 42.6℃) achieve the extraction yield of fucoxanthin to 0.443, and shows a higher extraction yield and lower process cost compare to other methods. Therefore, this method can be used in extraction of thermal sensitive materials.

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6. References

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