



RESPONSE SURFACE OPTIMIZATION OF MICROWAVE-ASSISTED SULFATED POLYSACCHARIDE EXTRACTION FROM PORPHYRA DENTATE

Yeu-Pyng Lin

Department of Food Nutrition, Chung Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.

Shao-Chi Wu

Department of Food Nutrition, Chung Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.

Jean-Yu Hwang

*Department of Food Nutrition, Chung Hwa University of Medical Technology, Tainan, Taiwan, R.O.C.,
zhenyu@mail.hwai.edu.tw*

Follow this and additional works at: <https://jmstt.ntou.edu.tw/journal>



Part of the [Aquaculture and Fisheries Commons](#)

Recommended Citation

Lin, Yeu-Pyng; Wu, Shao-Chi; and Hwang, Jean-Yu (2014) "RESPONSE SURFACE OPTIMIZATION OF MICROWAVE-ASSISTED SULFATED POLYSACCHARIDE EXTRACTION FROM PORPHYRA DENTATE," *Journal of Marine Science and Technology*. Vol. 22: Iss. 5, Article 15.

DOI: 10.6119/JMST-013-1218-2

Available at: <https://jmstt.ntou.edu.tw/journal/vol22/iss5/15>

This Research Article is brought to you for free and open access by Journal of Marine Science and Technology. It has been accepted for inclusion in Journal of Marine Science and Technology by an authorized editor of Journal of Marine Science and Technology.

RESPONSE SURFACE OPTIMIZATION OF MICROWAVE-ASSISTED SULFATED POLYSACCHARIDE EXTRACTION FROM *PORPHYRA DENTATE*

Yeu-Pyng Lin, Shao-Chi Wu, and Jean-Yu Hwang

Key words: *Porphyra denate*, sulfated polysaccharide, microwave-assisted extraction (MAE), response surface methodology (RSM).

ABSTRACT

An experiment was performed by adjusting the pH value (4-10) of a 10%-to-90% ethanol solution (v/v) and using it as an extraction solvent and, subsequently, using microwave-assisted extraction (MAE) to extract *Porphyra denate* solutions. The sulfated polysaccharides ($\mu\text{g/mL}$) of these extraction solutions were evaluated. The optimal treatments of *P. denate* extracts obtained using MAE were studied using response surface methodology. The study was performed using a three-level, four-factor design and aimed to determine the optimal combinations of the ethanol concentration (10%-90%, v/v, X_1), pH value of an ethanol solution (4-10, X_2), microwave power level (200-400 W, X_3), and heating time of a microwave field (1-5 min, X_4) that optimized the sulfated polysaccharide effect of a *P. denate* extract solution. The response variable of the sulfated polysaccharide effect was considerably affected by the ethanol concentration, and the pH value of the ethanol solution was at a 0.1% significant level. The optimal treatments were established by adjusting the pH value between 8 and 9 and the ethanol concentration between 30% and 50% and, subsequently, using a 1-min heating time in a 200-W microwave field.

I. INTRODUCTION

All seaweeds are rich sources of sulfated polysaccharides, including several that have become valuable additives in the food industry because of their rheological properties as gelling and thickening agents (e.g., carrageenan). In addition, sulfated

polysaccharides exhibit several biological activities including antioxidant, anticoagulant, antitumor, antiviral, immunomodulatory, and immunoinflammatory effects, as well as specific activities against kidney, liver, and urinary system disorders that may be relevant in nutraceutical and functional food as well as cosmetic, cosmeceutical, and pharmaceutical applications [2, 12]. Sulfated polysaccharides are found in the three main divisions of marine algal groups, Rhodophyta, Phaeophyta, and Chlorophyta [5]. Among these three groups, Phaeophyta has received most attention because they have a higher sulfate content than the other two groups do [7]. There has been little research on sulfated polysaccharides from *Porphyra* (Rhodophyta), and, thus, they deserve further exploration [19]. The *Porphyra* species are edible red algae that are abundantly cultivated in East and Southeast Asia [11]. The edible red seaweed, *P. dentate* (Bangiaceae), is widely distributed in East-Asian countries [15]. Porphyran, one of the main constituents of the *Porphyra* species, is a linear sulfated polysaccharide comprising a hot-water soluble portion of the cell wall and an intracellular matrix. It consists of D-galactose, 3,6-anhydro-L-galactose, 6-O-methyl-D-galactose, and L-galactose-6-sulphate [11]. The sulfated polysaccharides from the *Porphyra* species are worthy of further research.

Microwave-assisted extraction (MAE) has been reported as a novel method for extracting bioactive compounds. It involves using microwave energy to cause molecular movement and rotation in liquids by means of permanent or induced dipoles [21]. When a biological material with suitable dielectric properties (plant material along with a solvent for extraction) is placed in a microwave field, the molecules tend to align with the oscillating electromagnetic field, through either distortion or distribution of the electron cloud within the molecule or physical rotation of the molecular dipoles, both of which lead to a rapid heating of the solvent and sample matrix [6]. MAE is superior to conventional extraction techniques because of its high efficiency, short extraction time, rapid and volumetric heating of the absorption medium, low solvent consumption, high selectivity for target molecules, and high potential for automation [14].

Response surface methodology (RSM) is a statistical procedure frequently used in optimization studies. It involves using quantitative data based on an appropriate experimental design to determine the optimal conditions while solving multivariate problems. Several authors have used RSM in their optimization studies on the MAE of polysaccharides from *Catathelasma ventricosum* [26], *Cyclocarya paliurus* (Batal.) Iljinskaja [23], *Lilium davidii* var. *unicolor* Salisb [25], and *tremella* [4].

In our previous study, we focused on the effect of the MAE technology of polyphenols on the scavenging of free radicals and the ferrous chelating abilities of a *P. dentate* extract when various concentrations of *P. dentate*, mesh sizes of *P. dentate*, and ethanol percentages were used [16]. The objective of the present study was to study the effects of the ethanol concentration, pH value of the ethanol solution, microwave power, and heating time of the microwave field on sulfated polysaccharides from *P. dentate*. On the basis of RSM, second-order polynomial models were used to predict the effects of intermittent MAE treatments. The MAE scheme is a novel method for reducing the energy consumption of the extraction process while maintaining the desired product quality. This study provides a new approach for manufacturing seaweed applications.

II. MATERIALS AND METHODS

1. *Porphyra dentate* Powder Particles

Dried *P. dentate* was purchased from a traditional market in Penghu, Taiwan. The dried *P. dentate* alga (moisture 15.2%, crude protein 25.4%, crude lipid 0.55%, carbohydrate 41.35%, and ash 17.5%) was crushed and then screened using a standard screening sieve (Tokyo Garasu KiKai Co., Ltd., Tokyo, Japan). The *P. dentate* powder particles were screened through a 60-mesh sieve, and the diameter of the particles was smaller than 0.25 mm [22].

2. Microwave-Assisted Extraction

Focused microwave-assisted solvent extraction was performed using a modified method described by Soysa *et al.* [20]. A microwave-accelerated reaction system (MARS5, CEM Corporation, Matthews, North Carolina, U.S.A.) performed solvent extraction. The operation frequency of the magnetron was 2,455 MHz. The test suspensions that were used were 1.0% *P. dentate* powder particles mixed into 50-mL water-ethanol solutions in ratios of 90:10, 50:50, and 10:90 (v/v), and the pH value was adjusted to 4, 7, and 10, respectively. According to the experimental design (Table 1), the solutions were radiated in a microwave system, and the temperature was kept below 80°C. The infusions were allowed to cool down to room temperature and were then filtered and stored in a refrigerator at -20°C to determine the sulfated group contents of polysaccharides from *P. dentate*.

3. Quantification of the Sulfated Group Contents

1) Reagents

BaCl₂ buffer: The total volume of 10 mL of 2 M acetic acid, 2 mL of 5 μM BaCl₂, and 8 mL of 20 μM NaHCO₃ was adjusted to 100 mL by using absolute ethanol; Na-rhodizonate solution: 5 mg of Na-rhodizonate and 100 mg L-ascorbic acid were dissolved in 20 mL of distilled water. The volume was adjusted to 100 mL by using absolute ethanol. The reagents were light sensitive and, therefore, light protected. A standard curve was established using 2-12 μg/mL of Na₂SO₄ as the standard [8].

2) Sample Treatment

For all test samples, the total sugar content was adjusted to 10 mg/mL. Subsequently, 0.5 mL of 1 N HCl was added to 0.5 mL of a sample solution and boiled for 1 h. All of the solvent was removed using a vacuum concentrator at 60°C-65°C, and 0.5 mL of deionized water was then added [8].

3) Determination of the Sulfated Group Content

Two milliliters of absolute ethanol was added to 0.5 mL of a sample solution, and the insoluble portions were removed through centrifugation at 2,500 × g. Subsequently, 1 mL of the BaCl₂ buffer was added, followed by 1.5 mL of Na-rhodizonate. The reaction was conducted in the dark for 10 min, and A₅₂₀ was measured within 30 min [8].

4. Experimental Design

The response variables of the sulfated group contents of the polysaccharides of *P. dentate* were investigated. A three-level, four-parameter experimental design proposed by King and Lin [13] was used to evaluate the optimal treatment conditions. The experimental error was determined by performing the experimental procedure and measuring the center point three times.

Three mathematical functions of f_k were assumed to exist for η_k as follows:

$$\eta_k = f_k(\varepsilon_1, \varepsilon_2, \varepsilon_3, \varepsilon_4) \quad (1)$$

Where ε_1 is the ethanol concentration, ε_2 is the pH value of the ethanol solution, ε_3 is the microwave power, and ε_4 is the heating time of the microwave field. A second-order polynomial was used to express the function f_k as follows:

$$\eta_k = \beta_{k0} + \sum_{i=1}^4 \beta_{ki} X_i + \sum_{i=1}^4 \beta_{kii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{kij} X_i X_j \quad (2)$$

where β_{k0} , β_{ki} , β_{kii} , β_{kij} are regression coefficients and X_i represents the coded independent variables of ε_1 , ε_2 , ε_3 , and ε_4 . The values of the independent variables were coded within the range of -1 and +1, and the original independent variables, X_i , were normalized using the following equation:

$$X_i = \frac{2}{I_i}(\varepsilon_i - \bar{\varepsilon}_i) \quad (3)$$

Table 1. Process variables and their levels in the four variables-three levels response surface design.

Independent variables	Symbols		Levels		
	coded	uncoded	-1	0	+1
Ethanol concentration (% , v/v)	X_1	ε_1	10	50	90
pH value	X_2	ε_2	4	7	10
Microwave power (W)	X_3	ε_3	200	300	400
Heating time of microwave field (min)	X_4	ε_4	1	3	5

where ε_i is the current value of the variable, $\bar{\varepsilon}_i$ is the mean arithmetic value of the highest and lowest values of the set, and I_i is the greatest difference between those extremes.

5. Statistical Analysis

Contour and surface plots were determined using Sigmaplot software (Scientific Graph System, Version 7.00, SPSS Inc., 2001, U.S.A.). Analysis of an analysis of variance (ANOVA) table and the determination of the responses in those models was conducted using the PROC RSREG procedure of the SAS program, and the validity of the models was evaluated (SAS, Version 8.1, SAS Inc., 1999. U.S.A.).

III. RESULTS AND DISCUSSION

1. Effect of the Parameters

The experiments were performed according to a design with four variables and three levels for each variable (Box & Behnken, 1960). The independent variables were the ethanol concentration (X_1), the pH value of the ethanol solution (X_2), the microwave power (X_3), and the heating time of the microwave field (X_4). The experimental design in the coded and actual levels is shown in Table 1. The experimental design and the response surface analysis data of this study are shown in Table 2. The experiments were performed in random order to study the relationships between the dependent variable, which was the sulfated polysaccharide content from *P. dentate*, and the independent variables X_1 , X_2 , X_3 , and X_4 , which were the ethanol concentration, the pH value of the ethanol solution, the microwave power, and the heating time of the microwave field. ANOVA was performed to determine the lack of fit and significance of the linear, quadratic, and cross-product effects of the independent variables on the quality attributes (Table 3). The lack-of-fit test is a measure of the failure of a model used to represent data in the experimental domain at points that are not included in a regression [17]. The analysis of the lack of fit was performed for all dependent variables, and the results were nonsignificant. High coefficients of the determination values ($R^2 > 0.95$) were obtained for the significant-response surface models (i.e., the sulfated polysaccharide contents from *P. dentate*), indicating that a high proportion of the variability was explained by the data. The models developed in this study were proven to be adequate.

Table 2. The experimental design and data for the response surface analysis.

Treatment number ^a	X_1	X_2	X_3	X_4	Sulfated polysaccharides ($\mu\text{g/mL}$)
1	1 (90%)	1 (10)	0 (300W)	0 (3)	48.6
2	1 (90%)	-1 (4)	0 (300W)	0 (3)	23.3
3	-1 (10%)	1 (10)	0 (300W)	0 (3)	60.9
4	-1 (10%)	-1 (4)	0 (300W)	0 (3)	47.0
5	0 (50%)	0 (7)	1 (400W)	1 (5)	76.1
6	0 (50%)	0 (7)	1 (400W)	-1 (1)	75.1
7	0 (50%)	0 (7)	-1 (200W)	1 (5)	78.1
8	0 (50%)	0 (7)	-1 (200W)	-1 (1)	76.2
9	0 (50%)	0 (7)	0 (300W)	0 (3)	73.9
10	1 (90%)	0 (7)	0 (300W)	1 (5)	61.8
11	1 (90%)	0 (7)	0 (300W)	-1 (1)	59.7
12	-1 (10%)	0 (7)	0 (300W)	1 (5)	61.3
13	-1 (10%)	0 (7)	0 (300W)	-1 (1)	61.9
14	0 (50%)	1 (10)	1 (400W)	0 (3)	65.8
15	0 (50%)	1 (10)	-1 (200W)	0 (3)	70.0
16	0 (50%)	-1 (4)	1 (400W)	0 (3)	41.8
17	0 (50%)	-1 (4)	-1 (200W)	0 (3)	47.9
18	0 (50%)	0 (7)	0 (300W)	0 (3)	71.8
19	1 (90%)	0 (7)	1 (400W)	0 (3)	61.7
20	1 (90%)	0 (7)	-1 (200W)	0 (3)	58.9
21	-1 (10%)	0 (7)	1 (400W)	0 (3)	60.2
22	-1 (10%)	0 (7)	-1 (200W)	0 (3)	68.1
23	0 (50%)	1 (10)	0 (300W)	1 (5)	63.8
24	0 (50%)	1 (10)	0 (300W)	-1 (1)	73.4
25	0 (50%)	-1 (4)	0 (300W)	1 (5)	51.9
26	0 (50%)	-1 (4)	0 (300W)	-1 (1)	40.0
27	0 (50%)	0 (7)	0 (300W)	0 (3)	71.8

^aThe experimental runs were performed in random order.

Table 3. Analysis of variance for response variable.

Source	DF ^a	Sum of squares
		Sulfated polysaccharides ($\mu\text{g/mL}$)
Model	14	4310.05***
Linear	4	1625.39***
Quadratic	4	2505.06***
Cross product	6	179.60
Residual	12	199.25
Lack-of-fit	10	196.31 ^{NS}
Pure error	2	2.94
Independent variables		
Ethanol concentration (X_1)	5	977.84***
pH value (X_2)	5	3054.02***
Microwave power level (X_3)	5	70.38
Heating time of microwave field (X_4)	5	132.29
R^2		0.9558

*Significant at 5% level; **Significant at 1% level; ***Significant at 0.1% level.

^a: DF: Degree of freedom. ^{NS}: Not significant.

Table 4. Regression coefficients of the second order polynomial for response variable.

Coefficient ^a β_k	Sulfated polysaccharides ($\mu\text{g/mL}$)
β_{k0}	72.50***
β_{k1}	-3.78**
β_{k2}	10.88***
β_{k3}	-1.54
β_{k4}	0.56
β_{k11}	-11.81***
β_{k21}	2.85
β_{k22}	-16.68***
β_{k31}	2.68
β_{k32}	0.48
β_{k33}	1.51
β_{k41}	0.68
β_{k42}	-5.38*
β_{k43}	-0.23
β_{k44}	1.43

^a: These are the coefficients of Equation (2), and the numbers 1 to 4 in the subscripts refer to ethanol concentration, pH value, microwave power, and intermittency of microwave field, respectively.

*Significant at 5% level; **Significant at 1% level; ***Significant at 0.1% level.

An ANOVA was conducted to assess the significant effects of each independent variable on the responses and to analyze which responses were significantly affected by which treatment combination. As shown in Table 3, the optimal combination of the ethanol concentration and the pH value of the ethanol solution significantly affected sulfated polysaccharides at the 0.1% level.

Table 4 shows the regression coefficients for the second-order polynomial models of sulfated polysaccharide contents from *P. dentate* used to predict the values under optimal conditions. Contour plots were generated using significant parameters for each response. The responses of the sulfated polysaccharide contents from *P. dentate* were significantly affected by the independent variables (the ethanol concentration and the pH value of the ethanol solution). These responses were used to determine the optimal treatment conditions. The graphic superposition method was used for optimization.

2. Optimization Studies and Verification of the Models

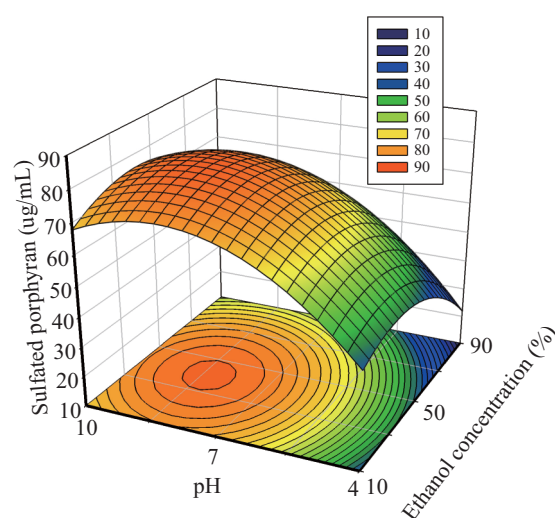
RSM is an approach that has been widely used in optimization studies. Optimal conditions can be determined by superimposing contour plots of relevant and statistically significant responses. An optimal area is generated, forming the basis for optimal treatments. Fig. 1 shows the regions of the optimal treatment conditions for adjusting the pH value between 8 and 9 and the ethanol concentration between 30% and 50% and, subsequently, using a 1-min heating time in a 200-W microwave field.

Table 5. Predicted and observed values for the response variables at optimum conditions.

Point	Coordinate (X_1, X_2, X_3, X_4)	Sulfated polysaccharides ($\mu\text{g/mL}$)	
		Predicted value	Observed value
1	(-0.25, 0, -1, -1)	77.21	77.15 ^{NS} \pm 0.97
2	(-0.25, 0.5, -1, -1)	80.57	80.23 ^{NS} \pm 0.69
3	(0, 0, -1, 1)	77.77	77.34 ^{NS} \pm 0.66

^{NS}: Not significant for *t*-test at 95% level confidence.

Results are of three replicates.

**Fig. 1. Response plot of sulfate polysaccharides ($\mu\text{g/mL}$) from *porphyra dentate* using microwave-assisted extraction with 1 min heating time at 200 W microwave field.**

The optimal operating conditions were determined when the sulfated polysaccharide content was higher than 80 $\mu\text{g/mL}$. The optimal treatment was achieved by applying a 1-min heating time in a 200-W microwave field through an operation that involved controlling the ethanol concentration within a range of 30%-50% and adjusting the pH value between 8 and 9. Verification tests were performed under optimal conditions (Points 1, 2, and 3) to determine the adequacy of the second-order polynomial model; the results are shown in Table 5. The experimental values were the averages of three replications and the differences among the predicted values were determined to be nonsignificant by conducting a *t* test at a 95% level of confidence, indicating that the second-order polynomial models that were generated were adequate. Optimal treatment conditions were recommended, and validation results were adequate and acceptable, demonstrating that the second-order polynomial models that were generated for the significant responses were valid.

3. Efficiency of Microwave Treatment

Zhao *et al.* [25] studied four parameters, namely extraction time, microwave power, extraction temperature, and the ratio of solids to water and optimized them by using the Box-

Behnken design with a quadratic regression model constructed by applying RSM to the MAE of polysaccharides from *Lilium davidii* var. *unicolor* Salisb (LP_{MAE}). Their results showed that when the ratio of solids to water increases, the microwave extraction efficiency of LP_{MAE} also increases. However, when the amount of solids decreases and that of water increases, the microwave power increases and the LP_{MAE} slightly decreases. Furthermore, their study showed that the lower the microwave power was, the higher the efficiency of the sulfated polysaccharides extraction became when the ratio of solids to liquid of a continuous phase was constant. However, the extraction time and the ratio of solids to water exhibited an optimal result for LP_{MAE}. The present study showed the same phenomenon of intermittency of sulfated polysaccharides, demonstrating an optimal extraction efficiency for a regular concentration of ethanol and pH value at various levels of microwave power.

The difference between our study and that of Zhao *et al.* [25] is that they extracted LP_{MAE} by adding *P. dentate* powder to 3,000 mL of liquid under unique MAE conditions and used an extraction time ranging from 30 to 90 min. However, in our research, we extracted sulfated polysaccharides by using 50 mL and an extraction time ranging from 0 to 60 sec, to save energy, time, and space. Consequently, the specific heat values of ethanol and water were 0.618 and 1.000 cal/g, respectively, and the dielectric constant of ethanol and water were 24.3 and 80.4, respectively. According to the equation of Gering *et al.* [10] and Faraji *et al.* [9], the dielectric constant of 10%, 50%, and 90% ethanol concentrations were 74.0, 52.6, and 29.2, respectively; this shows that moderate polarity is suitable for the MAE of sulfated polysaccharides.

4. Effect of Intermittent Microwave Treatment

In the present research, the ethanol concentration and pH value of the ethanol solution for the four parameters showed that MAE provides an advantage in extraction efficiency and microwave power, and that the heating time of the microwave field is superior to that of hot water extraction, ultrasonic assisted extraction, and Soxhlet extraction. MAE is also superior to the supercritical fluid extraction technique because it is easier and cheaper to operate [1]. The application of microwave energy has been proven to be an excellent alternative method that prevents uneven heating, improves the quality of a product, and reduces energy usage by allowing a redistribution of the temperature and moisture profiles within the product during off times through thermal diffusion [20]. Thus, MAE technology exhibited the advantages of energy conservation and a shorter time of bioactive substance extraction. However, the cold water extraction of sulfated polysaccharides involved using 68.1 µg/mL in 50% ethanol at a pH value of 7, and the same solvent condition used in MAE at 200 W involved using only 78.1 µg/mL, representing an increase of only 10 µg/mL. However, regarding the extraction time, using the MAE sulfated polysaccharides led to substantial savings, exceeding those of the cold water extraction method.

5. Extraction Ability of Ethanol Solution

Faraji *et al.* [9] indicated that water-ethanol mixtures are more basic media than pure water and that the values of the autoprotolysis constants (pK_{ap}) increase with the addition of ethanol. Aqueous organic solvent mixtures such as water-ethanol mixtures have been proven to be an appropriate reaction media in various fields of chemistry because of their specific properties as well as a superior ability to dissolve more compounds than pure solvents. The availability and diversity of these reaction media substantially improves the combination of pure water and alcohol solvents in binary mixtures. The pK_{ap} value of a solvent indicates the pH range of the media. This is crucial for the standardization of pH-value measurements of both organic solvents and aqueous organic solvent mixtures.

6. The pH Value

In their study of MAE alkaloids from the fruit of *Macleaya cordata* (Willd) R. Br, Zhang *et al.* [24] determined that the extraction efficiency of an acidic aqueous solution for alkaloids increases with the concentration of the acid. Shao *et al.* [18] used the MAE technique to extract flavonoids from *Perilla frutescens* leaves rapidly. Several influential parameters of the MAE procedure (microwave power, extraction cycle, solvent-to-material ratio, irradiation time, and pH value) were investigated for optimizing extraction using a single factor and the Box-Behnken experimental design. The influence of the pH value on the yield exhibited a considerable variation both above and below the optimal values. With an increase in the pH value, the yield initially increased but subsequently decreased. The extraction yield of flavonoids increased as the pH value increased from 7 to 8, but decreased when the pH value increased above 8, demonstrating that the extraction pH value substantially affects the extraction yield. The increased extraction yield under low pH-value conditions might be due to the inhibition of the enzymatic oxidation of phenolics and/or the fact that it maintains the stability of the extracted flavonoids. In addition, it is likely that alkaline soluble flavonoids were extracted. In our research, the MAE sulfated polysaccharides were affected by a low pH value.

IV. CONCLUSION

The aim of this study was to improve the effect of the ethanolic extraction treatment of *Porphyras dentate* by using MAE and to determine the optimal processing conditions. Satisfactory prediction equations were developed by using RSM to optimize the MAE of sulfated polysaccharides from *P. dentate*. Optimal conditions were established by adjusting the pH value between 8 and 9 and the ethanol concentration between 30%-50% and, subsequently, using a 1-min heating time in a 200-W microwave field.

REFERENCES

1. Afoakwah, A. N., Owusu, J., Adomako, C., and Teye, E., "Microwave

- assisted extraction (MAE) of antioxidant constituents in plant material.” *Global Journal of Bio-Science and Technology*, Vol. 1, pp. 132-140 (2012).
2. Ale, M. T., Mikkelsen, J. D., and Meyer, A. S., “Important determinants for fucoidan bioactivity: A critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds,” *Marine Drugs*, Vol. 9, pp. 2106-2130 (2011).
 3. Box, G. E. P. and Behnken, D. W., “Some new three level designs for the study of quantitative variables,” *Technometrics*, Vol. 2, pp. 455-475 (1960).
 4. Chen, Y. Z., Zhao, L., Liu, B. G., and Zuo, S., “Application of response surface methodology to optimize microwave-assisted extraction of polysaccharide from tremella,” *Physics Procedia*, Vol. 24, pp. 429-433 (2012).
 5. Costa, L. S., Fidelis, G. P., Cordeiro, S. L., Oliveira, R. M., Sabry, D. A., Câmara, R. B. G., Nobre, L. T. D. B., Costa, M. S. S. P., Almeida-Lima, J., Farias, E. H. C., Leite, E. L., and Rocha, H. A. O., “Biological activities of sulfated polysaccharides from tropical seaweeds,” *Biomedicine and Pharmacotherapy*, Vol. 64, pp. 21-28 (2010).
 6. Dandekar, D. V. and Gaikar, V., “Microwave assisted extraction of curcuminoids from *Curcuma longa*,” *Separation Science and Technology*, Vol. 37, pp. 2669-2690 (2002).
 7. de Jesus Raposo, M. F., de Morais, R. M. S. C., and de Morais, A. M. M. B., “Bioactivity and applications of sulphated polysaccharides from marine microalgae,” *Marine Drugs*, Vol. 11, pp. 233-252 (2013).
 8. Dodgson, K. S., “Determination of inorganic sulfate in studies on the enzymatic and non-enzymatic hydrolysis of carbohydrate and other sulfate esters,” *Journal of Biochemistry*, Vol. 78, pp. 312-319 (1961).
 9. Faraji, M., Farajtabar, A., and Gharib, F., “Determination of water-ethanol mixtures autoprotolysis constants and solvent effect,” *Journal of Applied Chemical Research*, Vol. 9, pp. 7-12 (2009).
 10. Gering, K. L., Lee, L. L., and Landis, L. H. A., “Molecular approach to electrolytes: Phase behavior and activity coefficients for mix-salt and multisolvent systems,” *Fluid Phase Equilibria*, Vol. 48, pp. 111-139 (1989).
 11. Jiang, Z. D., Hama, Y., Yamaguchi, K., and Oda, T., “Inhibitory effect of sulphated polysaccharide porphyran on nitric oxide production in lipopolysaccharide-stimulated RAW264.7 macrophages,” *Journal of Biochemistry*, Vol. 151, pp. 65-74 (2012).
 12. Jiao, G. L., Yu, G. L., Zhang, J. Z., and Ewart, H. S., “Chemical structures and bioactivities of sulfated polysaccharides from marine algae,” *Marine Drugs*, Vol. 9, pp. 196-223 (2011).
 13. King, V. A.-E. and Lin, Y. P., “Investigation of continuous and intermittent heating on far-infrared assisted freeze-drying,” *Transactions of the ASABE*, Vol. 52, pp. 1979-1988 (2009).
 14. Krishnaswamy, K., Orsat, V., Gariépy, Y., and Thangavel, K., “Optimization of microwave-assisted extraction of phenolic antioxidants from grape seeds (*Vitis vinifera*),” *Food Bioprocess Technology*, Vol. 6, pp. 441-455 (2013).
 15. Lee, J. H. and Yoon, J. M., “Genetic differences and variations in two *Porphyra* species (Bangiales, Rhodophyta),” *Journal of Aquaculture*, Vol. 19, pp. 67-76 (2006).
 16. Lin, Y. P., Huang, S. L., and Wu, S. C., “Effects of microwave-assisted extraction on the free radical scavenging and ferrous chelating abilities of *Porphyra dentate* extract,” *Journal of the Fisheries Society of Taiwan*, Vol. 39, pp. 35-46 (2011).
 17. Montgomery, D. C., *Design and Analysis of Experiments*, 2nd Ed., John Wiley and Sons, New York, USA (1984).
 18. Shao, P., He, J. Z., Sun, P. L., and Zhao, P. C., “Analysis of conditions for microwave-assisted extraction of total water-soluble flavonoids from *Perilla frutescens* leaves,” *Journal of Food Science and Technology*, Vol. 49, pp. 66-73 (2012).
 19. Silva, T. H., Alves, A., Popa, E. G., Reys, L. L., Gomes, M. E., Sousa, R. A., Silva, S. S., Mano, J. F., and Reis, R. L., “Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches,” *Biomatter*, Vol. 2, pp. 1-12 (2012).
 20. Soysal, Y., Ayhan, Z., Eştürk, O., and Arıkan, M. F., “Intermittent microwave-convective drying of red pepper: Drying kinetics, physical (colour and texture) and sensory quality,” *Biosystems Engineering*, Vol. 103, pp. 455-463 (2009).
 21. Sun, Y., Liao, X., Wang, Z., Hu, X., and Chen, F., “Optimization of microwave-assisted extraction of anthocyanins in red raspberries and identification of anthocyanin of extracts using high-performance liquid chromatography-mass spectrometry,” *European Food Research and Technology*, Vol. 225, pp. 511-523 (2007).
 22. Wu, S. C. and Pan, C. L., “Preparation of algal-oligosaccharide mixtures by bacterial agarases and their antioxidative properties,” *Fisheries Science*, Vol. 70, pp. 1164-1173 (2004).
 23. Xie, J. H., Xie, M. Y., Shen, M. Y., Nie, S. P., Li, C., and Wang, Y.-X., “Optimisation of microwave-assisted extraction of polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja using response surface methodology,” *Journal of the Science of Food and Agriculture*, Vol. 90, pp. 1353-1360 (2010).
 24. Zhang, F., Chen, B., Xiao, S., and Yao, S. Z., “Optimization and comparison of different extraction techniques for sanguinarine and chelerythrine in fruits of *Macleaya cordata* (Willd) R. Br.,” *Separation and Purification Technology*, Vol. 42, pp. 283-290 (2005).
 25. Zhao, B. T., Zhang, J., Guo, X., and Wang, J. L., “Microwave-assisted extraction, chemical characterization of polysaccharides from *Lilium davidii* var. *unicolor* Salisb and its antioxidant activities evaluation,” *Food Hydrocolloids*, Vol. 31, pp. 346-356 (2013).
 26. Zhao, L., Wang, K. Y., Wang, D. X., Liu, C., and Zhang, W., “Response surface methodology (RSM) for optimization of the microwave-assisted extraction of polysaccharides from *Catathelasma ventricosum*,” *Agricultural Journal*, Vol. 7, pp. 255-259 (2012).

