



EFFECTS OF MICROWAVE-ASSISTED EXTRACTION ON THE FREE RADICAL SCAVENGING AND FERROUS CHELATING ABILITIES OF MONOSTROMA NITIDUM EXTRACT

Yeu-Pyng Lin

Department of Food and Beverage Management, Tung-Fang Design University, Kaohsiung, Taiwan, R.O.C

Shao-Chi Wu

Department of Food and Beverage Management, Tung-Fang Design University, Kaohsiung, Taiwan, R.O.C.

Shih-Li Huang

Department of Baking Technology and Management, National Kaohsiung University of Hospitality and Tourism, Kaohsiung, Taiwan, R.O.C., hls@mail.nkust.edu.tw

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



Part of the [Life Sciences Commons](#)

Recommended Citation

Lin, Yeu-Pyng; Wu, Shao-Chi; and Huang, Shih-Li (2021) "EFFECTS OF MICROWAVE-ASSISTED EXTRACTION ON THE FREE RADICAL SCAVENGING AND FERROUS CHELATING ABILITIES OF MONOSTROMA NITIDUM EXTRACT," *Journal of Marine Science and Technology*. Vol. 21: Iss. 5, Article 15.

DOI: 10.6119/JMST-012-0912-1

Available at: <https://jmstt.ntou.edu.tw/journal/vol21/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.

EFFECTS OF MICROWAVE-ASSISTED EXTRACTION ON THE FREE RADICAL SCAVENGING AND FERROUS CHELATING ABILITIES OF *MONOSTROMA NITIDUM* EXTRACT

Yeu-Pyng Lin¹, Shao-Chi Wu¹, and Shih-Li Huang²

Key words: *Monostroma nitidum*, pulsed microwave-assisted extraction, DPPH radical scavenging effect, ferrous ion chelating capacity, response surface methodology.

ABSTRACT

The optimum treatments of *Monostroma (M.) nitidum* extract were studied using response surface methodology, and pulsed microwave-assisted extraction (MAE) method was applied in this study. The study was performed using a three-level, three-factor design and aimed at determining the optimum combinations of particle size of *M. nitidum* particle (X_1), concentration of *M. nitidum* (X_2), and ethanol percentage (X_3). The response variables of the α -diphenyl- α -picrylhydrazyl (DPPH) radical scavenging effect and ferrous ion chelating capacity were significantly affected by particle size (X_1) and the ethanol percentage (X_3) at a significance level of 5%. The coefficients of determination (R_2) of the response surface models of the DPPH radical scavenging effect and ferrous ion chelating capacity were found to be above 0.89. In this study the optimum treatments were established using the highest concentration of *M. nitidum* powder particles 1.5% with an ethanol percentage range of 10~30% and 0.38~0.25 mm particle size.

I. INTRODUCTION

Traditionally, seaweeds have been used in the treatment of various infectious diseases, and reports of many active compounds have been isolated. Among the features of marine

algae and their substances, several extracts have been shown to exhibit antioxidant capability [1]. Recently, there is a growing interest in the discovery of natural antioxidants, mainly for 2 reasons, one is the epidemical and clinical evidence suggesting that consumption of vegetables and fruits reduces the risk of developing chronic disease; the other is that phytochemicals are generally safer than synthetic chemicals [20].

The green alga, *M. nitidum*, are distributed around the shores of Hong Kong, Taiwan, the China sea, and around the Ryukyu islands of Japan. Many researchers have reported the anti-coagulant of *M. nitidum* and their sulfated polysaccharides play an important role on the anti-coagulant of *M. nitidum* [14-16, 30]. Further, reports on the antioxidative abilities of *M. nitidum* are very limited. Hence, this study will be focused on the antioxidative abilities of ethanol extract solutions of *M. nitidum* by MAE. The antioxidant activity of *M. nitidum* is concerned with polyphenols, Wu *et al.* [33] studied hot water extracts of *M. nitidum*, and results showed antioxidative properties in five *in vitro* antioxidative tests by reducing power, their chelating effect upon ferrous ions, scavenging of DPPH, inhibition effect upon the hemoglobin-catalyzed peroxidation of linoleic acid, and their scavenging capacity upon hydrogen peroxide. Saitoh *et al.* [21] observed the presence of pheophorbide a in the extract of a green seaweed, and *M. nitidum*. Pheophorbide a is a major colour constituent in green algal pigments [11]. Cho *et al.* [6] illustrate the strong antioxidant activity of the extract from *E. prolifera* is concerned with chlorophyll compound, pheophorbide, rather than phenolic compounds such as phlorotannins, which supported strong the DPPH radical scavenging activity.

Microwave-assisted extraction (MAE) appears to be particularly attractive due to the fast heating of aqueous samples [13]. The principal of the method lies in the fact that microwave energy is absorbed by the extract, which in turn transfers the energy to the sample in the form of heat. The partitioning of the analyses from the sample matrix to the extract depends mainly on the temperature and the nature of the extract [24].

Paper submitted 03/03/12; revised 07/22/12; accepted 09/12/12. Author for correspondence: Shih-Li Huang (e-mail: hls@mail.nkust.edu.tw).

¹ Department of Food and Beverage Management, Tung-Fang Design University, Kaohsiung, Taiwan, R.O.C.

² Department of Baking Technology and Management, National Kaohsiung University of Hospitality and Tourism, Kaohsiung, Taiwan, R.O.C.

Heating of microwave radiation offers several advantages over more conventional methods of heating, MAE can reduce both extraction time and solvent consumption as compared to conventional methods. Many cases have already proved that MAE is a viable alternative to conventional techniques for many kinds of samples [25].

The response surface methodology (RSM) is a statistical procedure frequently used in optimization studies. It uses quantitative data based on an appropriate experimental design to determine the optimum conditions while simultaneously solving multivariate problems. Several authors have used the RSM in optimization studies for free radical scavenging activity of shrimp protein hydrolysate, ultrasound-assisted extraction of the antioxidants of phenolic compounds from grape seeds and supercritical CO₂ extraction of γ -linolenic acid [5, 8, 22].

The objective of this work was to study the effects of particle size, concentration of the *M. nitidum* extract, and the ethanol percentage on the responses of the DPPH radical scavenging effect and the ferrous ion chelating capacity of *M. nitidum* ethanol-water extract, a green seaweed found in the waters off the coast of Taiwan. Based on the surface response methodology, second order polynomial models were obtained to predict the effects of MAE treatments over a wide. This study provides a new approach for the manufacturing process of seaweeds applications.

II. MATERIALS AND METHODS

1. *M. nitidum* Powder Particles

The *M. nitidum* was purchased from a traditional market in Penghu, Taiwan. The dried *M. nitidum* alga was crushed, and then screened by using a standard screening sieve (Tokyo Garasu KiKai Co., Ltd., Tokyo, Japan). The *M. nitidum* powder particles were screened for three sizes: (1) 40 mesh (0.42 mm), (2) 60 mesh (0.25 mm), (3) 80 mesh (0.08 mm) [31].

2. Pulsed Microwave-Assisted Extraction (MAE)

Pulsed microwave-assisted solvent extraction was performed using a modified method according to Han *et al.* [9]. A National 800 W microwave oven (NE-R30A, Taiwan, R.O.C.) was equipped with solvent extraction. According to the three-level-three-parameter experimental design (Table 1), the suspensions used were 0.5%, 1.0%, or 1.5% of *M. nitidum* powder particles of 0.42, 0.25, or 0.08 mm, respectively. These particles were then mixed into 100 mL of water-ethanol solutions, including 90:10, 50:50, and 10:90, (v/v). The solutions were radiated in a microwave oven at regular intervals (10 sec radiation and 30 sec off) to prevent the temperature from rising to above 80°C. The pulsed microwave-assisted extraction method was conducted with a cycle time of 40 sec, and total 15 periods were applied to an extract treatment. The infusions were allowed to cool down to room temperature, filtered and then stored in a refrigerator at 4°C to determine their antioxidant activities.

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

Independent variables	Symbols		Levels		
	coded	uncoded	-1	0	+1
Particle size (mm)	X_1	ε_1	0.42	0.25	0.08
<i>M. nitidum</i> extract concentration (%)	X_2	ε_2	0.5	1.0	1.5
Ethanol percentage (%)	X_3	ε_3	10	50	90

3. α -diphenyl- α -picrylhydrazyl (DPPH) Assay

The DPPH free radical scavenging capacities of the *M. nitidum* extraction solution extracted by MAE were measured using a method reported previously [32]. Samples were mixed with 0.1 mM DPPH (Sigma Chemical Co., MO, USA) ethanol solution and 50 mM Tris-HCl buffer (pH 7.4) solution. Methanol (Panreac, Barcelona, Spain) was used as a control. After 30 min, the reduction of the DPPH free radicals was measured at 517 nm absorbance. The concentration of L-ascorbic acid (Sigma) at 1 mg/mL was used as a positive control. The inhibition ratio was calculated from Eq. (1) as follows.

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample}) / \text{absorbance of control}] \times 100. \quad (1)$$

4. Chelating Effect on Ferrous Ions

The chelating effect on ferrous ions was determined according to a previous method [32]. The *M. nitidum* extraction solution extracted by MAE was mixed with methanol and 400 μ M FeCl₂ (Merck, Hessen, Germany), followed by the addition of 2 mM ferrozine (Sigma). After 10 min, the absorbance of the mixture was determined to be 562 nm. Trolox was used as a positive control. The chelating effect (%) was calculated by Eq. (1).

5. Experimental Design

The response variables of the DPPH radical scavenging effect and ferrous ion chelating capacity were investigated. A three-level-three-parameter experimental design as reported by King and Lin [12] was used to evaluate the optimum treatment conditions. The experimental error was estimated by performing the experimental procedure measuring the center point three times.

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

$$\eta_k = f_k(\varepsilon_1, \varepsilon_2, \varepsilon_3) \quad (2)$$

where ε_1 is the mesh pore size, ε_2 is the concentration of *M. nitidum* extract, and ε_3 is the ethanol percentage (%). A second order polynomial is used to express the function f_k as follows:

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

Treatment number ^a	X_1	X_2	X_3	Ferrous ion chelating capacity (%)	Free radical scavenging effect (%)
1	1	1	0	20.6	6.4
2	1	-1	0	23.6	13.2
3	-1	1	0	29.1	18.7
4	-1	-1	0	20.7	8.9
5	1	0	1	19.1	4.6
6	1	0	-1	31.0	15.5
7	-1	0	1	34.1	8.4
8	-1	0	-1	20.2	22.4
9	0	1	1	21.8	7.3
10	0	1	-1	40.5	22.6
11	0	-1	1	31.1	17.8
12	0	-1	-1	31.0	23.8
13	0	0	0	32.0	11.9
14	0	0	0	35.3	10.7
15	0	0	0	31.6	12.8

^a: The experimental runs were performed in random order.

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

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

$$X_i = \frac{2}{I_i} (\varepsilon_i - \bar{\varepsilon}_i) \quad (4)$$

Where ε_i is the current value of the variable, $\bar{\varepsilon}_i$ is the mean arithmetic value of the largest and the smallest value of the set, and I_i is the greatest difference between those extremes.

6. 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 the ANOVA table and estimation 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 Parameters

Experiments were performed according to a design with three variables and three levels of each variable [4]. The

Table 3. Analysis of variance for response variables.

Source	DF ^a	Ferrous ion chelating capacity (%)	Free radical scavenging effect (%)
Model	9	545.32*	511.67*
Linear	3	50.37	319.98**
Quadratic	3	207.69*	98.78
Cross product	3	287.26*	92.92
Residual	5	64.92	31.76
Lack-of-fit	3	56.67	29.54
Pure error	2	8.25	2.22
Independent variables			
Particle size (X_1)	4	404.90*	139.49*
<i>M. nitidum</i> concentration (X_2)	4	143.19	124.46
Ethanol percentage (%) (X_3)	4	289.71*	336.06**
R^2		0.8936	0.9416

^a: DF: Degree of freedom.

*: Significant at the 5% level; **: Significant at the 1% level.

independent variables were mesh pore size (X_1), concentration of the *M. nitidum* extract (X_2) and the ethanol percentage (X_3). The experimental design of the coded and actual levels is shown in Table 1. The experimental design and the response surface analysis data in this study are shown in Table 2. The experiments were performed in random order to study the relationships of the dependent variables, including the responses, such as the DPPH radical scavenging effect and the ferrous ion chelating capacity, to the independent variables X_1 , X_2 and X_3 , which include particle size, *M. nitidum* concentration, and ethanol percentage. An analysis of variance was performed to determine the lack-of-fit and the 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 to represent data in the experimental domain at points not included in the regression [18]. The analysis of the lack-of-fit was performed on all of the dependent variables, and the results obtained were insignificant. In the case of the models, high coefficients of the determination values ($R^2 > 0.89$) were obtained for the significant response surface models, i.e. ferrous ion chelating capacity and free radical scavenging effect. This indicated that a high proportion of variability was explained by the data. Thus the models developed were proven to be adequate.

An analysis of variance was conducted to assess the significant effects of each independent variable on the responses and to analyze which responses were significantly affected by the various treatment combinations. As shown in Table 3, particle size (X_1) and ethanol percentage (X_3) significantly affected the ferrous ion chelating capacity and the free radical scavenging effect at the 5% level.

Table 4 shows the regression coefficients for the second-order polynomial models of the ferrous ion chelating capacity

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

Coefficient ^a β_k	Ferrous ion chelating capacity (%)	Free radical scavenging effect (%)
β_{k0}	32.97***	11.80***
β_{k1}	-2.13	-2.34*
β_{k2}	0.70	-1.09
β_{k3}	-2.08	-5.78**
β_{k11}	-7.23**	-2.58
β_{k21}	-2.85	-4.15*
β_{k22}	-2.23	2.58
β_{k31}	-6.45*	0.78
β_{k32}	-4.70*	-2.33
β_{k33}	0.37	3.50*

^a: These are coefficients of Eq. (3), and the numbers 1 to 3 in the subscripts refer to Particle size, *M. nitidum* concentration, and ethanol percentage (%), respectively.

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

and the free radical scavenging effect used to predict the values at optimal conditions. Contour plots were generated using significant parameters for each response. The responses of the DPPH radical scavenging effect and ferrous ion chelating capacity were affected significantly by independent variables (particle size and ethanol percentage). These responses were also used to determine the optimal treatment conditions. The graphical superimposition method was used for optimization.

2. α -diphenyl- α -picrylhydrazyl (DPPH) Free Radical Scavenging Activity

Equalization DPPH has been used extensively as a free radical to evaluate reducing substances [7], Proton radical-scavenging has been reported to be an important mechanism for antioxidation. The decrease in absorbance of DPPH radicals is caused by antioxidants through the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radicals by hydrogen donation. This is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the activity of antioxidants [2]. The reduction in the concentration of DPPH allows researchers to monitor the decrease in absorbance at a characteristic wavelength when proton-radical scavengers are encountered [34]. The DPPH radical scavenging effects of *M. nitidum* using various particle sizes and *M. nitidum* concentrations with different percentage ethanol-water solutions that were extracted by pulsed MAE are shown in Fig. 1, the highest DPPH radical scavenging effect of *M. nitidum* was observed using the lowest mesh pore size and lowest ethanol percentage. The hot water extract of *M. nitidum* from our previous paper [31] processed 6.4% of the scavenging effect on the DPPH radicals, and the *M. nitidum* extract using MAE treatment processed over 18% in this study.

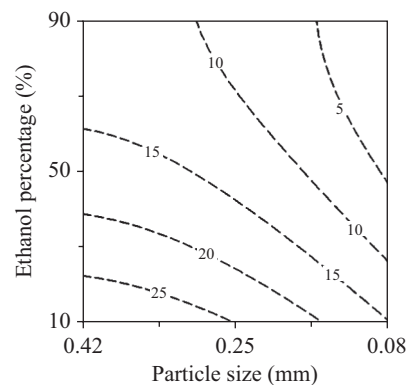


Fig. 1. Contour plots of the DPPH free radical scavenging effect of ethanol *M. nitidum* extract at a *M. nitidum* extract concentration of 1.5%.

These results indicate that the MAE treatment improves the extraction ability of the DPPH radical scavenging effect. Pan *et al.* [19] compared longan (*Dimocarpus longan* Lour.) peel extracted with 95% ethanol employing either the microwave-assisted extraction or the Soxhlet extraction method. Both the microwave-assisted extract of longan peel (MAEL) and the Soxhlet extract of longan peel (SEL) showed excellent antioxidative activity in the DPPH radical scavenging assay compared to the synthetic antioxidant 2,6-di-ter-butyl-4-methylphenol (BHT), and the antioxidative activities of MAEL were all superior to those of SEL. Pan *et al.* [19] also found that the antioxidative activity of MAEL was superior to that of SEL, suggesting that the microwave-assisted method is superior compared to the Soxhlet extraction method. Shao *et al.* [23] studied the *Perilla frutescens* leaves extract and found that the extract by the MAE method had a higher scavenging effect on DPPH radicals than the extract by the Soxhlet method. The lower activity of the Soxhlet extract could be the result the extended extraction time, which results in exposure to the unfavorable conditions of light and oxygen. When the concentration increased from 0.10 to 0.80 mg/mL, the scavenging effects of the flavonoids extract by the MAE method increased significantly. When the scavenging rate achieved 62.3%, it remained in a steady state, even when the concentration increased. Hayat *et al.* [10] studied the effects of the MAE method on the extraction of citrus mandarin pomace. The results showed that the free fraction of phenolic acids increased, whereas the bound fractions was reduced and the DPPH radical scavenging activity was increased. Wang *et al.* [29] applied the MAE technique for the extraction of polysaccharides of *Potentilla anserine*, and the results showed stronger antioxidant activities compared to using hot water extraction by DPPH radical scavenging assay, and the molecular weights played a more important role in antioxidant activities.

3. Ferrous Ion Chelating Capacity

Iron, the most abundant transition-metal ion in the human

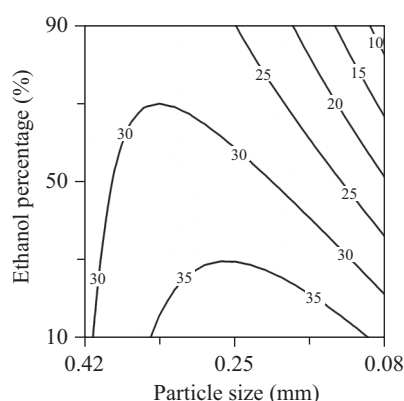


Fig. 2. Contour plots of ferrous ion chelating capacity of ethanol *M. nitidum* extract at a *M. nitidum* extract concentration of 1.5%.

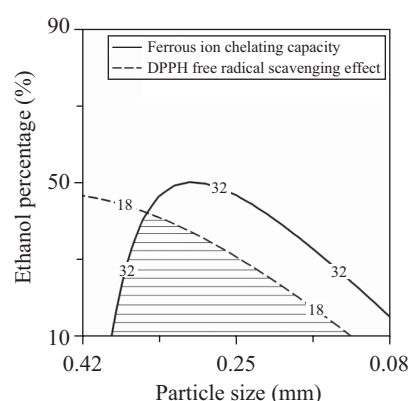


Fig. 3. Superimposed contour plots of antioxidative effect on ethanol *M. nitidum* extract at a *M. nitidum* extract concentration of 1.5%.

body, may work as a catalyst for the generation of reactive oxygen species under pathological conditions [26]. In the presence of low concentrations of transition-metal ions, hydroxyl radicals are formed from hydrogen peroxide via the Fenton reaction [3]. As shown in Fig. 2, a saddle point was observed at the lowest mesh pore size and highest ethanol percentage, and the ferrous ion chelating capacity exceeded 30% with decreasing ethanol percentage below 50%. In our previously paper [31], ferrous ion chelating capacity of the hot water extract from *M. nitidum* was 7.4%, and was lower than the *M. nitidum* extract in this study. Wang *et al.* [28] studied the extraction of polysaccharides of *Artemisia sphaerocephala* by MAE technique. Their results showed that stronger antioxidant activities were obtained by MAE treatment than by hot water extraction, and that the molecular weights played a more important role in the antioxidative activities. Michel *et al.* [17] extracted by the MAE method and compared them those from other common extraction techniques such as pressing, maceration and pressurized liquid extraction. The MAE method resulted in the extract with the most active and richest phenolic content and included molecules such as quercetin and isorhamnetin that were not extracted with other techniques. Zhu *et al.* [35] found that microwave-assisted extraction with 70% ethanol had the highest extraction efficiency for flavonoids from *Portulaca oleracea* L. compared to ultrasonic extraction, Soxhlet extraction, condensing reflux extraction, and marinated extraction. Compared with the Soxhlet extraction, the extraction time of MAE was shortened by 97%, and compared with the reflux extraction, the extraction time was shortened by 94%. Wang *et al.* [27] showed that for the extraction of aloe-emodin in aloe samples using the MAE technique, the extraction time and solvent consumption are much less than when using either the Soxhlet or the ultrasonic extraction. When it came to extraction time, MAE was the fastest extraction method, requiring only 3 min, whereas the Soxhlet extraction method required 360 min and the ultrasonic extraction method required 30 min. Thus, the MAE

method demonstrated to be an efficient method allowing higher yields of aloe-emodin from aloe in a shorter time.

4. Optimization Studies and Verification of the Models

Response surface methodology is an approach widely used in optimization studies. Optimum conditions can be determined by superimposing contour plots of relevant and statistically significant responses. An optimum area is generated, forming the basis for optimum treatments. Fig. 3 shows the regions of the optimum treatment conditions in this study for ferrous ion chelating capacity and the free radical scavenging effect.

The optimum operating conditions were determined using DPPH radical scavenging effects higher than 18%, and an ferrous ion chelating capacity of at least 32%, respectively. The optimum treatment was established with a concentration of *M. nitidum* powder particles of 1.5% with the operation controlling the ethanol percentage within the range of 10~30% and particle sizes between 0.25~0.38 mm, which were derived from the superimposed contour plots of the free radical scavenging effect and the ferrous ion chelating capacity. Verification tests were performed at the optimum conditions (point 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. The differences among the predicted values were found to be insignificant by a *t*-test at a 95% level of confidence, indicating that the second order polynomial models generated were adequate. Optimum treatment conditions were recommended and validation results were adequate and acceptable, demonstrating that the second order polynomial models generated for the significant responses were valid.

IV. CONCLUSIONS

To The aim of this study was to improve the effect of the ethanol extraction treatment of *M. nitidum* with microwave-assisted extraction and to find the optimum processing

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

Point	Coordinate(X_1, X_2, X_3)	Ferrous ion chelating capacity (%)		Free radical scavenging effect (%)	
		Predicted value	Observed value	Predicted value	Observed value
1	(0, 1, -0.8)	37.13	37.12 ^{ns} ± 0.47	22.02	22.62 ^{ns} ± 0.82
2	(0.25, 1, -0.8)	36.91	37.41 ^{ns} ± 0.85	20.08	19.48 ^{ns} ± 0.74
3	(0, 1, -1)	38.62	38.42 ^{ns} ± 0.60	24.90	25.71 ^{ns} ± 0.42

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

The results are of three replicates.

conditions. Satisfactory prediction equations were derived using the response surface methodology for the optimization of the free radical scavenging effect and the ferrous ion chelating capacity. The optimum conditions were established with the concentration of *M. nitidum* powder particles set at 1.5%. Optimum processing was found with an ethanol percentage range of 10~30% and a particle size of 0.25~0.38 mm.

REFERENCES

- Al-Amoudi, O. A., Mutqwie, H. H., Patel, A. V., and Blun, G., "Chemical composition and antioxidant activities of Jeddah corniche algae, Saudi Arabia," *Saudi Journal of Biological Sciences*, Vol. 16, pp. 23-29 (2009).
- Alkan, M., Yüksek, H., Gürsoy-Kol, Ö., and Calapoğlu, M., "Synthesis, acidity and antioxidant properties of some novel 3,4-disubstituted-4,5-dihydro-1h-1,2,4-triazol-5-one derivatives," *Molecules*, Vol. 13, pp. 107-121 (2008).
- Al-Omar, M. A., Beedham, C., and Alsarra, I. A., "Pathological roles of reactive oxygen species and their defence mechanisms," *Saudi Pharmaceutical Journal*, Vol. 12, pp. 1-18 (2004).
- 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).
- Cao, W. H., Zhang, C. H., Hong, P. Z., and Ji, H. W., "Optimising the free radical scavenging activity of shrimp protein hydrolysate produced with alcalase using response surface methodology," *International Journal of Food Science & Technology*, Vol. 44, pp. 1602-1608 (2009).
- Cho, M. L., Lee, H. S., Kang, I. J., Wond, M. H., and You, S. G., "Antioxidant properties of extract and fractions from *Enteromorpha prolifera*, a type of green seaweed," *Food Chemistry*, Vol. 127, pp. 999-1006 (2011).
- Cotelle, N., Bemier, J. L., Cateau, J. P., Pommery, J., Wallet, J. C., and Gaydou, E. M., "Antioxidant properties of hydroxyl flavones," *Free Radical Biology and Medicine*, Vol. 20, pp. 35-43 (1996).
- Ghaffoor, K., Choi, Y. H., Yeong, J. J., and Jo, I. H., "Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from grape (*Vitis vinifera*) seeds," *Journal of Agricultural and Food Chemistry*, Vol. 57, pp. 4988-4994 (2009).
- Han, C., Cao, X., Yu, J. J., Wang, X. R., and Shen, Y., "Arsenic speciation in *Sargassum fusiforme* by microwave-assisted extraction and LC-ICP-MS," *Chromatographia*, Vol. 69, pp. 587-591 (2009).
- Hayat, K., Zhang, X. M., Farooq, U., Abbas, S., Xia, C. S., Jia, H. H., Zhong, F., and Zhang, J., "Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace," *Food Chemistry*, Vol. 123, pp. 423-429 (2010).
- Jeffrey, S. W., "A report of green algal pigments in the central North Pacific Ocean," *Marine Biology*, Vol. 37, pp. 33-37 (1976).
- King, V. A.-E. and Lin, Y. P., "Investigation of continuous and intermittent heating on far-infrared assisted freeze-drying," *Transactions of ASABE*, Vol. 52, pp. 1979-1988 (2009).
- Lu, Y., Yue, X. F., Zhang, Z. Q., Li, X. X., and Wang, K., "Analysis of *Rodgersia aesculifolia* Batal. rhizomes by microwave-assisted solvent extraction and GC-MS," *Chromatographia*, Vol. 66, pp. 443-446 (2007).
- Maeda, M., Uehara, T., Harada, N., Sekiguchi, M., and Hiraoka, A., "Heparinoidactive sulfated polysaccharides from *Monostroma nitidum* and their distribution in the Chlorophyta," *Phytochemistry*, Vol. 30, pp. 3611-3614 (1991).
- Mao, W. J., Fang, F., Li, H. Y., Qi, X. H., Sun, H. H., Chen, Y., and Guo, S. D., "Heparinoid-active two sulfated polysaccharides isolated from marine green algae *Monostroma nitidum*," *Carbohydrate Polymers*, Vol. 74, pp. 834-839 (2008).
- Mao, W. J., Li, H. Y., Li, Y., Zhang, H., Qi, X. H., and Sun, H. H., "Chemical characteristics and anticoagulant activity of the sulfated polysaccharide isolated from *Monostroma latissimum* (Chlorophyta)," *International Journal of Biological Macromolecules*, Vol. 44, pp. 70-74 (2009).
- Michel, T., Destandau, E., and Elfakir, C., "Evaluation of a simple and promising method for extraction of antioxidants from sea buckthorn (*Hippophaë rhamnoides* L.) berries: Pressurised solvent-free microwave assisted extraction," *Food Chemistry*, Vol. 126, pp. 1380-1386 (2011).
- Montgomery, D. C., *Design and Analysis of Experiments*, 2nd Ed., John Wiley and Sons, New York, USA (1984).
- Pan, Y. M., Wang, K., Huang, S. Q., Wang, H. S., Mu, X. M., He, C. H., Ji, X. W., Zhang, J., and Huang, F. J., "Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel," *Food Chemistry*, Vol. 106, pp. 1264-1270 (2008).
- Patra, J. K., Rath, S. K., and Jena, K., "Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract: A Study on inhibition of glutathione-s-transferase activity," *Turkish Journal of Biology*, Vol. 32, pp. 119-125 (2008).
- Saitoh, K., Awaka, I., and Suzuki, N., "Determination of chlorophylls by reversed-phase high-performance liquid chromatography with isocratic elution and the column-switching technique," *Journal of Chromatography A*, Vol. 693, pp. 176-180 (1995).
- Sajilata, M. G., Singhal, R. S., and Kamat, M. Y., "Supercritical CO₂ extraction of γ -linolenic acid (GLA) from *Spirulina platensis* ARM 740 using response surface methodology," *Journal of Food Engineering*, Vol. 84, pp. 321-326 (2008).
- Shao, P., He, J. H., 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 (2011).
- Shen, Y., Han, C., Liu, J. D., Liu, A., Ji, X. W., and Liu, C. P., "Analysis of volatile components of *Pseudostellaria heterophylla* (Miq.) Pax by microwave-assisted solvent extraction and GC-MS," *Chromatographia*, Vol. 68, pp. 679-682 (2008).
- Shen, Y., Liu, A. L., Ye, M. D., Wang, L., Chen, J. H., Wang, X. R., and Han C., "Analysis of biologically active constituents in *Centella asiatica* by microwave-assisted extraction combined with LC-MS," *Chromatographia*, Vol. 70, pp. 431-438 (2009).
- Toyokuni, S., "Iron and carcinogenesis: From Fenton reaction to target genes," *Redox Report*, Vol. 7, pp. 189-197 (2002).
- Wang, G. H., Su, P., Zhang, F., Hou, X. Y., Yang, Y., and Guo, Z. K., "Comparison of microwave-assisted extraction of aloe-emodin in aloe with Soxhlet extraction and ultrasound-assisted extraction," *Science China Chemistry*, Vol. 54, pp. 231-236 (2011).
- Wang, J. L., Zhang, J., Wang, X. F., Zhao, B. T., Wu, Y. Q., and Yao, J., "A comparison study on microwave-assisted extraction of *Artemisia sphaero-*

- cephala* polysaccharides with conventional method: Molecule structure and antioxidant activities evaluation," *International Journal of Biological Macromolecules*, Vol. 45, pp. 483-492 (2009).
29. Wang, J. L., Zhang, J., Zhao, B. T., Wang, X. F., Wu, Y. Q., and Yao, J., "A comparison study on microwave-assisted extraction of *Potentilla anserina* L. polysaccharides with conventional method: Molecule weight and antioxidant activities evaluation," *Carbohydrate Polymers*, Vol. 80, pp. 84-93 (2010).
 30. Wijesekara, I., Pangestuti, R., and Kim, S. K., "Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae," *Carbohydrate Polymers*, Vol. 84, pp. 14-21 (2011).
 31. 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).
 32. Wu, S. C., Wang, F. J., and Pan, C. L., "The comparison of antioxidative properties of seaweed oligosaccharides fermented by two lactic acid Bacteria," *Journal of Marine Science and Technology*, Vol. 18, pp. 537-545 (2010).
 33. Wu, S. C., Wen, T. N., and Pan, C. L., "The antioxidative properties of algal-oligosaccharide-lysates hydrolyzed by two bacterial agarases," *Fisheries Science*, Vol. 71, pp. 1149-1159 (2005).
 34. Yamaguchi, T., Takamura, H., Matoba, T., and Terao, J., "HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl," *Bioscience, Biotechnology, and Biochemistry*, Vol. 62, pp. 1201-1204 (1998).
 35. Zhu, H. B., Wang, Y. Z., Liu, Y. X., Xia, Y. L., and Tang, T., "Analysis of flavonoids in *Portulaca oleracea* L. by UV-Vis spectrophotometry with comparative study on different extraction technologies," *Food Analytical Methods*, Vol. 3, pp. 90-97 (2010).