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Extraction Yields and Functional Properties of Garlic Extracts by Response Surface Methodology

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Abstract Extraction characteristics of garlic and functional properties of corresponding extract were monitored by response surface methodology (RSM). Maximum extraction yield of 26.41% was obtained at microwave power of 146.29 W, ethanol concentration of 63.31%, and extraction time of 5.88 min. At microwave power, ethanol concentration, and extraction time of 114.84 W, 58.83%, and 1.42 min, respectively, maximum electron-donating ability (EDA) was 72.86%. Maximum nitrite-scavenging ability was 94.62% at microwave power, ethanol concentration, and extraction time of 81.83 W, 2.65%, and 3.83 min, respectively. Superoxide dismutase (SOD) showed maximum pseudo-activity of 49.12% at microwave power of 34.23 W, ethanol concentration of 33.11%, and extraction time of 4.40 min. Based on superimposition of 4-dimensional RSM with respect to extraction yield, electron-donating ability, nitrite-scavenging ability, and pseudo-activity of SOD, optimum ranges of extraction conditions were microwave power of 0-100 W, ethanol concentration of 40-70%, and extraction time of 2-8 min.

Keywords: garlic, yield, functionality, monitoring, response surface methodology (RSM)

Introduction

As geriatric diseases such as high blood pressure, arteriosclerosis, and heart disease increase with the increase in national income and changes in eating habits, importance of functional foods is keenly recognized within the framework of prevention and control of these diseases. Consumers' preference for natural substances, with their safety and beneficial effects on the human body as well as various physiological efficacies being demonstrated, is on the growing trend. With the changes in the modern life style, the first consideration of the consumers has been given to convenience, and a number of drink-type medical supplies or natural foods have been developed by extracting soluble from natural substances to utilize the effective components of these natural products. From the nutritional point of view, cruciferous vegetables such as broccoli, kale, and cabbage are rich in vitamins and minerals, as well as sulfur compounds such as isothiocyanates, nitriles, and gortin, which give off characteristic smell (1). These compounds are known to prevent some cancers and repress mutation in human body. In addition, extracts from cruciferous vegetables have been found to repress oxidative damage of DNA and exhibit antioxidant power against lipid peroxidation within microsome (1-5). Garlic extract exhibits antioxidant action by increasing the levels of cellular antioxidant enzyme, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, and scavenging reactive oxygen species (ROS) (6), and thus might have some benefit in diseases in which ROS play a part. The overall objective of this study was to establish the optimum extraction condition of functional materials from garlic. Response surface analysis was used to monitor functionality and extraction characteristic of the effective components of garlic under various experimental conditions such as microwave power, ethanol concentration, and extraction time.

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Materials and Methods

Preparation of ground garlic Fresh garlic, grown harvested in Korea, were purchased from Garak market in Seoul, Korea. After cleaning, the garlic was cut into 0.5 cm pieces and dried at 40°C in a hot-air drier. The dried garlic pieces were then ground to less than 0.5 mm pieces using a grinder (KFN-400S; Kaiser Co., Yangsan, Korea) and stored in a sealed 0.2-mm polyethylene (PE) film bag at −20°C.

Experimental design for response surface methodology To optimize the extraction condition, response surface methodology (RSM) (7) was applied to monitor the extraction characteristics as affected by various extraction conditions. Experimental design for extraction conditions was made by control composite design (8), and statistical analysis system (SAS) (9) was used for RSM. Independent parameters in the extraction, namely, the microwave power (30-150 W, X_1), ethanol concentration (0-100%, X_2), and extraction time (1-9 min, X_3), were assigned numbers (−2, −1, 0, 1, 2), and 16 intervals were set on the basis of the central composite design for the extraction experiment (Table 1). The dependent parameters (Y_n) such as yield, electron-donating ability, nitrite-scavenging ability, and pseudo-activity of SOD as affected by the independent parameters were determined 3 times, and their average values were used for the regression analysis.

Determination of extraction yield The garlic extracts were concentrated in a rotary vacuum evaporator (Rotavapor R-123; Buchi, Flawil, Switzerland) and dried at 105°C in

Table 1. Yield, electron-donating ability, nitrite-scavenging ability, and superoxide dismutase (SOD)-like activity of garlic by central composite design for response surface analysis

Exp. No.[1)]	Independent variables			Response variables			
	Microwave power (W)	Ethanol concentration (%)	Extraction time (min)	Yield (%)	Electron-donating ability (%)	Nitrite-scavenging ability (%)	SOD-like activity (%)
1	60(-1)	25(-1)	3(-1)	14.95	25.65	88.34	40.25
2	60(-1)	25(-1)	7(1)	13.25	33.60	85.72	36.82
3	60(-1)	75(1)	3(-1)	14.50	42.44	60.30	32.12
4	60(-1)	75(1)	7(1)	15.75	51.87	63.28	28.09
5	120(1)	25(-1)	3(-1)	17.05	37.63	88.52	29.49
6	120(1)	25(-1)	7(1)	15.85	13.20	83.91	34.48
7	120(1)	75(1)	3(-1)	20.35	55.81	66.71	33.21
8	120(1)	75(1)	7(1)	22.45	22.99	64.88	30.71
9	90(0)	50(0)	5(0)	21.05	53.91	70.67	40.64
10	90(0)	50(0)	5(0)	22.85	57.20	74.34	42.45
11	30(-2)	50(0)	5(0)	20.95	46.29	73.45	49.99
12	150(2)	50(0)	5(0)	25.00	34.57	72.01	38.34
13	90(0)	0(-2)	5(0)	4.33	12.12	89.43	35.83
14	90(0)	100(2)	5(0)	14.60	40.11	56.29	25.49
15	90(0)	50(0)	1(-2)	19.35	69.27	77.12	33.99
16	90(0)	50(0)	9(2)	21.70	46.24	74.52	30.12

[1)]The number of experimental condition by central composite design.

an oven (Forced convection oven; Jeico Tech, Gimpo, Korea) until constant mass was reached. The yields were expressed in terms of solid content in the dried product per solid content in the dried garlic powder used on dry basis (%).

Determination of electron-donating ability Electron-donating ability (EDA) of the garlic extracts was determined in terms of reducing power of DPPH in each extract according to a modified method of Kang *et al.* (10). One mL of each extract was mixed with 1 mL of 4×10^{-4} M DPPH dissolved in 99.9% ethanol to make total volume of 2 mL. After shaking the mixtures on a vortex mixer for 10 sec and holding at room temperature for 30 min, absorbances were measured at 525 nm using a ultra violet (UV)/VIS spectrophotometer (SSE-343; Jasco, Hachioji, Japan). EDA was expressed in percent using the following equation:

$$\text{EDA (\%)}=\left(1-\frac{A}{B}\right)\times100$$

where A and B are absorbances at 525 nm with/without test sample, respectively. All data represent means of 3 values measured separately.

Determination of nitrite-scavenging activity A procedure described by Lim *et al.* (11) was used to measure nitrite-scavenging activity (NSA). One mL of 1 mM $NaNO_2$ solution was added to 1 mL of each garlic extract, and pH values of the resulting mixtures were adjusted to 1.2 using 8 mL of buffer solutions: 0.1 N HCl for pH 1.2. Final volume of each sample was made to 10 mL. The samples were allowed to react at 37°C for 1 hr and, 1 mL each of the samples were taken from the solutions, mixed thoroughly with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent, and kept at 20°C for 15 min. Just prior to usage, Griess reagent was prepared by mixing equal amounts of 1% sulfanilic acid and 1% naphthylamine, which were made with 30% acetic acid. Residual nitrite content was determined by measuring the absorbance at 520 nm. The NSA was also expressed in percent using the following equation:

$$\text{NSA (\%)}=\{1-(A-C)/B\}\times100$$

where A is absorbance of the mixture of sample and 1 mM $NaNO_2$ after 1 hr reaction, and B is an absorbance of mixture of distilled water and 1 mM $NaNO_2$ after 1 hr reaction; and C is absorbance of garlic extracts.

Antioxidant activity of superoxide dismutase Pseudo-activity of SOD was measured by a modified method of Kim *et al.* (12). After vacuum concentration of each extract, pH of each sample was adjusted to 8.5 using Tris-HCl buffer [50 mM tris (hydroxymethyl) amino-methane+ 10 mM EDTA, pH 8.5]. Three mL of the Tris-HCl buffer and 0.2 mL of 7.2 mM pyrogallol were added to 0.2 mL of each sample. The mixtures were held at 25°C for 10 min before stopping the reaction by adding 1 mL of 1 N HCl, and the absorbances were determined at 420 nm using a UV/VIS spectrometer. Pseudo-activity of SOD was expressed in percent using the following equation:

$$\text{Pseudo-activity of SOD (\%)}=\left(1-\frac{A}{B}\right)\times100$$

where A is the absorbance difference between treated sample and control, and B is the absorbance difference between untreated sample and control.

Table 2. Polynomial equations calculated by RSM program for extraction conditions of garlic

Response variables	Second order polynomials[1)]	R^2	Significance
Yield	$Y_Y = 10.005-0.077917X_1-0.38475X_2+0.017188X_3+0.000285X_1^2+0.001308X_1X_2-0.004994X_2^2+0.002812X_1X_3+0.015625X_2X_3-0.089063X_3^2$	0.9271	0.0086
Electron-donating ability	$Y_{EDA} = -81.430938+1.494042X_1+1.66785X_2+10.796563X_3-0.004201X_1^2-0.001182X_1X_2-0.011776X_2^2-0.155479X_1X_3-0.017275X_2X_3+0.1375X_3^2$	0.8999	0.0205
Nitrite-scavenging ability	$Y_{PHI} = 106.309062-0.013458X_1-0.65755X_2-2.196875X_3+0.0000625X_1^2+0.001607X_1X_2+0.000142X_2^2-0.014167X_1X_3+0.02095X_2X_3+0.207187X_3^2$	0.9614	0.0014
SOD-like activity	$Y_{SOD} = 40.377812-0.2345X_1+0.1904X_2+4.992188X_3+0.000728X_1^2+0.001135X_1X_2-0.004354X_2^2-0.000104X_1X_3+0.004775X_2X_3-0.593125X_3^2$	0.8551	0.0548

[1)]X_1, microwave power (W); X_2, ethanol concentration (%); X_3, extraction time (min).

Table 3. Predicted levels of extraction condition for the maximum responses of variables by the ridge analysis

Responses variables	Independent variables[1)]			Maximum	Morphology
	X_1	X_2	X_3		
Yield (%)	146.29	63.31	5.88	26.41	Saddle point
Electron-donating ability (%)	114.84	58.83	1.42	72.86	Saddle point
Nitrite-scavenging ability (%)	81.83	2.65	3.83	94.62	Saddle point
SOD-like activity (%)	34.23	33.11	4.40	49.12	Saddle point

[1)]X_1, microwave power (W); X_2, ethanol concentration (%); X_3, extraction time (min).

Prediction of optimum extraction condition The optimum ranges of extraction conditions were predicted by superimposing the response surfaces regarding extraction yield, EDA, NSA, and pseudo-activity of SOD. Random points selected within the optimum ranges were applied to repression equation to determine optimum extraction values.

Results and Discussion

Changes in yields Table 1 shows extraction yields under 16 extraction conditions set by the central composite design. The regression equations for response surface are listed in Table 2. R^2 for the regression equation was 0.9271 with significance of less than 10% being recognized. The predicted peak point led to the highest yield of 26.41% with corresponding independent parameters being microwave power of 146.29 W, ethanol concentration of 63.31%, and extraction time of 5.88 min (Table 3). Four-dimensional response surface obtained for yields as influenced by each extraction condition is shown in Fig. 1, indicating the yield to increase with the ethanol concentration. The most predominant effect was observed with the ethanol concentration, while the effects of the microwave power and extraction time were less significant. Similar result has been reported by Park *et al.* (13), who found out that the soluble solid content of ethanol extracts was more influenced by ethanol concentration than the microwave power and extraction time.

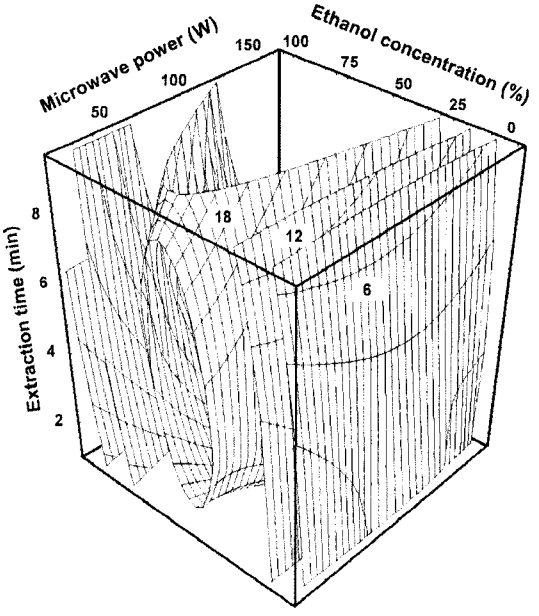

Fig. 1. Response surface plot for extraction yield in garlic extract at constant values (yield: 6-12-18%) as a function of microwave power, ethanol concentration, and extraction time.

Changes in electron-donating ability EDA of natural products provides electron to free radical and suppresses lipid oxidation in foods as well as delays aging process in human body (10). Removal of free radicals plays an important role in preventing diseases and aging of our body. DPPH method has been used to measure hydrogen-donating ability, which acts as an antioxidant by reducing physiologically active compounds having antioxidation activity such as tocopherol, ascorbate, flavonoid compounds, aromatic amines, Maillard-type browning materials, and some peptides (14). EDAs of the garlic under various extraction conditions are listed in Table 1, and Fig. 2 shows 4-dimensional response surface for EDA. The regression equation of changes in EDA calculated by RSM program for various extraction conditions (microwave power, ethanol concentration, and extraction time) is shown in Table 2 with R^2 being 0.8999 with less than 10% significance level

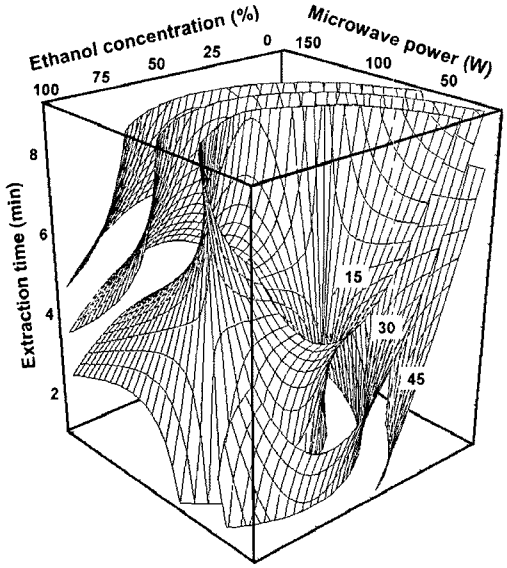

Fig. 2. Response surface plot for electron-donating ability (EDA) in garlic extract at constant values (EDA: 15-30-45%) as a function of microwave power, ethanol concentration, and extraction time.

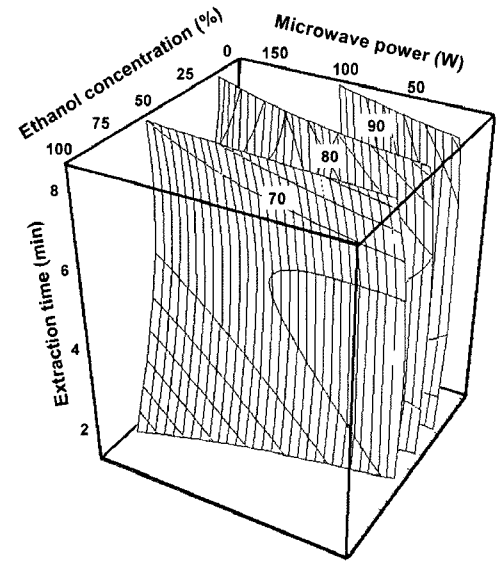

Fig. 3. Response surface plot for nitrite-scavenging ability (NSA) in garlic extract at constant values (NSA: 70-80-90%) as a function of microwave power, ethanol concentration, and extraction time.

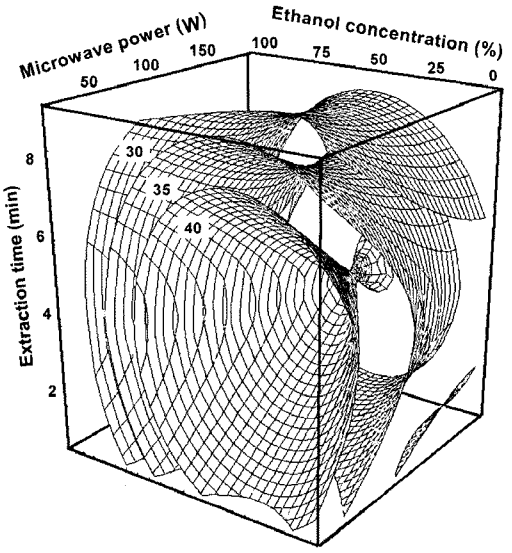

Fig. 4. Response surface plot for SOD-like activity in garlic extract at constant values (SOD-like activity: 30-35-40%) as a function of microwave power, ethanol concentration, and extraction time.

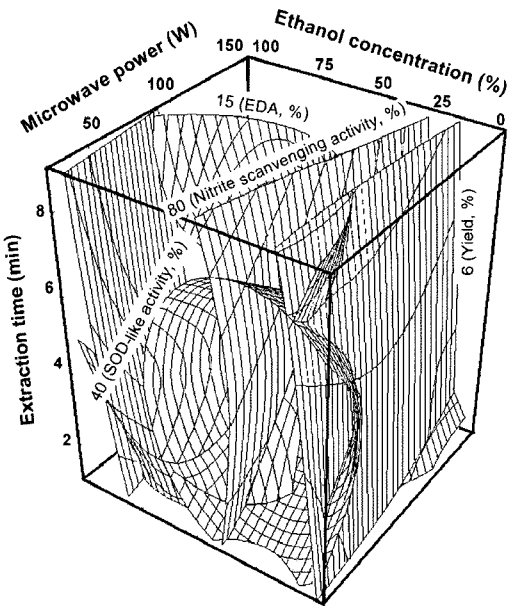

Fig. 5. Superimposed response surface plot for optimization of yield (6%), electron-donating ability (15%), nitrite-scavenging ability (80%), and SOD-like activity (40%) of extract from garlic.

recognized. EDA of garlic extracts was at the maximum level of 72.86% with the microwave power, ethanol concentration, and extraction time being 114.84 W, 58.83%, and 1.42 min, respectively (Table 3). In general, EDA decreased as the extraction conditions were out of the optimum level (Fig. 2). As in the case of yield, the EDA of garlic extracts was strongly influenced by the extraction conditions (Table 4). Similar result was reported by Yoon *et al.* (15) with optimum ethanol concentration ranging from 30 to 40%.

Change in nitrite-scavenging ability The use of nitrites results in the occurrence of very low levels of nitrosamines, which has been known to be carcinogenesis in laboratory animals at higher levels (16). NSA of the garlic under various extraction conditions are listed in Table 1. The regression equation for NSA showed R^2 as 0.9614 and its significance was confirmed within a significance level of 10% (Table 2). The estimated maximum NSA was 94.62% under the conditions of microwave power 81.83 W, ethanol concentration 2.65%, and extraction time 3.83 min (Table 3). The 4-dimensional response surface for NSA is presented in Fig. 3. NSA was highly affected by ethanol concentration while the microwave power and extraction time had negligible effect (Table 4).

Table 4. Regression analysis for regression model of physiochemical properties in extraction condition of garlic[1)]

Extraction condition	F-ratio			
	Yield	Electron-donating ability	Nitrite-scavenging ability	SOD-like activity
Microwave power	2.64	4.13*	0.43	2.17
Ethanol concentration	12.57***	6.80**	36.70***	4.48*
Extraction time	0.47	4.51**	0.784	2.16

[1)]Significant *at 10% level ; **at 5% level ; ***at 1% level.

Table 5. Optimum extraction condition for response variables yielding the optimum response by superimposing of the 4-dimensional response surface

Extraction condition	Range of predicted condition
Microwave power (W)	0-100
Ethanol concentration (%)	40-70
Extraction time (min)	2-8

Table 6. Predicted values of response variables at a given condition[1)] within the range of optimum extraction conditions

Response variables	Predicted value
Yields (%)	22.99
Electron-donating ability (%)	49.72
Nitrite-scavenging ability (%)	70.73
SOD-like ability (%)	41.31

[1)]Microwave power 50 W, ethanol concentration 55%, and extraction time 5 min.

Antioxidant activity of superoxide dismutase SOD is associated with removal of superoxide in the living body (17). Numerous natural materials having SOD-like activity have been under investigation, because the active oxygen formed in the body is supposed to cause oxidative hindrance. Less than 1% of significance level was noticed in SOD-like activity of garlic extract with R^2 being 0.8551. Maximum SOD-like activity predicted was 49.12% when the microwave power, ethanol concentration, and extraction time were 34.23 W, 33.11%, and 4.40 min, respectively (Table 3). Response surface regarding SOD-like activity is presented in Fig. 4. SOD-like activity was highly affected by ethanol concentration and extraction time, while the microwave power had negligible effect (Table 4).

Prediction of optimum extraction conditions The optimum ranges for extraction conditions of garlic was predicted by superimposing the 4-dimensional response surfaces with respect to extraction yield, EDA, NSA, and SOD-like activity obtained under various conditions, and the optimum extraction ranges to maximize the quality characteristics of garlic were established at the microwave power of 0-100 W, ethanol concentration of 40-70%, and extraction time of 2-8 min (Fig. 5, Table 5). At the random conditions (50 W, 55%, and 5 min) within the optimum extraction ranges, extraction yield, elctron-donating ability (EDA), nitrite-scavenging ability (NSA), and superoxide dismutase (SOD)-like activity were predicted as 22.99, 49.72, 70.73, and 41.31%, respectively (Table 6).

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