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Monitoring the Functional Properties of *Pleurotus eryngii* Extracts Using Response Surface Methodology

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Abstract Response surface methodology was employed to optimize extraction conditions for finding the maximal functional properties of *Pleurotus eryngii*. Based on central composite design, the study plan was established with variations of microwave power (30-150 W), ethanol concentration (0-99.9%), and extraction time (1-9 min). Regression analysis was applied to obtain a mathematical model. A maximal yield of 47.86% was obtained when the microwave power, ethanol concentration, and extraction time were set at 122.7 W, 42.14%, and 8.3 min, respectively. A maximized electron donating ability of 93.32% was found under the following conditions: a microwave power of 144.19 W, an ethanol concentration of 49.52%, and an extraction time of 6.7 min. When the microwave power, ethanol concentration, and extraction time were set at 125.43 W, 40.54%, and 8.1 min, respectively, the maximum nitrite-scavenging ability was 80.47%. The optimum ranges of the extraction conditions, superimposed by the response surface methodology, could predicate a microwave power of 110-150 W, ethanol concentration of 0-45%, and extraction time of 7-9 min.

Keywords: *Pleurotus eryngii,* yield, total polyphenol, electron donating ability, nitrite-scavenging ability.

Introduction

Economic growth has been accompanied by dietary changes, which have resulted in increased adult diseases such as hypertension, arteriosclerosis, stroke, and diabetes. Therefore, many people have begun to show interest in functional foods that may prevent these adult diseases. One of the various factors that can cause such diseases is oxidative damage caused by free radicals (1-3). Our body possesses an antioxidant defense system against these free radicals, however, because this defensive system is insufficient to prevent oxidative damage, antioxidants contained in foods can help reduce oxidative damage (4-6). Recently, antioxidant research has focused on natural substances used in daily life, such as herbs, spices, and teas, and on the discovery of effective compounds and their safety for human use (7-10). Supplements that contain the effective components of these natural products continue to be developed.

From a nutritional point of view, mushrooms are not rich in fat and contain appreciable amounts of dietary fiber (11), which regulates various physiological functions. In addition, their abilities to lower cholesterol, modulate the immune system, and inhibit tumoral growth have resulted in recent highlights of the functional compounds of mushrooms (12-16). Among the major naturally occurring antioxidant components found in mushrooms are phenolic substances, which have radical scavenging effects (17, 18). In particular, *Pleurotus eryngii,* which belongs to the white-rot fungi group, is known to be rich in protein, amino acids, and minerals; it also possesses anti-aging and anti-tumour properties (19), lowers blood glucose (20), and inhibits the proliferation of human colon cancer cells (21). However, edible mushrooms are characterized by a short shelf life (1-3 days at room temperature) that is linked to the occurrence of post-harvest changes (22). These changes are due to the high activities of enzymes such as protease and polyphenol oxidase, which are responsible for protein and sugar decreases and browning reactions during storage (23). Therefore, if the nutritional and functional compounds of mushrooms could be extracted and commercialized as drinks or teas, a more effective use of these functional compounds would be available without seasonal constraints. Phenolic substances are some of the functional compounds of *P. eryngii* that could be used for healthcare products and processed foods because they are easily soluble in water (24).

The objective of the current study was to establish the optimum extraction conditions for maximizing the effective components of *P. eryngii* under various experimental conditions, including microwave power, ethanol concentration, and extraction time. To accomplish this, a central composite design was employed for the experimental design, and response surface methodology (RSM) was used to find the optimum ranges.

Materials and Methods

Samples *P. eryngii* mushrooms, cultivated in the Suwon area of Korea, were purchased from Garak Market in Seoul on June 5, 2006. After cleaning, the *P. eryngii* were cut into 0.5 cm pieces and dried at 40°C in a hot-air drier. The dried *P. eryngii* pieces were then ground to less than 0.5 mm using a grinder (KFN-400S; Kaiser Co., Korea) and stored in a sealed 0.2 mm PE film bag at -20°C.

Preparation of sample extracts Dried and pulverized 2.5 g portions of *P. eryngii* were extracted with 50 mL of 0 -99.9% ethyl alcohol using a microwave (Soxwave; Prolabo, Fontenay-sous-Bios, France) under the extraction

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conditions described in Table 1. The extracts were filtered with Whatman filter paper No. 2. The filtered extracts were then evaporated under reduced pressure and redissolved in 25 mL of H_2O for the biological experiments.

Experimental design for RSM RSM was applied to obtain the optimized extraction conditions that yield the highest antioxidant properties of *P. eryngii* (25). As shown in Table 1, the experimental design for the extraction conditions was set by a central composite design (26). Independent variables such as microwave power (X_1: 30-150 W), ethanol concentration (X_2: 0-99.9%), and extraction time (X_3: 1-9 min) were assigned as numbers (-2, -1, 0, 1, 2), and 16 different intervals for the extraction experiment were set based on the central composite design. The dependent variables (Y_n) selected were yield, electron donating ability, total polyphenol content, inhibitory effect on tyrosinase, and nitrite-scavenging ability, which were determined 3 times and then their average values were used for the regression analysis. Statistical analysis system (SAS) (27) was used for the regression equations to describe the response surface.

Determination of extraction yield The *P. eryngii* extracts were concentrated in a rotary vacuum evaporator (Rotavapor R-123; Buchi, Flawil, Switzerland) and dried at 105°C in an oven (Forced convection oven, Jeico Tech, Gimpo, Korea) until a constant mass was reached. The yields were expressed in terms of the solid content of the dried product per solid content of the dried *P. eryngii* powder that was used on a dry basis (%) (28).

Determination of electron donating ability The electron donating ability (EDA) of the *P. eryngii* extracts was determined in terms of the reducing power of α, α-diphenyl-picrylhydrazyl (DPPH) in each extract according to a modified method by Kang *et al.* (29). One mL of each extract was mixed with 1 mL of 4×10^{-4} M DPPH dissolved in 99.9% ethanol to make a total volume of 2 mL. After shaking the mixtures in a vortex mixer for 10 sec and maintaining them at room temperature for 30 min, the absorbances were measured at 525 nm using a UV/VIS spectrophotometer (Jasco, Hachioji, Japan). EDA was expressed as a percentage using the following equation:

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

where, A is the absorbance of a sample treated with the extract and B is the absorbance of an untreated sample. All data reported in this paper represent means of 3 values measured separately.

Inhibitory effect on tyrosinase The inhibitory effect on tyrosinase was measured using a method reported by Wong *et al.* (30). A crude tyrosinase solution was prepared by dissolving mushroom tyrosinase (110 units/mL, T7755; Sigma Co., St, Louis, MO, USA) in 50 mM sodium phosphate buffer (pH 7.0). Subsequently, 0.2 mL of the crude tyrosinase solution and 0.1 mL of each *P. eryngii*

Table 1. Yield, electron donating ability, tyrosinase inhibition, total polyphenol content, and nitrite-scavenging ability of *Pleurotus eryngii* by central composite design for response surface analysis

Exp. No.[1)]	Microwave power (W)	Ethanol concentration (%)	Extraction time (°C)	Yield (%)	Electron donating ability (%)	Tyrosinase inhibition (%)	Total polyphenol content (%)	Nitrite scavenging ability (g)
1	60 (-1)	25 (-1)	3 (-1)	40.13	60.44	26.66	27.00	62.69
2	60 (-1)	25 (-1)	7 (1)	37.07	55.14	28.92	26.36	56.90
3	60 (-1)	75 (1)	3 (-1)	31.07	50.93	27.70	20.97	65.27
4	60 (-1)	75 (1)	7 (1)	33.20	43.01	27.48	19.86	57.07
5	120 (1)	25 (-1)	3 (-1)	39.07	52.24	32.57	26.84	53.04
6	120 (1)	25 (-1)	7 (1)	41.33	70.60	32.38	30.82	70.07
7	120 (1)	75 (1)	3 (-1)	30.53	55.95	30.58	19.61	55.61
8	120 (1)	75 (1)	7 (1)	37.33	70.12	28.61	24.59	64.50
9	90 (0)	50 (0)	5 (0)	34.93	57.88	30.24	23.11	58.89
10	90 (0)	50 (0)	5 (0)	34.93	57.37	30.93	23.71	59.03
11	30 (-2)	50 (0)	5 (0)	36.93	80.23	29.37	24.76	60.59
12	150 (2)	50 (0)	5 (0)	42.53	85.03	31.20	33.02	61.32
13	90 (0)	0 (-2)	5 (0)	40.62	63.66	28.68	40.59	58.68
14	90 (0)	99.9 (2)	5 (0)	15.33	30.40	26.23	15.89	40.58
15	90 (0)	50 (0)	1 (-2)	39.73	52.02	31.68	24.01	59.08
16	90 (0)	50 (0)	9 (2)	45.87	67.75	31.30	33.55	72.61

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

extract were added to 2.8 mL of a 10 mM catechol solution. The absorbances of the resulting mixtures were measured at 420 nm using a UV/VIS spectrometer to determine tyrosinase activity. The inhibitory effects on tyrosinase were calculated by measuring the changes in the absorbances per unit time as follows:

$$\text{Inhibitory effect (\%)} = \left(1-\left(\frac{A-B}{C}\right)\right)\times 100$$

where, A is the change in absorbance of samples treated by the enzyme solution, B is the change in absorbance of samples treated by a buffer solution *in lieu* of the enzyme solution, and C is the change in absorbance of samples treated by distilled water *in lieu* of the extracts.

Determination of total polyphenol content The total polyphenol content was measured by the Folin-Denis method (31). Solutions containing 0.1 mL of garlic extract, 8.4 mL of distilled water, and 0.5 mL of 2 N Folin reagents were allowed to sit for 3 min before adding 1 mL of a 20% Na_2CO_3 solution. After holding the mixed solution for 1 hr, the absorbance was measured at 765 nm using a UV/VIS spectrometer.

Determination of nitrite-scavenging ability Nitrite-scavenging ability was measured by the method of Gray and Dugan (32), where 0.1 mM $NaNO_2$ was added to 2 mL of each extract and mixed with 7 mL of 0.1 N HCl (pH 2.0) and 0.2 N citric acid (pH 3.0, 4.2, and 6.0). This mixture yielded a 10 mL total volume. After incubating the mixed solution for 1 hr at 37°C, 1 mL of this incubated solution was mixed with 0.4% Griess reagent (prepared by mixing equal volumes of 1% sulfanylic acid and 0.1% naphthylamin in 2% acetic acid). After standing at room temperature for 15 min, the absorbance of the mixture was measured at 520 nm using a UV/VIS spectrometer. A control experiment was also carried out in a similar manner using distilled water in place of the extracts. The nitrite scavenging effect was expressed in a percent using the following formula:

$$N(\%) = \left(1-\left(\frac{A-B}{C}\right)\right)\times 100$$

where, A is the absorbance of a sample treated with 1 mM $NaNO_2$ and then incubated for 1 hr, B is the absorbance of an untreated sample, and C is the absorbance of a sample treated with distilled water.

Prediction of the optimum extraction conditions The optimum ranges of the extraction conditions were predicted by superimposing the response surfaces for the extraction yield, electron donating ability, total polyphenol content, inhibitory effect on tyrosinase, and nitrite-scavenging ability. Random points selected within the optimum ranges were applied to a repression equation to determine the optimum extraction values.

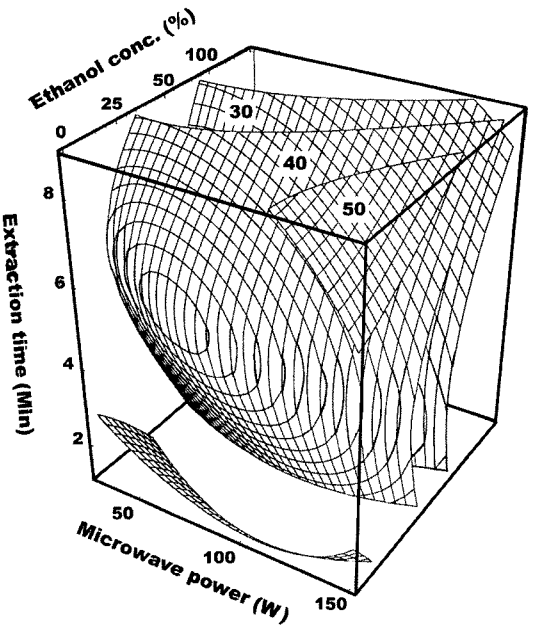

Fig. 1. Response surface for yield in *Pleurotus eryngii* extracts at constant values (yield: 30-40-50%) as a function of microwave power, ethanol concentration, and extraction time.

Results and Discussion

The various nutritional components of *P. eryngii* used in the present experiment were as follows: 5.60% moisture, 0.80% fat, 18.37% protein, 5.05% ash content, and 29.14% dietary fiber on a dry basis.

The results from the different extraction conditions set by central composite design are shown in Table 1. The regression equations to describe the response surfaces were calculated by SAS and are listed in Table 2. The R^2 value of the regression equation was 0.9397 at a 0.005 level of significance. A saddle point, which was recognized in terms of the contour line for yield, led to the highest yield of 47.86% under microwave conditions of 122.71 W, an ethanol concentration of 42.14%, and an extraction time of 8.3 min (Table 3). As shown in Fig. 1, the response surface for the yield increased with a decrease in ethanol concentration and shorter extraction time. Table 4 indicates that the yield of the *P. eryngii* extracts was most affected by changes in ethanol concentration, and to a lesser extent by changes in the extraction time.

The R^2 value of the regression equation for total polyphenol content was 0.8594 with a *p*-value of 0.0506 (Table 2). The predicted minimum of the total polyphenol content was 38.36%, which was obtained with a microwave power, ethanol concentration, and extraction time of 119.91 W, 13.81%, and 6.9 min, respectively (Table 3). Figure 2 shows that the four-dimensional response surface for total polyphenol content had a tendency to increase in accordance with increasing microwave power and decreasing ethanol concentration. This increased polyphenol content with decreasing ethanol concentration seems to be due to the ready solubility of polyphenols in water (24). As seen in Table 4, the total

Table 2. Polynomial equations calculated by the RSM program for the extraction conditions of *Pleurotus eryngii*

Response	Second order polynomial equation[1)]	R^2	Significance
Yield	Y_Y=69.897187-0.309833X_1-0.047150X_2-7.370312X_3+0.001333X_1^2+0.000065X_2X_1 -0.00272X_2^2+0.020813X_3X_1+0.024325X_3X_2+0.491875X_3^2	0.9397	0.005
Total polyphenol content	Y_{TP}=58.003438-0.327417X_1-0.37435X_2-4.609062X_3+0.001522X_1^2 -0.000155X_2X_1+0.001932X_2^2 +0.022313X_3X_1+0.001325X_3X_2+0.335625X_3^2	0.8594	0.0506
Electron donating ability	Y_{EDA}=155.362813-1.832000X_1-0.07645X_2-7.552813X_3+0.006946X_1^2+0.004145X_2X_1 -0.004238X_2^2 +0.095312X_3X_1-0.017025X_3X_2+0.141250X_3^2	0.8941	0.0239
Tyrosinase inhibitory effect	Y_T=15.603437+0.138917X_1+0.2312X_2+0.726875X_3-0.000083333X_1^2-0.000893X_2X_1 -0.001252X_2^2 -0.00875X_3X_1-0.01065X_3X_2+0.056562X_3^2	0.8709	0.0405
Nitrite scavenging activity	Y_{NSA}=92.295-0.524333X_1+0.5002X_2-9.249062X_3+0.000971X_1^2-0.000958X_2X_1 -0.003732X_2^2 +0.083146X_3X_1-0.026375X_3X_2+0.430312X_3^2	0.8663	0.0470

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

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

Response[1)]	X_1	X_2	X_3	Maximum	Morphology
Yield (%)	122.71	42.14	8.3	47.86	Saddle point
Total polyphenol content (%)	119.91	13.81	6.9	38.36	Minimum
Electron donating ability (%)	144.19	49.52	6.7	93.32	Saddle point
Tyrosinase inhibition (%)	138.35	38.66	2.8	33.44	Saddle point
Nitrite-scavenging ability (%) pH 1.2	125.43	40.54	8.1	80.47	Saddle point

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

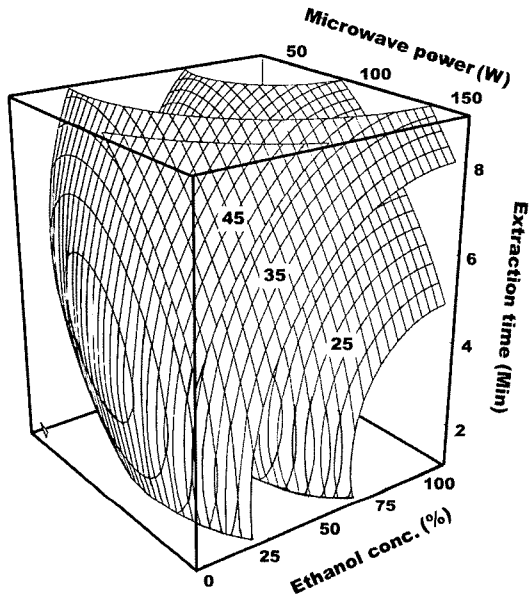

Fig. 2. Response surface for total polyphenol content in *Pleurotus eryngii* extracts at constant values (polyphenol content: 25-35-45%) as a function of microwave power, ethanol concentration, and extraction time.

polyphenol content was strongly affected by changes in ethanol concentration, while the microwave power and extraction time had no affect.

Electron donating ability (EDA) is a measure of the ability to donate electrons to free radicals. Therefore, the EDA method has been used as an index of lipid oxidation suppression in foods, as well as for delayed aging, which is activated by free radicals in the human body (29). In Table 2, the R^2 value of the regression equation for EDA is 0.8941 at a significance level of $p<0.05$. The predicted value, a maximum electron donating ability of 93.32%, was obtained at a microwave power of 144.19 W, an ethanol concentration of 49.52%, and an extraction time of 6.7 min (Table 3). Among the extraction conditions, the most effective factor on EDA was microwave power, followed by ethanol concentration. However, the extraction time had no significant affect on the EDA (Table 4). Considering that the yield, total polyphenol content, and EDA had a similar tendency to be significantly affected by the ethanol concentration, this result would seem to be due to the good water solubility of polyphenols, as mentioned above (24), and their antioxidant effects by the removal of free radicals (18).

Tyrosinase is a copper-containing enzyme that uses polyphenol substrates known as polyphenol oxidase (PPO), polyphnolase, and cresolase. In addition, tyrosinase is known to induce enzymatic browning during the storage and processing of foods (33). As shown in Table 2, the R^2 value of the regression equation for the inhibitory effect on tyrosinase was 0.8709 and there was a significant difference in the extraction conditions at $p<0.05$. From the contour line for the inhibitory effect on tyrosinase, the highest value for the predicted saddle point was 33.44% when the microwave power, ethanol concentration, and extraction time were 138.35 W, 38.66%, and 2.8 min, respectively (Table 3). The four-dimensional response surface for the inhibitory effects of the *P. eryngii* extracts on tyrosinase had the same tendency as the EDA response surface (Fig. 4). Similar to EDA, the inhibitory effects of

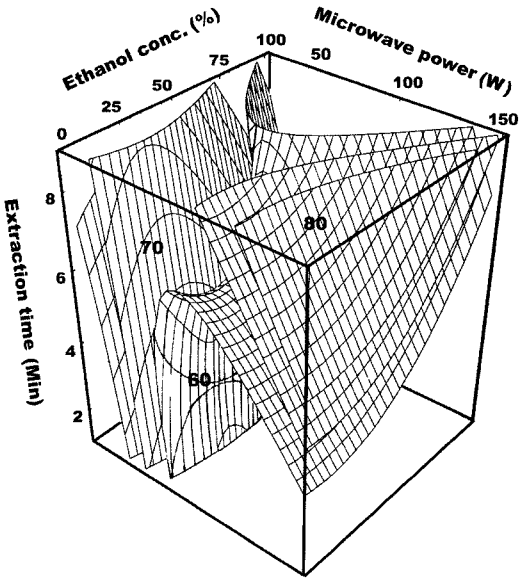

Fig. 3. Response surface for electron donating ability in *Pleurotus eryngii* extracts at constant values (electron donating ability: 60-70-80%) as a function of microwave power, ethanol concentration, and extraction time.

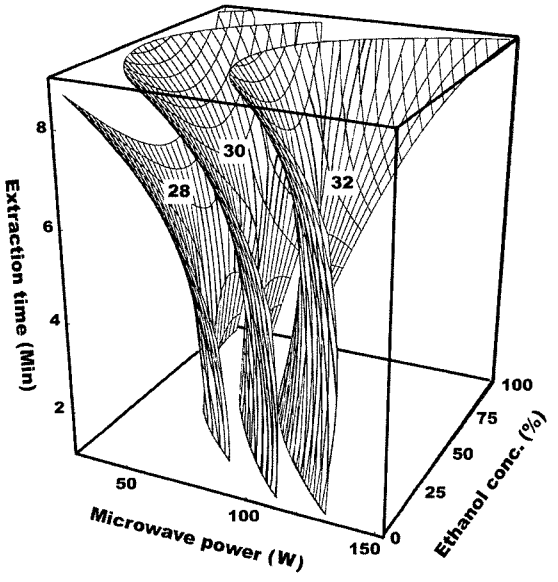

Fig. 4. Response surface for tyrosinase inhibition in *Pleurotus eryngii* extracts at constant values (tyrosinase inhibition: 28-30-32%) as a function of microwave power, ethanol concentration and, extraction time.

the *P. eryngii* extracts on tyrosinase were affected by the microwave power and ethanol concentration, but not the extraction time (Table 4); in particular, the microwave power had more influence on the tyrosinase inhibitory effects than the ethanol concentration.

The R^2 value for the scavenging ability of nitrite against *P. eryngii* extract that was adjusted to a pH 1.2 was 0.8663 at a significance level 0.05 (Table 2). In the predicted value of the saddle point, the maximum nitrite-scavenging ability was estimated as 80.47% under the following conditions: a microwave power of 125.43 W, an ethanol concentration of 40.54%, and an extraction time of 8.1 min (Table 3). Figure 5 presents the response surface with regard to nitrite-scavenging ability, which tended to increase as the ethanol concentration decreased. Similarly, Kim *et al.* (34) reported that mushrooms contain total phenols that might be related to EDA, and to antioxidant and nitrite-scavenging abilities. As shown in Table 4, nitrite-scavenging ability was highly affected by changes in the extraction time. However, neither the microwave power nor ethanol concentration significantly contributed to the nitrite-scavenging ability.

Figure 6 shows the optimum ranges for the maximum

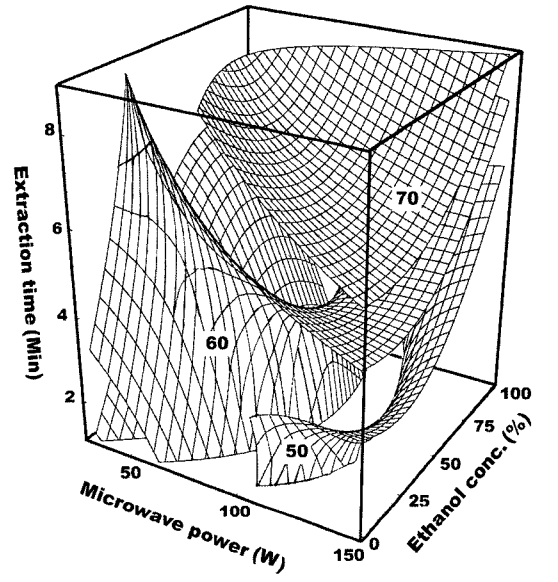

Fig. 5. Response surface for nitrite-scavenging ability (pH 1.2) in *Pleurotus eryngii* extracts at constant values (nitrite-scavenging ability: 50-60-70%; pH 1.2) as a function of microwave power, ethanol concentration, and extraction time.

Table 4. Regression analysis for the regression model of physiochemical properties in the extraction conditions of *Pleurotus eryngii*[1)]

Extraction condition	F-ratio				
	Yield	Total polyphenol content	Electron donating ability	Tyrosinase inhibition	Nitrite-scavenging ability (pH 1.2)
Microwave power (W)	1.91	1.51	5.76**	4.67**	3.01
Ethanol concentration (%)	14.55***	7.07**	3.34*	4.52*	2.57
Extraction time (min)	3.84*	1.61	2.24	1.04	4.86**

1) $^{*}p<0.1$; $^{**}p<0.5$; $^{***}p<0.01$.

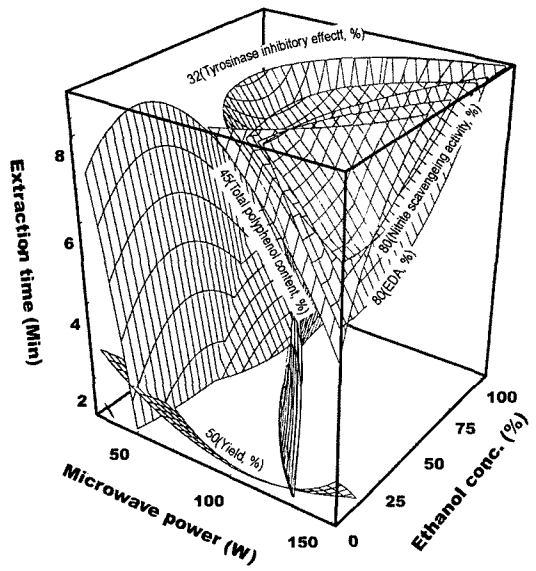

Fig. 6. Superimposed response surface for optimization of yield (50%), electron donating ability (80%), total polyphenol content (45%), tyrosinase inhibition (32%), and nitrite-scavenging ability (80%) (pH 1.2) of extracts from *Pleurotus eryngii*.

functional properties of *P. eryngii*. These optimum ranges were predicted using response surface methodology and then marked by a dark zone. The optimum extraction ranges obtained by superimposing the four-dimensional response were: a microwave power of 110-150 W, an ethanol concentration of 0-45%, and an extraction time of 7-9 min (Table 5). In addition, the yield, EDA, total polyphenol content, tyrosinase inhibition effect, and nitrite-scavenging ability were predicted at the given conditions (130 W, 22.5%, and 8 min) within the optimum extraction ranges, and then an experiment was conducted under the same conditions. The validity of the regression equations could be verified by comparing the predicted values with those obtained experimentally (Table 6).

In conclusion, the objective of this study was to establish the optimum extraction conditions for the maximum functional properties of *P. eryngii* using response surface methodology (RSM). A central composite design was also applied to investigate the effects of microwave power (30, 60, 90, 120, 150 W), ethanol concentration (0, 25, 50, 75, 99.9%), and extraction time (1, 3, 5, 7, 9 min). A maximal yield of 47.86% occurred under a microwave power of 122.7 W, an ethanol concentration of 42.14%, and an extraction time of 8.3 min. The maximal electron donating ability was 93.32% when the microwave power, ethanol concentration, and extraction time were set at 144.19 W, 49.52%, and 6.7 min, respectively. A maximal polyphenol content of 38.36% occurred with a microwave power of 119.61 W, an ethanol concentration of 13.81%, and an extraction time of 6.9 min. The highest tyrosinase inhibitory effect was 33.44% and occurred at a microwave power of 138.35 W, ethanol concentration of 38.66%, and extraction time of 2.8 min. The maximal nitrite-scavenging ability was 80.47% with a microwave power, ethanol concentration, and extraction time of 144.19 W, 49.52%, and 6.7 min, respectively. Finally, the predicted optimum conditions were a microwave power of 130 W, an ethanol concentration of 22.5%, and an extraction time of 8 min within the optimal extraction ranges (110-150 W, 0-45%, 7-9 min).

Table 5. Optimum extraction conditions for the maximum responses of yield, electron donating ability (EDA), total polyphenol content, tyrosinase inhibitory effect, and nitrite-scavenging ability of *Pleurotus eryngii* by superimposing their contour maps

Extraction condition	Range of optimum condition	Optimum condition
Microwave power (W)	110-150	130
Ethanol concentration (%)	0-45	22.5
Extraction time (min)	7-9	8

Table 6. Predicted and observed values of the response variables at a given condition within the range of optimum extraction conditions

Response variable	Predicted value[1)]	Experimental value[2)]
Yield (%)	46.53	44.07
Electron donating ability (%)	79.92	74.43
Nitrite scavenging activity (%)	78.76	73.18
Total polyphenol content (%)	39.03	35.18
Tyrosinase inhibition (%)	32.35	36.26

[1)]Given conditions of independent variables: microwave power 130 W, ethanol concentration 22.5%, and extraction time 8 min.
[2)]Mean values of triplicate determinations.

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