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Monitoring on Extraction Yields and Functional Properties of *Brassica oleracea var. capita* Extracts

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Abstract Extraction characteristics of Bonus species of *Brassica oleracea var. capita* and functional properties of corresponding extract were monitored by response surface methodology (RSM). Maximum extraction yield of 44.07% was obtained at ratio of solvent to sample of 27.94 mL/g, ethanol concentration of 24.35%, and extraction temperature of 55.21°C. At ratio of solvent to sample, ethanol concentration, and extraction temperature of 21.11 mL/g, 58.53%, and 68.83°C, respectively, maximum electron-donating ability was 48.44%. Maximum inhibitory effect on tyrosinase was 68.94% at ratio of solvent to sample, ethanol concentration, and extraction temperature of 24.08 mL/g, 10.49%, and 78.71°C, respectively. Superoxide dismutase (SOD) showed maximum pseudo-activity of 24.78% at ratio of solvent to sample of 22.66 mL/g, ethanol concentration of 45.69%, and extraction temperature of 93.81°C. Based on superimposition of four-dimensional RSM with respect to extraction yield, electron-donating ability, and pseudo-activity of SOD, optimum ranges of extraction conditions were ratio of solvent to sample of 20-30 mL/g, ethanol concentration of 35-65%, and extraction temperature of 50-80°C.

Keywords: cabbage, yield, functionality, monitoring, RSM, tyrosinase, antioxidant

Introduction

As adult 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 solubles 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). Cabbage has been used traditionally to treat stomach-related disease, headache, and hangover. Although cabbage contains relatively low amounts of vitamins and minerals, it can still meet the required daily allowances for adults due to its fairly high consumption (6). Phytochemicals of cabbage are hydroxycinnamates, including caffeic acid, chlorogenic acid, ferulic acid, and ñ-coumaric acid, having antioxidant activity (7, 8). The overall objective of this study was to establish the optimum extraction condition of functional materials from cabbage (Bonus). Response surface analysis was used to monitor functionality and extraction characteristic of the effective components of cabbage under various experimental conditions such as ratio of solvent to sample content, ethanol concentration, and extraction temperature.

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Materials and Methods

Preparation of ground cabbage A variety of Bonus of fresh cabbage (*Brassica oleracea* L.) harvested in October, 2003 was supplied by the Nongwoo Bio Co. (Yeoju, Korea). After cleaning, the cabbage was cut into 0.5 cm piecesand dried at 40°C in a hot-air drier. The dried cabbage pieces were then ground to less than 0.5 mm pieces using a grinder (Kaiser Co., KFN-400S, City, Korea) and stored in a sealed 0.2-mm PE film bag at -20 °C.

Experimental design for response surface methodology To optimize the extraction condition, response surface methodology (RSM) (9) was applied to monitor the extraction characteristics as affected by various extraction conditions. Experimental design for extraction conditions was made by control composite design (10), and statistical analysis system (SAS) (11) was used for RSM. Independent parameters in the extraction, namely, the ratio of solvent to sample (10~30 mg/L, X_1), ethanol concentration (0~100%, X_2), and extraction temperatures (35~95

°C, 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. The dependent parameters (Y_n) such as yield, electron-donating ability, inhibitory effect on tyrosinase, and pseudo-activity of superoxide dismutase as affected by the independent parameters were determined three times, and their average values were used for the regression analysis.

Determination of extraction yield The cabbage 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, Kimpo, 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 cabbage powder used on dry basis (%).

Determination of electron-donating ability Electron-donating ability (EDA) of the cabbage extracts was determined in terms of reducing power of α, α-diphenyl-picrylhydrazyl (DPPH) in each extract according to a modified method of Kang *et al.* (13). One milliliter 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 UV/VIS spectrophotometer. EDA was expressed in percent using the following equation:

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

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

Tyrosinase inhibitory acivity Inhibitory effect on tyrosinase was measured by a method reported by Wong *et al.* (14), and the crude tyrosinase solution was prepared by dissolving mushroom tyrosinase (Sigma, T7755, 110 units/mL) in 50 mM sodium phosphate buffer (pH 7.0). Subsequently, 0.2 mL crude tyrosinase solution and 0.1 mL cabbage extract were added to 2.8 mL of 10 mM catechol solution, and the absorbances of the resulting mixtures were then measured at 420 nm by a UV/VIS spectrometer (Jasco, Hachioji, Japan) for determination of tyrosinase activities. Inhibitory effects on tyrosinase were calculated by measuring changes in absorbances per unit time as follows:

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

where A is absorbance at 420 nm with test sample and enzyme; B and C are absorbance at 420 nm without enzyme and test sample, respectively.

Antioxidant activity of superoxide dismutase Pseudo-activity of superoxide dismutase (SOD) was measured by a modified method (15) of Marklund. 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 milliliters 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:

$$\text{Pseudo-activity of SOD(\%)} = \left(1 - \frac{A}{B}\right) \times 100$$

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

Prediction of optimum extraction condition The optimum ranges of extraction conditions were predicted by superimposing the response surfaces regarding extraction yield, EDA, 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 0.8177 with significance of less than 10% being recognized. The predicted peak point led to the highest yield of 44.07% with corresponding independent parameters being ratio of solvent to sample of 27.94 mL/g, ethanol concentration of 24.35%, and extraction temperature of 55.21°C (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 ratio of solvent to sample. The most predominant effect was observed with the ethanol concentration, while the effects of the ratio of solvent to sample and extraction temperature were less significant. Similar result has been reported by Park *et al.* (16), who found out that the soluble solid content of ethanol extracts were more influenced by ethanol concentration than the ratio of solvent to sample 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 (13). 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 (17). EDAs of the cabbage (Bonus) under various extraction conditions are listed in Table 1, and Fig.

Table 1. Experi-mental data on yield, electron donating ability, tyrosinase inhibition and superoxide dismutase (SOD)-like Activity of cabbage (Bonus) by central composite design for response surface analysis

Exp. No.[1)]	Independent variables			response variables			
	Ratio of solvent to sample content (mL)	Ethanol concentration (%)	Extraction Temperature (°C)	Yield (%)	Electron donating ability (%)	Tyrosinase inhibition (%)	SOD-like activity (%)
1	15 (-1)	25 (-1)	50 (-1)	31.000	20.915	51.267	0.000
2	15 (-1)	25 (-1)	80 (1)	31.250	31.152	62.416	15.009
3	15 (-1)	75 (1)	50 (-1)	30.125	37.054	40.791	17.671
4	15 (-1)	75 (1)	80 (1)	37.250	46.350	43.999	22.424
5	25 (1)	25 (-1)	50 (-1)	39.875	27.884	63.601	0.000
6	25 (1)	25 (-1)	80 (1)	37.500	34.638	66.141	15.251
7	25 (1)	75 (1)	50 (-1)	31.500	41.891	38.733	14.938
8	25 (1)	75 (1)	80 (1)	41.875	48.650	52.967	19.135
9	20 (0)	50 (0)	65 (0)	40.125	45.420	49.003	17.307
10	20 (0)	50 (0)	65 (0)	40.875	45.315	46.449	18.820
11	10 (-2)	50 (0)	65 (0)	27.500	27.739	35.676	16.787
12	30 (2)	50 (0)	65 (0)	44.125	42.481	51.889	21.373
13	20 (0)	0 (-2)	65 (0)	39.125	21.339	61.113	0.000
14	20 (0)	100 (2)	65 (0)	23.000	25.864	32.787	12.874
15	20 (0)	50 (0)	35 (-2)	36.750	28.641	45.668	0.000
16	20 (0)	50 (0)	95 (2)	39.855	32.321	56.682	27.215

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

Table 2. Polynomial equations calculated by RSM program for extraction conditions of cabbage (Bonus)

Response variables	Second order Polynomials[1)]	R^2	Significance
Yield	Y_Y=-0.749271+2.943229X_1+0.056979X_2+0.059438X_3-0.046875X_1^2-0.009125X_1X_2-0.003775X_2^2+0.001042X_1X_3+0.006542X_2X_3-0.002442X_3^2	0.8177	0.0978
Electron donating ability	Y_{EDA}=-134.737347+5.509517X_1+1.128295X_2+2.534897X_3-0.102575X_1^2-0.003318X_1X_2-0.008706X_2^2-0.010033X_1X_3-0.000312X_2X_3-0.016541X_3^2	0.8177	0.0979
Tyrosinase inhibition	Y_{TI}=37.263618+2.465446X_1-0.176262X_2-0.419810X_3-0.039435X_1^2-0.009149X_1X_2-0.000310X_2^2+0.004028X_1X_3+0.001251X_2X_3+0.003832X_3^2	0.8899	0.0265
SOD-like activity	Y_{SOD}=-76.140069-0.016983X_1+1.226197X_2+1.405194X_3+0.010165X_1^2-0.006264X_1X_2-0.004651X_2^2-0.000523X_1X_3-0.007103X_2X_3-0.005001X_3^2	0.9520	0.0026

[1)]X_1 : ratio of solvent to sample content (mL/g), X_2 : ethanol concentration (%), X_3 : extraction temperature (°C)

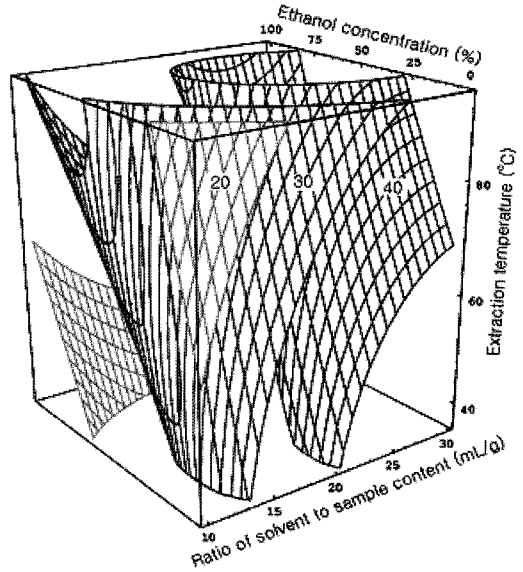

Fig. 1. Response surface plot for extraction yield in cabbage (Bonus) extract at constant values (yield: 20-30-40%) as a function of ratio of solvent to sample content, ethanol concentration and extraction temperature.

2 shows four-dimensional response surface for EDA. The regression equation of changes in EDA calculated by RSM program for various extraction conditions (ratio of solvent to sample content, ethanol concentration, and extraction time) is shown in Table 2 with R^2 being 0.8177 with less than 10% significance level recognized. EDA of cabbage extracts was at the maximum level of 48.44% with the ratio of solvent to sample, ethanol concentration, and extraction temperature being 21.11 mL/g, 58.53%, and 68.8°C, 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 cabbage extracts was strongly influenced by the extraction conditions (Table 4). Similar result was reported by Yoon *et al.* (18) with optimum ethanol concentration ranging from 30 to 40%.

Tyrosinase inhibitory acivity Inhibitory effect of cabbage extracts on tyrosinase (dihydroxy-L-phenylalanine oxygen oxidoreductase, EC 1.14.18.1), which induces enzymatic browning during storage and processing of foods (19), was relatively high (Table 1). Figure 3 shows four-dimensional response surface for tyrosinase inhibition in cabbage

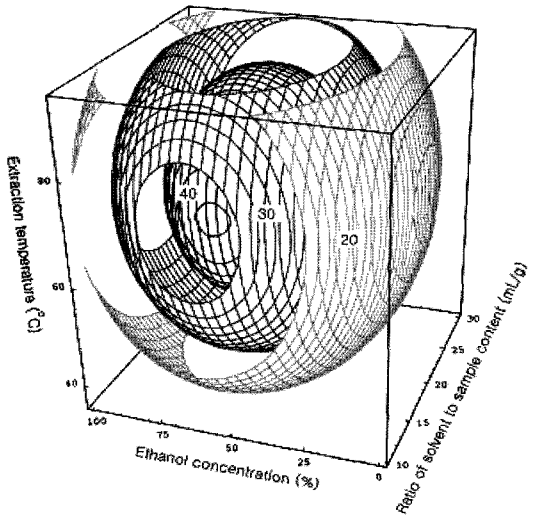

Fig. 2. Response surface plot for electron donating ability in cabbage (Bonus) extract at constant values (electron donating ability: 20-30-40%) as a function of ratio of solvent to sample content, ethanol concentration and extraction temperature.

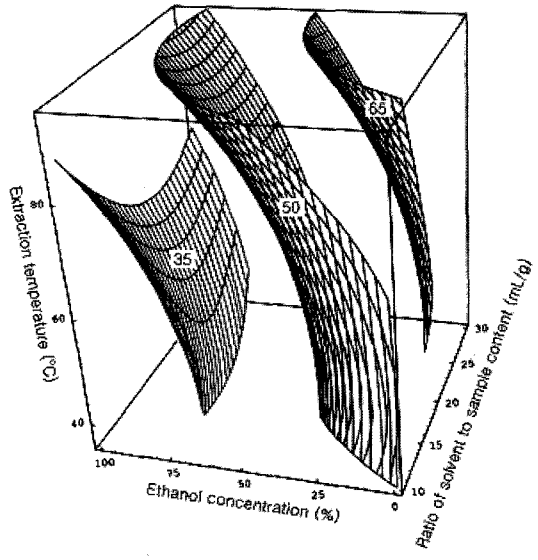

Fig. 3. Response surface plot for tyrosinase inhibition in cabbage (Bonus) extract at constant values (tyrosine inhibition: 35-50-65%) as a function of ratio of solvent to sample content, ethanol concentration and extraction temperature.

(Bonus) extract. R^2 of the regression equation for the inhibitory effect on tyrosinase was 0.8899 with significance level of less than 5%. The highest inhibitory effect of 68.94% was obtained with ratio of solvent to sample, ethanol concentration, and extraction temperature of 24.08 mL/g, 10.49%, and 78.7°C, respectively (Table 3). The inhibitory effect tended to increase as ethanol concentration decreased and the ratio of solvent to sample increased (Fig. 3). As in the case of yield and EDA, the ethanol concentration was the most influential factor among the extraction conditions in terms of inhibitory effect.

Antioxidant activity of superoxide dismutase SOD is associated with removal of superoxide in the living body (20). Numerous natural materials having SOD-like activity are 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 cabbage extract with R^2 being 0.9520. Maximum SOD-like activity predicted was 24.78% when the ratio of solvent to sample, ethanol concentration, and extraction temperature were 22.66 mL/g, 45.69%, and 93.8°C, 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 temperature, while the ratio of solvent to sample content had negligible effect (Table 4).

Prediction of optimum extraction conditions The optimum ranges for extraction conditions of cabbage (Bonus) was predicted by superimposing the four-dimensional response surfaces with respect to yield, EDA, and SOD-like activity obtained under various conditions, and the optimum extraction ranges to maximize the quality

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

Responses variables	Independent variables			Maximum	Morphology
	X_1[1)]	X_2[2)]	X_3[3)]		
Yield (%)	27.94	24.35	55.21	44.07	Saddle point
Electron donating ability (%)	21.11	58.53	68.83	48.44	Maximum
Tyrosinase inhibition (%)	24.08	10.49	78.71	68.94	Saddle point
SOD-like activity (%)	22.66	45.69	93.81	24.78	Saddle point

[1)]Ratio of solvent to sample content (mL/g)
[2)]Ethanol concentration (%)
[3)]Extraction temperature (°C)

Table 4. Regression analysis for regression model of physiochemical properties in extraction condition of cabbage (Bonus)

Extraction condition	F-Ratio			
	Yield	Electron donating ability	Tyrosinase inhibition	SOD-like activity
Ratio of solvent to sample content	3.39	1.61	1.90	0.18
Ethanol concentration	3.25*	4.97**	8.43**	13.39***
Extraction temperature	1.28	2.12	1.66	16.68***

*Significant at 10% level ; **significant at 5% level ; ***significant at 1% level

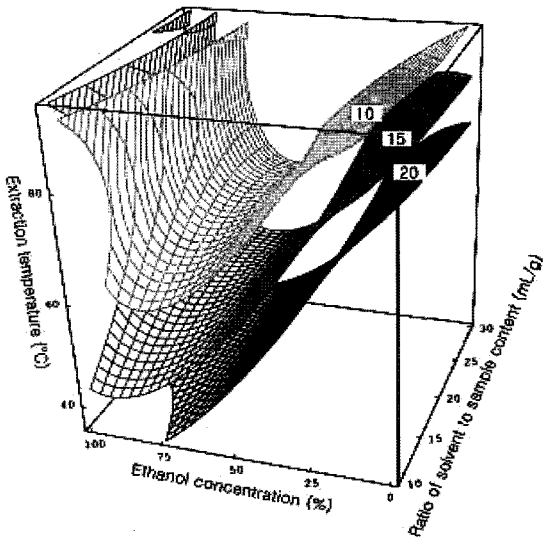

Fig. 4. Response surface plot for SOD-like activity in cabbage (Bonus) extract at constant values (SOD-like activity: 10-15-20%) as a function of ratio of solvent to sample content, ethanol concentration and extraction temperature.

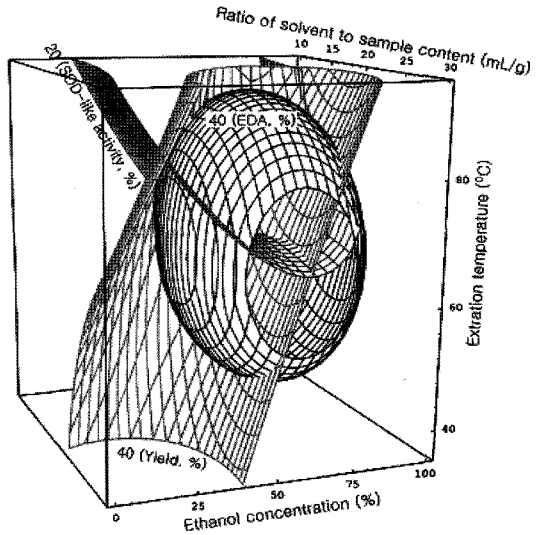

Fig. 5. Superimposed response surface plot for optimization of yield (40%), electron donating ability (40%) and SOD-like activity (20%) of extract from cabbage (Bonus).

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

Condition	Range of predicted condition
Ratio of solvent to sample content (mL/g)	20~30
Ethanol concentration (%)	35~65
Extraction temperature (°C)	50~80

characteristics of cabbage (Bonus) were established at the ratio of solvent to sample content of 20~30 mL/g, ethanol concentration of 35~65%, and extraction temperature of 50~80°C (Fig. 5, Table 5). At the random conditions (25 mL/g, 50%, and 70°C) within the optimum extraction ranges, yield, EDA, and SOD-like activity were predicted as 42.46, 47.14, and 19.72%, respectively (Table 6).

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

Response variables	Predicted value
Yields (%)	42.46
Electron donating ability (%)	47.14
Tyrosinase inhibition (%)	54.05
SOD-like ability (%)	19.72

[1]Given conditions: 25 mL/g in ratio of solvent to sample content, 50% in ethanol concentration, 70°C in extraction temperature

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