Research article

Effect of Korean Red Ginseng extraction conditions on antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content: optimization using response surface methodology

Jin Woo Lee [1], Eun Jin Mo [1], Ji Eun Choi [1], Yang Hee Jo [1], Hari Jang [1], Ji Yeon Jeong [1], Qinghao Jin [1], Hee Nam Chung [2], Bang Yeon Hwang [1], Mi Kyeong Lee [1,*]

[1] College of Pharmacy, Chungbuk National University, Cheongju, Korea
[2] Dasom Co., Ltd, Cheongju, Korea

ABSTRACT

Background: Extraction conditions greatly affect composition, as well as biological activity. Therefore, optimization is essential for maximum efficacy.
Methods: Korean Red Ginseng (KRG) was extracted under different conditions and antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content evaluated. Optimized extraction conditions were suggested using response surface methodology for maximum antioxidant activity and extraction yield.
Results: Analysis of KRG extraction conditions using response surface methodology showed a good fit of experimental data as demonstrated by regression analysis. Among extraction factors, such as extraction solvent and extraction time and temperature, ethanol concentration greatly affected antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content. The optimal conditions for maximum antioxidant activity and extraction yield were an ethanol concentration of 48.8%, an extraction time 73.3 min, and an extraction temperature of 90°C. The antioxidant activity and extraction yield under optimal conditions were 43.7% and 23.2% of dried KRG, respectively.
Conclusion: Ethanol concentration is an important extraction factor for KRG antioxidant activity and extraction yield. Optimized extraction conditions provide useful economic advantages in KRG development for functional products.

1. Introduction

Panax ginseng Meyer (Araliaceae), commonly known as Korean Ginseng, is one of the most widely used traditional medicines. *P. ginseng* roots are used as a tonic to enhance immune response and consequent health and longevity [1,2]. Diverse beneficial effects, such as anticancer, anti-diabetic, neuroprotective, and anti-inflammatory activities have also been reported [3–6].

To increase useful components and biological activities of Korean Ginseng, various preparation methods have been investigated. Drying after steaming, which produces Korean Red Ginseng (KRG), is well known for the production of new active constituents [7–10]. Fermentation or treatment in acidic conditions is also suggested for production of and/or increasing active constituents [11–14].

In order to use *P. ginseng* in traditional medicine or for development as functional foods, appropriate extraction procedures are indispensable. Extraction procedures are also important in determining extract efficacy. Many factors, such as extraction solvent, extraction time and temperature, and solid–liquid ratios, affect extract composition, as well as biological activity [15–17].

* Corresponding author. College of Pharmacy, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Korea
  E-mail address: mklee@chungbuk.ac.kr (M.K. Lee).

Therefore, optimization of extraction conditions is required for maximum efficacy. Response surface methodology (RSM) is a useful statistical tool that can derive optimal conditions by considering several factors simultaneously. RSM consists of mathematical and statistical methods and derives optimal conditions based on experimental data obtained from rationally designed experiments [18–20]. Therefore, RSM is an effective method for optimization of extraction conditions, especially in cases involving multiple variables.

Oxidative stress describes an imbalance between the production of reactive oxygen species and antioxidant defenses. It is a major contributor to age-related symptoms and pathogenesis of many diseases, such as cancer, diabetes, atherosclerosis, neurodegenerative diseases, and osteoporosis [21,22]. Consumption of antioxidant-rich fruits or botanical extracts minimizes senescence and chronic disease [23–25]. KRG reportedly exhibits beneficial effects against various diseases through enhancing antioxidant defense [26–29].

In the present study, we investigated the impact of KRG extraction conditions on antioxidant activity using RSM. Given the importance of extraction efficiency for further product development, the extraction yield was also compared. Additionally, ginsenoside Rg1 and phenolic content were also measured. Ultimately, optimized extraction conditions for maximum antioxidant activity and maximum extraction yield using RSM are suggested.

2. Materials and methods

2.1. Plant material

KRG was purchased from a local herbal market in Chungbuk, Korea, in September 2014. They were identified by the herbarium of College of Pharmacy at Chungbuk National University, where a voucher specimen was deposited (CBNU201409-KRG). Ginsenoside Rg1 was purchased from Baoji Herbest Bio-Tech Co., Ltd (Baoji, Shaanxi, China).

2.2. Preparation of KRG extract

Powdered KRG (500 mg) was weighed and extracted with 10 mL extraction solvent as indicated in Table 1. The solvent was evaporated and the extract analyzed for antioxidant activity. For HPLC

analysis, each sample solution was filtered through a 0.45 μm membrane filter.

2.3. Antioxidant activity

KRG antioxidant activity was evaluated by measuring free-radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, extracts prepared from different extraction conditions were mixed with freshly prepared DPPH solution. After shaking, the reaction mixtures were allowed to stand for 30 min at room temperature in a dark environment. The radical scavenging activity was determined by measuring the absorbance at 517 nm. The relative radical scavenging activity (%) was calculated as [1 − absorbance of solution with sample and DPPH / absorbance of solution with DPPH] × 100.

2.4. Experimental design for RSM

A Box-Behnken design (BBD) with three variables and three levels was used to optimize the extraction conditions of KRG. Target responses were selected as antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content. The independent extraction variables for extraction solvent (ethanol) (X_1), extraction time (X_2), and extraction temperature (X_3) were chosen for this study and their ranges determined based on a preliminary single-factor experiment. As shown in Table 1, the complete design consisted of 15 experimental points, including three replicates of the center points (all variables were coded as zero).

Regression analysis was performed according to the experimental data. The mathematical model is described by the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{1 \leq i \leq j}^{3} \beta_{ij} X_i X_j$$

where Y is the response, β_0 is the constant coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient. The statistical significance of the coefficients in the regression equation was checked by analysis of variance (ANOVA). The fitness of the polynomial model equation to the responses was evaluated with the coefficients of R^2 and lack of fit was evaluated using an F-test.

Table 1
A Box-Behnken design for independent variables and their responses

Run	Coded variables			Actual variables			Observed values			
	X_1	X_2	X_3	EtOH (%)	Time (min)	Temperature (°C)	Antioxidant activity (%)	Extraction yield (%)	Rg1 (mg/g extract)	Phenolics (mg GAE /g extract)
1	1	0	−1	100	60	30	7.9	1.6	23.0	2.8
2	0	0	0	50	60	60	40.9	19.7	3.3	7.4
3	0	−1	−1	50	30	30	36.2	18.2	3.7	7.3
4	0	0	0	50	60	60	40.3	20.1	3.3	8.7
5	0	1	1	50	90	90	35.6	27.9	2.2	10.2
6	1	−1	0	100	30	60	18.9	5.4	14.3	3.7
7	0	0	0	50	60	60	40.1	18.2	3.4	7.3
8	1	1	0	100	90	60	25.6	5.4	21.5	4.0
9	−1	0	1	0	60	90	20.2	27.0	1.3	7.8
10	0	−1	1	50	30	90	39.7	23.1	2.5	9.8
11	−1	0	−1	0	60	30	29.2	24.0	1.7	8.8
12	−1	1	0	0	90	60	25.0	21.3	1.8	6.6
13	−1	−1	0	0	30	60	20.2	24.5	2.0	8.3
14	0	1	−1	50	90	30	36.3	16.3	3.7	8.8
15	1	0	1	100	60	90	32.8	8.0	16.0	5.2

EtOH, ethanol.

2.5. HPLC conditions for the quantitation of ginsenoside Rg1

Analysis was performed using a Waters HPLC system (Waters Corp., Milford, MA, USA) equipped with Waters 515 pumps, a 2996 photodiode array detector, and Waters Empower software using YMC J'sphere ODS-H80 [YMC America, Inc., Allentown, PA, USA; (4 μm, 150 mm × 4.6 mm)] for quantitation. Chromatographic separation was accomplished using a gradient solvent system of acetonitrile−water (ratio range, 20:80 to 50:50) for 30 min at a flow rate of 1.0 mL/min. Molecule detection was achieved using an evaporative light-scattering detector (Waters Corp., Milford, MA, USA) (Fig. 1).

Stock standard solution of ginsenoside Rg1 was prepared in methanol at a concentration of 1.0 mg/mL. Standard working solutions were prepared with serial dilutions of 0.01, 0.02, 0.10, 0.25, and 0.50 mg/mL, and used to generate calibration curves. A good linearity of calibration curves for ginsenoside Rg1 was achieved with a correlation coefficient of 0.9998.

2.6. Measurement of total phenolic content

The total phenolic content was measured using a Folin-Ciocalteau assay. Folin-Ciocalteau's phenol reagent was added to the 96-well plate containing the test samples. After 5 min of incubation with gentle shaking, 7% Na_2CO_3 was added to the reaction mixture, and the mixture was left in the dark for 90 min at room temperature. The absorbance was measured at 630 nm using a microplate reader and total phenolic content expressed as gallic acid equivalents using gallic acid as a standard.

3. Results

3.1. Model fitting

To evaluate the multiple effects of extraction factors on antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content, a BBD with a three-level factor was employed. The ranges of these variables were determined as extraction solvent (X_1, ethanol concentration at 0%, 50%, or 100%), extraction time (X_2, 30, 60, or 90 min), and extraction temperature (X_3, 30, 60, or 90°C) based on a preliminary single-factor experiment. The variables were coded at three levels (−1, 0, and 1) and the complete design consisted of 15 experimental points, including three replicates of the center points (all variables were coded as zero), as shown in Table 1.

Table 1 shows that antioxidant activity, extraction yields and ginsenoside Rg1 and phenolic content varied depending on extraction conditions. Second-order polynomial regression equations were established by RSM to evaluate the relationship between variables and responses. The linear (X_1, X_2, and X_3), quadratic (X_1^2, X_2^2, and X_3^2), and interaction coefficients (X_1X_2, X_2X_3, and X_1X_3) were calculated and the significance of each coefficient determined using t test and p values (Table 2). Larger coefficients with a smaller p value ($p < 0.05$) indicated the considerable effect of these coefficients on the respective responses. Correlations between three independent variables and each response were also estimated by multiple determination (R^2). The value of R^2 was 0.959, 0.982, and 0.986 for antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content, respectively, which demonstrated the effectiveness of this model. The validity of the models was also confirmed using lack-of-fit testing (Table 3). an insignificant p value for lack of fit ($p > 0.05$) for three responses indicated the adaptability of this model to experimental data. Relationships between every two variables for antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content are shown in three-dimensional response surface plots based on regression equations
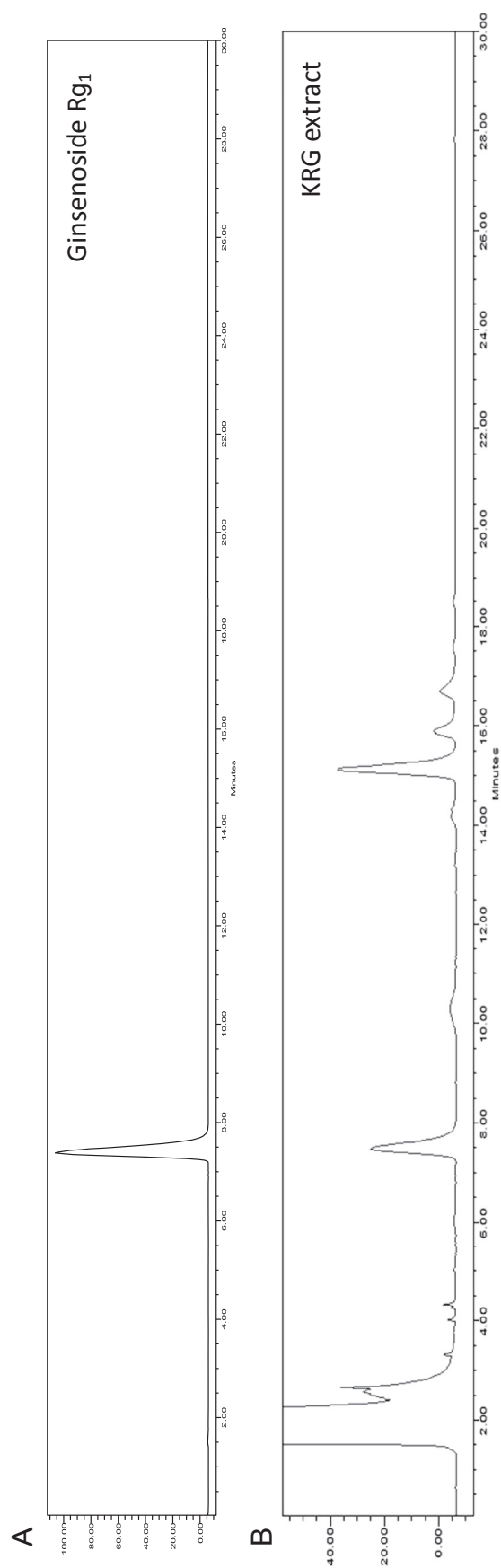

Fig. 1. (A) HPLC chromatogram of ginsenoside Rg1 and (B) HPLC chromatogram of Korean Red Ginseng extract prepared using extraction conditions from center point ($X_1 = 0$, $X_2 = 0$, and $X_3 = 0$). Chromatographic separation was accomplished using a gradient solvent system with acetonitrile-water (ratios from 20:80 to 50:50) for 30 min at a flow rate of 1.0 mL/min using an evaporative light-scattering detector. HPLC, high-performance liquid chromatography.

Table 2
Regression coefficients and their significance in the second-order polynomial regression equation

	Coefficient	Standard error	t	p
[Antioxidant activity]				
Intercept	40.133	1.912	20.995	< 0.001
X_1	−1.063	1.171	−0.908	0.406
X_2	0.988	1.171	0.844	0.437
X_3	2.225	1.171	1.901	0.116
X_1^2	−15.917	1.723	−9.238	< 0.001
X_2^2	−1.767	1.723	−1.025	0.352
X_3^2	−1.392	1.723	−0.808	0.456
X_1X_2	0.500	1.655	0.302	0.775
X_1X_3	8.275	1.655	4.999	0.004
X_2X_3	−1.025	1.655	−0.619	0.563
[Extraction yield]				
Intercept	19.333	1.098	17.610	< 0.001
X_1	−9.550	0.672	−14.205	< 0.001
X_2	−0.038	0.672	−0.056	0.958
X_3	3.238	0.672	4.816	0.005
X_1^2	−5.704	0.990	−5.764	0.002
X_2^2	0.521	0.990	0.526	0.621
X_3^2	1.521	0.990	1.537	0.185
X_1X_2	0.800	0.951	0.841	0.438
X_1X_3	0.850	0.951	0.894	0.412
X_2X_3	1.675	0.951	1.762	0.138
[Ginsenoside Rg1]				
Intercept	2.990	0.890	3.361	0.020
X_1	8.504	0.545	15.611	< 0.001
X_2	0.886	0.545	1.627	0.165
X_3	−1.210	0.545	−2.221	0.077
X_1^2	7.244	0.802	9.034	< 0.001
X_2^2	−0.336	0.802	−0.419	0.692
X_3^2	0.251	0.802	0.313	0.767
X_1X_2	1.858	0.770	2.411	0.061
X_1X_3	−1.640	0.770	−2.129	0.087
X_2X_3	−0.185	0.770	−0.240	0.820
[Phenolics]				
Intercept	7.783	0.488	15.937	< 0.001
X_1	−1.992	0.299	−6.662	0.001
X_2	0.055	0.299	0.184	0.861
X_3	0.668	0.299	0.232	0.076
X_1^2	−2.509	0.440	−5.700	0.002
X_2^2	0.366	0.440	0.831	0.444
X_3^2	0.881	0.440	2.001	0.102
X_1X_2	0.485	0.423	1.147	0.303
X_1X_3	0.865	0.423	2.045	0.096
X_2X_3	−0.275	0.423	−0.650	0.544

Table 3
ANOVA for response surface regression equation

	Sum of square	Degree of freedom	Mean square	F	p
[Antioxidant activity]					
Regression	1,329.35	9	147.705	12.37	0.006
Linear	62.35	3	20.782	1.74	0.274
Square	972.50	3	324.166	27.14	0.002
Interaction	294.50	3	98.167	8.22	0.022
Residual error	59.71	5	11.943	–	–
Lack-of-fit	59.33	3	19.777	12.88	0.100
Pure error	0.38	2	0.192	–	–
Total	1,389.06	14	–	–	–
$R^2 = 0.959$, adjusted $R^2 = 0.884$					
[Yield]					
Regression	967.07	9	107.452	29.72	0.001
Linear	813.48	3	271.161	74.99	< 0.001
Square	136.91	3	45.637	12.62	0.009
Interaction	16.67	3	5.557	1.54	0.314
Residual error	18.08	5	3.616	–	–
Lack-of-fit	16.08	3	5.358	5.34	0.162
Pure error	2.01	2	1.003	–	–
Total	985.14	14	–	–	–
$R^2 = 0.982$, adjusted $R^2 = 0.949$					
[Ginsenoside Rg1]					
Regression	818.13	9	90.903	38.29	< 0.001
Linear	596.51	3	198.835	83.76	< 0.001
Square	196.93	3	65.642	27.65	0.002
Interaction	24.70	3	8.232	3.47	0.107
Residual error	11.87	5	2.374	–	–
Lack-of-fit	11.36	3	3.788	14.96	0.063
Pure error	0.506	2	0.253	–	–
Total	830.00	14	–	–	–
$R^2 = 0.986$, adjusted $R^2 = 0.960$					
[Phenolics]					
Regression	67.98	9	7.55	10.56	0.009
Linear	35.35	3	11.78	16.47	0.005
Square	28.40	3	9.46	13.23	0.008
Interaction	4.24	3	1.41	1.97	0.236
Residual error	3.58	5	0.72	–	–
Lack-of-fit	2.42	3	0.81	1.40	0.443
Pure error	1.16	2	0.58	–	–
Total	71.56	14	–	–	–
$R^2 = 0.950$, adjusted $R^2 = 0.860$					

ANOVA, analysis of variance.

(Figs. 2–4). Collectively, this model adequately fits the experimental data and is suitable for optimization.

3.2. Effect of extraction variables on antioxidant activity

Multiple regression analysis on the experiment data yielded the second-order polynomial regression equation for coded values as follows:

$$\text{Antioxidant activity (\%)} = 40.13 - 1.06X_1 + 0.99X_2 + 2.23X_3$$
$$- 15.92X_1^2 - 1.77X_2^2 - 1.39X_3^2$$
$$+ 0.50X_1X_2 + 8.28X_1X_3 - 1.03X_2X_3$$

Table 2 shows that the quadratic term for ethanol concentration (X_1^2) had the most significant effect on antioxidant activity, followed by interaction terms for ethanol concentration and extraction temperature (X_1X_3). Other variables, however, were not significant in this model.

The fitness of the predicted model was supported by $F = 12.37$ and $p = 0.006$. An insignificant lack-of-fit value of $p = 0.100$ also indicated that the model adequately fit the experimental data. Overall, statistical analysis supported good fits between experimental and predicted values and the suitability of this polynomial model for further optimization.

Three-dimensional response surfaces describing antioxidant activity are shown in Fig. 2. Figs. 2A and 2B showed the quadratic effects of ethanol concentration on antioxidant activity. Antioxidant activity slightly improved with increasing ethanol concentrations up to a certain level, but diminished thereafter. Extraction temperature showed linear effects on antioxidant activity, as antioxidant activity improved with increasing extraction temperatures (Fig. 2B). However, antioxidant activity showed minimal changes relative to extraction time (Figs. 2A and 2C).

Taken together, response surface analysis, as well as statistical analysis, indicated that KRG antioxidant activity was greatly impacted by ethanol concentration changes, whereas little effect was observed related to extraction temperature and time.

3.3. Effect of extraction variables on extraction yield

A second-order polynomial regression equation for extraction yield using coded values was derived from multiple regression analysis on the experimental data as follows:

$$\text{Extraction yield (\%)} = 19.33 - 9.55X_1 - 0.04X_2 + 3.24X_3$$
$$- 5.70X_1^2 + 0.52X_2^2 - 1.52X_3^2$$
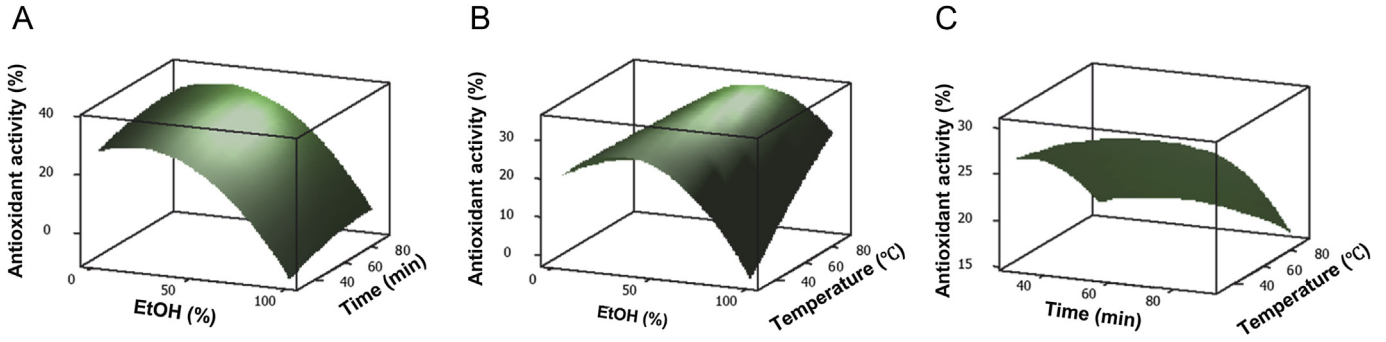$$+ 0.80X_1X_2 + 0.85X_1X_3 + 1.68X_2X_3$$

Fig. 2. Response surface plot analysis of KRG extraction solvent, extraction time, and extraction temperature on antioxidant activity. The fixed variables were set to coded value 0 as (A) 60°C, (B) 60 min, and (C) 50% ethanol. KRG, Korean Red Ginseng.
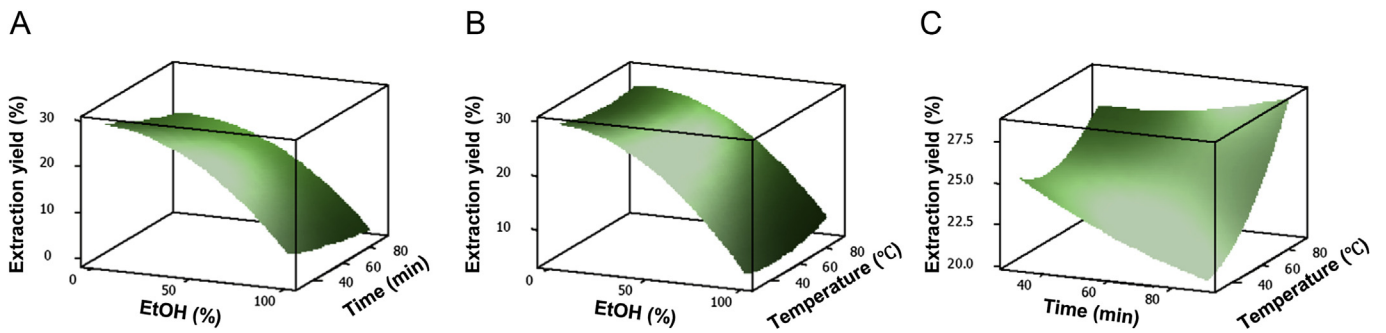

Fig. 3. Response surface plot analysis of KRG extraction solvent, extraction time, and extraction temperature of KRG on extraction yield. The fixed variables were set to coded value 0 as (A) 60°C, (B) 60 min, and (C) 50% ethanol. KRG, Korean Red Ginseng.
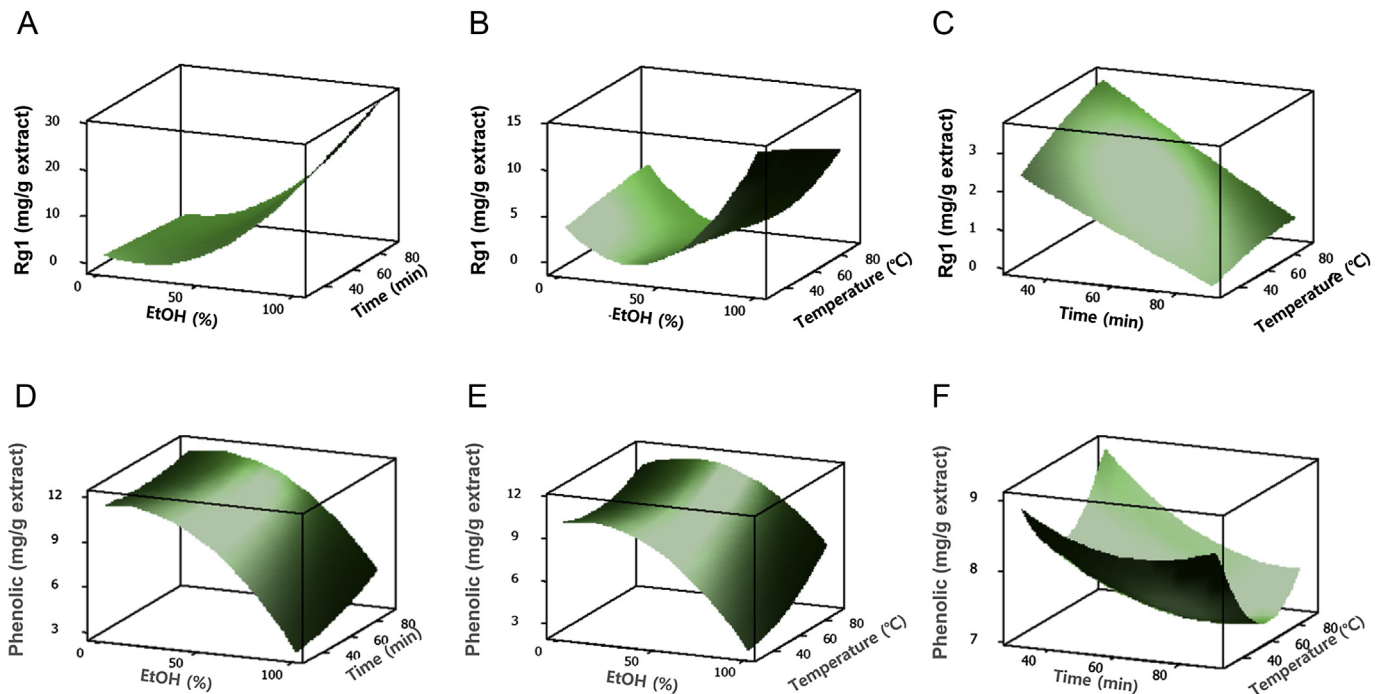

Fig. 4. Response surface plot analysis of KRG extraction solvent, extraction time, and extraction temperature on ginsenoside Rg1 and phenolic content. The fixed variables were set to coded value 0 as (A,D) 60°C, (B,E) 60 min, and (C,F) 50% ethanol. KRG, Korean Red Ginseng.

The linear (X_1) and quadratic (X_1^2) terms for ethanol concentration exhibited the most significant effects on extraction yield, with $p < 0.001$ and $p < 0.002$, respectively (Table 2). The linear term of extraction time (X_3) also showed significant effect, however, other variables did not show significant effects on extraction yield. Values of $F = 29.72$ and a $p = 0.001$ demonstrated the fitness of the predicted model. The coefficient determination (R^2) and the adjusted coefficient determination (adj. R^2) were 0.982 and 0.949, respectively, and the lack-of-fit value was $p = 0.162$. These results supported the good fit of experimental values and predicted ones.

Three-dimensional response surface plots for extraction yield are shown in Fig. 3. Consistent with regression analysis results, the linear effect of ethanol concentration was inversely proportional to extraction yield (Figs. 3A and 3B). Extraction temperature showed linear effect on extraction yield and as extraction temperature increased, extraction yield also increased (Fig. 3C). Extraction time showed mixed effects that were dependent upon other variables.

Collectively, response surface analysis, as well as statistical analysis, indicated that extraction yield was noticeably affected by ethanol concentration to a greater degree than extraction temperature.

3.4. Effect of extraction variables on ginsenoside Rg1 and phenolic content

Multiple regression analysis of the experimental data yielded the second-order polynomial regression equation for coded values as follows:

Table 4
Predicted and observed values of maximum antioxidant activity and extraction yield under optimized conditions

Extraction condition			Antioxidant activity (%)		Extraction yield (%)	
EtOH (%)	Time (min)	Temperature (°C)	Predicted	Observed	Predicted	Observed
46.8	73.0	90.0	40.7	43.7	24.9	23.2

EtOH, ethanol.

$$Rg1\ content\ (mg/g\ extract) = 2.99 - 8.50X_1 + 0.87X_2$$
$$- 1.21X_3 + 7.24X_1^2 - 0.34X_2^2$$
$$+ 0.25X_3^2 + 1.86X_1X_2$$
$$- 1.64X_1X_3 - 0.19X_2X_3$$

Phenolic content (mg/g extract)
$$= 7.78 - 1.99X_1 + 0.06X_2 + 0.67X_3 - 2.51X_1^2 + 0.37X_2^2$$
$$+ 0.88X_3^2 + 0.49X_1X_2 + 0.87X_1X_3 - 0.28X_2X_3$$

As given in Table 2, the linear (X_1) and quadratic (X_1^2) terms for ethanol concentration had the most significant effect on ginsenoside Rg1 and phenolic content. Other variables, however, did not show any significant effect.

Values of $F = 38.29$ and $F = 10.56$, together with $p < 0.001$ and $p < 0.009$, for ginsenoside Rg1 and phenolic content, respectively, supported the fitness of the model. Additionally, insignificant lack-

A

R² = 0.1781

B

R² = 0.2818

C

R² = 0.4089
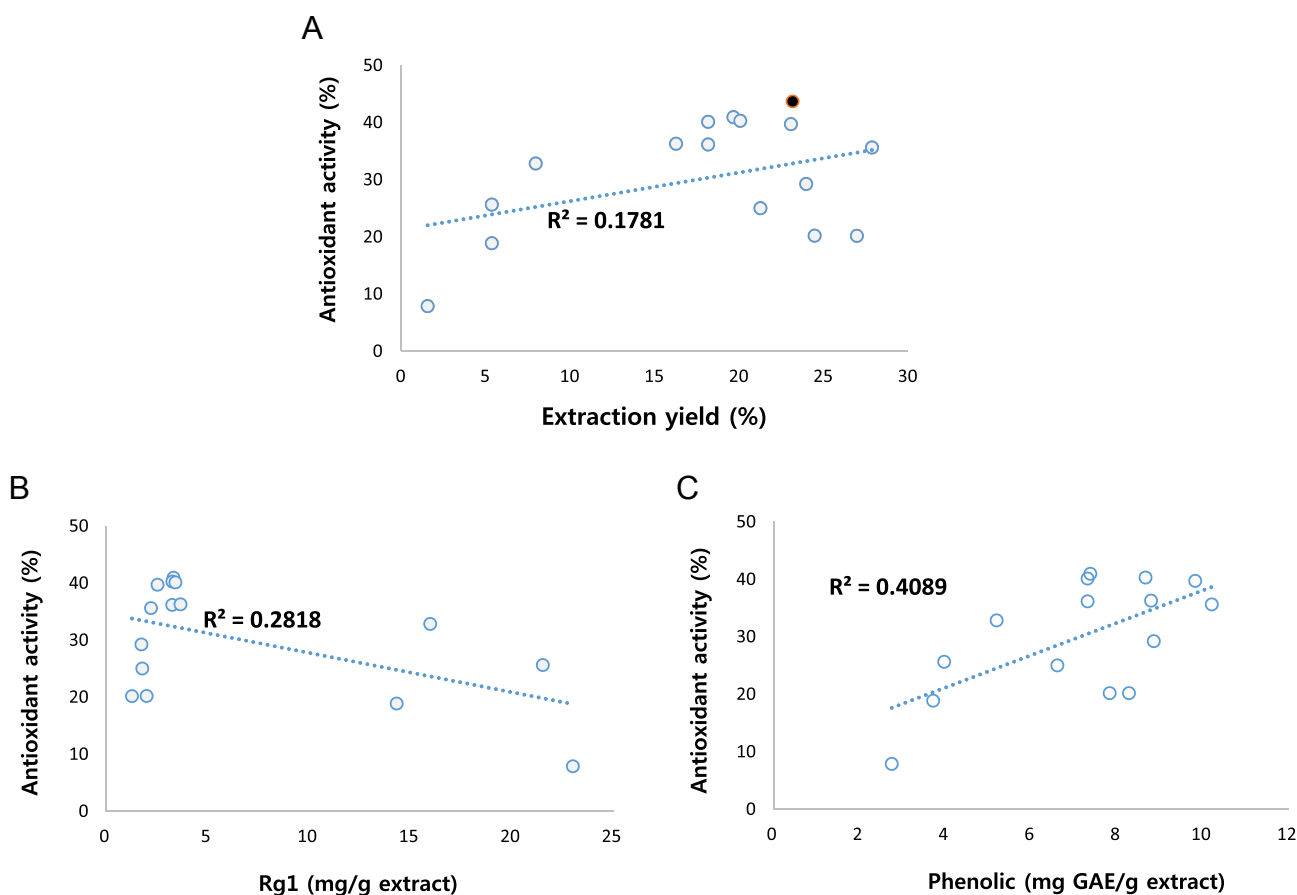
Fig. 5. Correation between (A) antioxidant activity and extraction yield and (B) antioxidant activity and ginsenoside Rg1 content (C) and antioxidant activity and phenolic content. Open dots indicate the actual values of experimentatal data and closed dots indicate the optimized values.

of-fit values of $p = 0.063$ and $p = 0.443$ for ginsenoside Rg1 and phenolic content, respectively, also indicated that the model adequately fit the experimental data. Overall, statistical analysis supported the suitability of this polynomial model for further optimization.

Three-dimensional response surface plots for ginsenoside Rg1 content also showed the dramatic effect of ethanol concentration on ginsenoside Rg1 content. Using a fixed temperature of 60°C, ethanol concentration exerted a linear effect on ginsenoside Rg1 content (Fig. 4A), as ginsenoside Rg1 content increased with increasing ethanol concentration. Using a fixed time of 60 min, ethanol concentration exhibited a quadratic effect on ginsenoside Rg1 content (Fig. 4B). Ginsenoside Rg1 content began to decrease slightly up to a certain ethanol concentration, but increased thereafter. However, ginsenoside Rg1 content displayed minimal changes relative to extraction time and temperature as compared to ethanol concentration (Figs. 4A–4C).

Three-dimensional response surface plots for phenolic content also showed the dramatic effects associated with ethanol concentration. However, contrary to ginsenoside Rg1 content, phenolic content decreased with increasing ethanol concentration (Figs. 4D and 4E). Phenolic content showed minimal changes relative to extraction time and temperature as compared to ethanol concentration (Fig. 4F).

3.5. Correlation between antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content

In the present study, KRG extracts prepared from 15 different extraction conditions were evaluated for antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content. As shown in Table 1, responses varied greatly depending on extraction conditions. Therefore, correlations between each response were investigated. First, correlation between antioxidant activity and extraction yield was analyzed. Little correlation was observed between antioxidant activity and extraction yield, as demonstrated by the value $R^2 = 0.178$ (Fig. 5A). Next, correlation between antioxidant activity and ginsenoside Rg1 and phenolic content was analyzed. Ginsenosides are characteristic saponins of ginseng known to play an important role in ginseng pharmacological activity [30–33]. Antioxidant mechanism of ginsenosides, including Rg1, is involved in diverse biological activity [32–34]. However, antioxidant activity was not proportional to ginsenoside Rg1 content and little correlation was observed, as $R^2 = 0.281$ (Fig. 5B). Analysis of correlation between antioxidant activity and phenolic content showed that antioxidant activity was slightly proportional to phenolic content, with $R^2 = 0.409$ (Fig. 5C). Ginseng contains diverse constituents, including ginsenosides and phenolic, as well as oligosaccharides and polysaccharides [35–38]. Our present study suggests that antioxidant activity was achieved by the combinatorial actions of diverse ginseng extract components.

3.6. Optimization of extraction parameters and verification

We next optimized extraction conditions to achieve maximum antioxidant activity and extraction yield. Based on our results, an optimized extraction condition for maximum antioxidant activity and extraction yield was determined at ethanol concentration of 46.8%, an extraction time of 73.0 min, and a temperature of 90.0°C, which predicted 40.7% of antioxidant activity and a 24.9% extraction yield. KRG extract prepared under this condition exhibited 43.7% antioxidant activity and 23.2% extraction yield, correlating with predicted values (Table 4). Thus, this model is suitable for optimizing the KRG extraction process.

KRG is widely developed as functional ingredients for diverse activities. Antioxidant activity is a representative effect of KRG and contributes to diverse pharmacological uses, such as anti-fatigue, immunomodulatory, anticancer, and metabolic disorder medication. Therefore, maximum antioxidant activity is essential in the delivery of a high quality product. As shown in Fig. 5A, the antioxidant activity of KRG extract prepared under optimized extraction conditions was 43.7%, which is a stronger result relative to 15 other extraction conditions. Therefore, KRG extract prepared under these conditions will be more effective to applications involving human health. For the development of KRG as a product, economic efficiency is also required. Although higher extraction yields can be achieved from other extraction conditions (Fig. 5A), effectiveness is more important than extraction yield for the development of functional products. Therefore, the optimized conditions were preferentially focused on antioxidant activity in our present study. These optimized extraction conditions provide adequate extraction yields as compared to 15 other extraction conditions.

In conclusion, efficacy and extraction yields are greatly impacted by extraction conditions, especially extraction solvent. Our present study provides optimized extraction conditions for maximum antioxidant activity and extraction yield. This will provide useful information for KRG development that offers not only maximum efficacy, but also economic efficiency.

Conflicts of interest

The authors declare no conflicts of interest.

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