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Optimization of Accelerated Solvent Extraction of Ginsenosides from Cultivated Wild Ginseng Using Response Surface Methodology

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ABSTRACT: This study's aim is to apply response surface methodology (RSM) to model and optimize the accelerated solvent extraction (ASE) technique for extracting the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenosides from cultivated wild ginseng. To extract ginsenosides from cultivated wild ginseng, a new ASE-based method, combined with RSM modeling and optimization, was developed. The RSM method, which was based on a five-level, three-factor central composite design, was used to obtain the optimal combination of extraction conditions. Briefly, the optimal extraction conditions for the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside were as follows: 88.64% ethanol for each extraction solvent, 105.98°C and 129.66°C of extraction temperature, 28.77 and 15.92 min of extraction time, extraction pressure of 1,500 psi, nitrogen purge of 60 s, flush volume of 60%, and one extraction cycle. A 3D response surface plot and contour plot derived from the mathematical models were applied to obtain the optimal conditions. Under the above conditions, the experimental extraction yields of the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside content were 7.45 and 32.82 mg/g, respectively, which closely agrees with the model's prediction values.

Keywords: accelerated solvent extraction, cultivated wild ginseng, ginsenosides, response surface methodology

INTRODUCTION

Ginseng (*Panax ginseng* C.A. Meyer) belongs to the Genus *Panax* (Araliaceae family) and has been widely used as a source of natural medicine in East Asia for thousands of years, particularly in China, Korea, and Japan (Yun, 2001). Owing to its important medicinal properties, ginseng is highly valued and vigorously promoted. Pharmacologically active substances include ginsenosides, flavonoids, and polysaccharides, among which ginsenosides are the main bioactive compounds in ginseng (Kim and Park, 2011). Ginseng saponins, also known as ginsenosides, have many functional properties, including anticancer, antioxidant, metabolism-enhancing, immune function-related, cardiovascular disease-preventive, and anti-obesity (Leung and Wong, 2010; Wee et al., 2011).

Cultivated wild ginseng is found in mountainous regions, whereas mountain-cultivated ginseng, which mimics mountain wild ginseng, is grown in forests and mountains. However, cultivated wild ginseng is considered superior to regular-cultivated ginseng because it contains higher amounts of certain ginsenosides. Although ginsenoside levels are consistently low in more intensively cultivated crops, their growth rates are high (Lim et al., 2005). The major bioactive compounds in cultivated wild ginseng are saponins, such as ginsenosides, and nonsaponins, such as panacen, polyacetylene derivatives, and phenol. However, only saponins have notable pharmacological efficacy (Gillis, 1997).

Accelerated solvent extraction (ASE), introduced by the Dionex Corporation in 1995, is a green technique for plant material sample preparation prior to chromatographic analysis (Heng et al., 2013). ASE is also known as pressurized liquid extraction, pressurized solvent extraction, or enhanced solvent extraction. When water is used as the solvent, it is referred to as pressurized hot water extraction, subcritical water extraction, or superheated water extraction (Mustafa and Turner, 2011). ASE uses elevated pressure (500～2,000 psi) and temperature (40～200°C) for a relatively short time to accelerate the extraction rate. Greater pressure allows the extraction cell to be filled more quickly and effectively by injecting solvents into the matrix, which can maintain solvents in liquid form, even at high temperatures. Elevated temperatures increase solvation ability by decreasing the solvent's viscosity and surface tension, resulting in an accel-

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erated diffusion rate and mass transfer of the analyte into the solvent, thereby improving the recovery of the compounds of interest (Ameer et al., 2017).

Response surface methodology (RSM), introduced by Box and Wilson (1951), is an effective statistical and mathematical technique for experimental parameter optimization using experimental designs, such as the Box-Behnken design (BBD), central composite design (CCD), and Doehlert's design (Zolgharnein et al., 2013). An RSM model, including these designs, was used, and the levels of the experimental parameters were identified and optimized to achieve an optimal response while performing minimal iterations (Jentzer et al., 2015). Several researchers have used CCD, which is the most popular form of RSM, to optimize various food processing methods, such as milling (Ghodke et al., 2009), extraction (Huang et al., 2008), and fermentation (Dhandhukia and Thakkar, 2008). The extraction process is less laborious and time consuming than other methods. Owing to these advantages, CCD is widely employed to optimize the extraction of natural components, including phenolics (Yang et al., 2009), chromones (Li et al., 2011), saponins (Kwon et al., 2003), and polysaccharides (Xie et al., 2010).

This study's aim is to apply RSM to model and optimize ASE techniques for extracting ginsenosides from cultivated wild ginseng. Several important factors, such as extraction solvent, extraction time, and extraction temperature, were systemically analyzed using CCD.

MATERIALS AND METHODS

Materials

Five-year-old cultivated wild ginseng roots were collected (August 5, 2019) from the experimental field of Jinsaengbio Farm Association Co. (Hamyang-gun, Gyeongnam, Korea). The standards of ginsenoside−Rb1, Rg1, Re, Rf, Rh1, Rc, Rb2, Rd, Rg6, F4, Rk3, Rh4, Rg3, Rk1, and Rg5−were purchased from the Ambo Institute (Daejeon, Korea). J.T. Baker (Phillipsburg, NJ, USA) supplied acetonitrile and water. All other chemicals used were of analytical grade.

Accelerated solvent extraction procedure

Pressurized liquid extraction was performed using an ASE 350 System (Dionex, Sunnyvale, CA, USA) with a stainless-steel extraction cell. Approximately 5 g of cultivated wild ginseng sample was placed into an extraction cell after being uniformly mixed with a similar weight of diatomaceous earth. To prevent the powder from penetrating the extraction bottle, a frit and filter (Dionex) were positioned at the cell's end. The ASE conditions were as follows: static cycles, 1; solvent flush %, 60 volumes; nitrogen purge, 60 s; and pressure, 1,500 psi. Extraction solvent, temperature, and static time were used as extraction variables for the ASE of ginsenosides. The extract was evaporated to dryness using a rotary evaporator at 50°C, which was freeze-dried.

Determination of ginsenosides by high-performance liquid chromatography

The ginsenosides were determined using the method described by Dong et al. (2011), with slight modifications. Analyses were performed using an Agilent 1260 liquid chromatograph (Hewlett Packard, Wilmington, NC, USA) equipped with a quaternary gradient pump and multiple wavelength detector operating at 203 nm. The samples were separated on a Zorbax Eclipse XDB-C18 column (4.6 mm×150 mm, 5 μm; Agilent Technologies, Inc., Santa Clara, CA, USA) at 35°C with a sample injection volume of 10 μL. The mobile phase comprised a gradient of water (A) and acetonitrile (B). The following gradient was used: 20% B (0 min), 20% (0～10 min), 32% (10～40 min), 50% (40～55 min), 65% (55～70 min), 90% (70～82 min), and 80% (82～90 min). Data analysis was performed using Chemstation software (Hewlett Packard). The flow rate of the mobile phase was 0.9 mL/min.

Preparation of standard solutions

Stock solutions of ginsenosides, Rb1, Rg1, Re, Rf, Rh1, Rc, Rb2, Rd, Rg6, F4, Rk3, Rh4, Rg3, Rk1, and Rg5, were prepared in methanol. A series of standard operating solutions of different concentrations was obtained by diluting the standard stock solutions.

Experimental design and statistical analytic

RSM was used to optimize ginsenoside extraction from cultivated wild ginseng. The three selected factors were ethanol concentration (X_1), extraction temperature (X_2), and extraction time (X_3). Six center points in CCD with three factors were recommended. A five-level three-factor CCD (MINITAB Statistical Software, Release 21 for Windows, Minitab Inc., State College, PA, USA) was used to determine the best combination of extraction varia-

Table 1. Factors and their adopted (uncoded) values at different coded levels

Factor (Y)	Symbol	−2	−1	0	+1	+2
Ethanol concentration (%)	X_1	21	35	55	75	89
Extraction temperature (°C)	X_2	53	81	122	163	191
Extraction time (min)	X_3	5	10	17	24	29

bles to maximize the yield of ginsenosides extracted from cultivated wild ginseng. The factorial, center, and axial points in the CCD method constitute an experimental design with five levels for each factor and three replicates, summing up to 60 runs [$3(2^k+2k+m)=3(8+6+6)=60$]. Table 1 lists the experimental plan with the coded and uncoded levels of design factors. The low, middle, and high levels of each factor were coded as −1, 0, and +1, respectively, whereas the lowest and highest levels were coded as −2 and +2, respectively. The mathematical relationship of the *Y* response to the corresponding factors is expressed by the following second-order polynomial equation:

$$Y = \beta_0 + \sum_{j=l}^{k}\beta_j X_j + \sum_{j=l}^{k}\beta_{jj} X_j^2 + \sum\sum_{i<j}\beta_{ij} X_i Y_j$$

where *Y* is the estimated response; β_0, β_j, β_{jj}, and β_{ij} are the regression coefficients for the intercept, linearity, square, and interaction, respectively; X_i and X_j are independent coded variables. MINITAB Statistical Software (Minitab Inc.) was used to estimate the response of each experimental design and the set of optimized conditions. The fitness of the polynomial model equation is expressed by the coefficient R^2. The *F*- and *P*-values were used to check the significant level ($P<0.05$) of the regression coefficients. The data are expressed as the mean of three replicates.

RESULTS AND DISCUSSION

Optimization of ASE by RSM

To evaluate the multiple effects of extraction factors on the sum of ginsenosides (Rg1, Rb1, and Rg3) and the total ginsenoside, a five-level and three-factor BBD was employed. The ranges of these variables were determined as extraction solvent (X_1: ethanol concentration at 21%, 35%, 55%, 75%, and 89%), extraction temperature (X_2: 53, 81, 122, 163, or 191°C), and extraction time (X_3: 5, 10, 17, 24, or 29 min). The variables were coded at five levels (−2, −1, 0, 1, and 2), and the complete design comprised 60 experimental points, including three replicates of the center points (all variables were coded as zero), as shown in Table 1.

Notably, the sum of ginsenosides (Rg1, Rb1, and Rg3) and the total ginsenoside content varied depending on the extraction conditions (Table 1). Second-order polynomial regression equations were established using 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 was determined using *t*-test and *P*-values (Table 2). Larger coefficients with a smaller *P*-value ($P<0.05$) indicated that these coefficients significantly affected the respective responses. Correlations between the three independent variables and each response were also estimated using multiple determinations (R^2). The values of R^2 were

Table 2. Analysis of mean square deviation of regression equation for the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside of cultivated wild ginseng

Parameter	Sum of ginsenosides Rg1, Rb1, and Rg3					Total ginsenoside[1)]				
	SS	DF	MS	*F*-value	Prob>*F*	SS	DF	MS	*F*-value	Prob>*F*
Model	89.7429	11	8.1584	92.15	<0.0001	2,035.57	11	185.05	189.83	<0.0001
Linear										
X_1[2)]	17.5917	1	17.5917	198.69	<0.0001	552.86	1	552.86	567.12	<0.0001
X_2	33.7091	1	33.7091	380.73	<0.0001	131.97	1	131.97	135.37	<0.0001
X_3	1.0624	1	1.0624	12.00	0.001	13.51	1	13.51	13.86	0.001
Quadratic										
X_1^2	0.7092	1	0.7092	8.01	0.007	13.60	1	13.60	13.95	<0.0001
X_2^2	25.1550	1	25.1550	284.12	<0.0001	1,177.38	1	1177.38	1207.76	<0.0001
X_3^2	0.1502	1	0.1502	1.70	0.199	54.86	1	54.86	56.28	<0.0001
Interaction										
X_1X_2	3.5376	1	3.5376	39.96	<0.0001	107.11	1	107.11	109.88	<0.0001
X_1X_3	1.6837	1	1.6837	19.02	<0.0001	0.42	1	0.42	0.43	0.513
X_2X_3	0.9030	1	0.9030	10.20	0.002	0.79	1	0.79	0.81	0.371
Residual	4.2498	48	0.0885	–	–	46.79	48	0.97	–	–
Lack of fit	2.6786	22	0.1218	2.01	0.144	23.81	22	1.08	1.22	0.308
Pure error	1.5712	26	0.0604	–	–	22.98	26	0.88	–	–
Cor total	93.9927	59	–	–	–	2,082.36	59	–	–	–
			R^2=0.9548					R^2=0.9775		

[1)]Sum of individual ginsenoside content (Rb1 + Rg1 + Re + Rf + Rh1 + Rc + Rb2 + Rd + Rg6 + F4 + Rk3 + Rh4 + Rg3 + Rk1 + Rg5).
[2)]Factors are as described in Table 1.
SS, sum of squares; DF, degree of freedom; MS, mean square; *F*-value, Fischer test value; Prob, probability; R^2, determination coefficient.

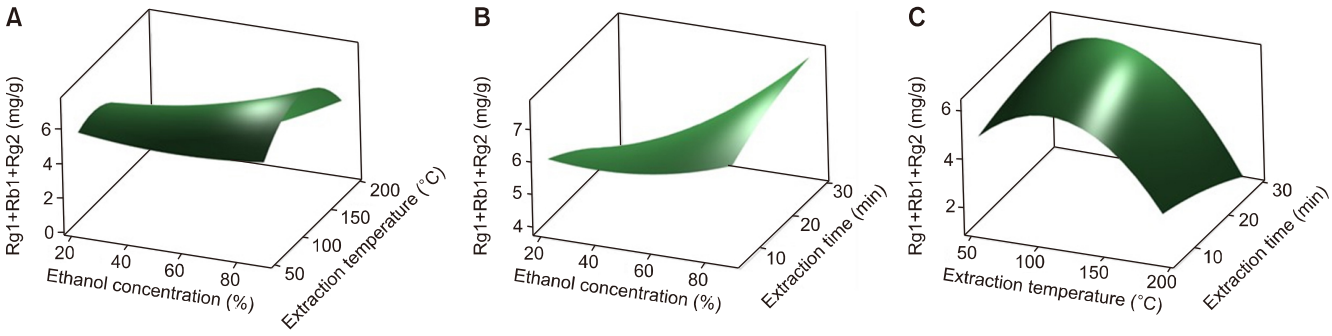

Fig. 1. Response surface plot analysis of ethanol concentration, extraction temperature, and extraction time on the sum of ginsenosides Rg1, Rb1, and Rg3. The fixed variables were set to coded value 0 as 17 min (A), 122°C (B), and 55% ethanol (C).

0.9548 and 0.9775 for the sum of ginsenosides (Rg1, Rb1, and Rg3) and the total ginsenoside content, respectively, demonstrating the effectiveness of this model. The validity of the model was confirmed using a lack-of-fit test (Table 2). An insignificant *P*-value for the lack of fit ($P>0.05$) for the three responses indicated that this model was adaptable to the experimental data. Relationships between every two variables for the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside content are shown in three-dimensional response surface plots based on regression equations (Fig. 1). Collectively, this model adequately fits the experimental data and is suitable for optimization.

Effect of extraction variables on the sum of ginsenosides Rg1, Rb1, and Rg3

Table 2 shows the design matrix and corresponding results of the RSM experiments, which were established to determine the effects of the three independent variables: ethanol concentration, extraction temperature, and extraction time. Through multiple regression analysis of the experimental data, the predicted response *Y* for the sum of ginsenosides Rg1, Rb1, and Rg3 can be expressed in terms of coded values using the following second-order polynomial equation:

$$Y=4.67-0.0944X_1+0.07412X_2-0.0038X_3+0.000324X_1^2 -0.000458X_2^2-0.001219X_3^2+0.000483X_1X_2 +0.001923X_1X_3-0.000687X_2X_3$$

where *Y* is the sum of ginsenosides Rg1, Rb1, and Rg3 (mg/g), and X_1, X_2, and X_3 are the coded variables for ethanol concentration, extraction temperature, and extraction time, respectively.

Statistical testing of the model was performed using analysis of variance (ANOVA). Table 3 lists the ANOVA results for the fitted quadratic polynomial model of the sum of the ginsenosides Rg1, Rb1, and Rg3. The quadratic regression model demonstrated a determination coefficient (R^2) of 0.9548 with no significant lack of fit at $P>0.05$, implying that the calculated model could justify 95.48% of the results. The results indicated that the model used to fit the response variable was significant ($P<0.0001$) and adequate to represent the relationship between the response and independent variables (Hossain et al., 2012). The significance of the model was also determined by an *F*-test, suggesting that the model had a high *F*-value ($F=92.15$). R^2 adj (adjusted determination coefficient) is a correlation measure used to test the goodness-of-fit of the regression equation (Kim et al., 2012). The R^2 adj value of this model was 0.9444, indicating that only 5.56% of the total variation was not explained by the model. The significance of each coefficient was determined using the *F*-value and *P*-value (Table 2). As observed, all three extraction parameters (X_1, X_2, $P<0.001$ or X_3, $P<0.05$) significantly affected the sum of ginsenosides Rg1, Rb1, and Rg3. In addition, all quadratic parameters (X_1^2 and X_2^2) were significant at the level of $P<0.05$ or $P<0.0001$, whereas the X_3^2 was insignificant ($P>0.1$). Moreover, the interaction quadratic parameters (X_1X_2, X_1X_3, and X_2X_3) were significant at $P<0.0001$ or $P<0.05$.

Effect of extraction variables on total ginsenosides

A second-order polynomial regression equation for the total ginsenoside using coded values was derived from multiple regression analysis of the experimental data as follows:

$$Y=-11.03+0.0003X_1+0.5621X_2+0.577X_3-0.001420X_1^2 -0.003131X_2^2-0.02329X_3^2+0.002656X_1X_2 +0.00096X_1X_3+0.000644X_2X_3$$

where *Y* is the total ginsenoside content and X_1, X_2, and X_3 are the coded variables for ethanol concentration, extraction temperature, and extraction time, respectively.

Notably, the experimental model was adequate ($P<0.0001$) and the lack of fit was not significant ($P>0.05$) (Table 2). The determination coefficient (R^2) of the total ginsenosides was 0.9775, indicating that the model could explain 97.75% of the variation. The model was highly significant and fit the experimental data well. The posi-

Table 3. Experimental data on the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside of cultivated wild ginseng under different conditions based on central composite design for response surface methodology

Run[1)]	Factor[2)]			Sum of ginsenosides Rg1, Rb1, and Rg3 (mg/g)	Total ginsenoside (mg/g)[3)]
	X1	*X2*	*X3*		
1	21	122	17	5.55±0.08	23.03±0.23
2	55	122	17	5.80±0.05	30.25±0.30
3	55	122	17	5.48±0.06	28.74±0.29
4	75	163	24	5.52±0.06	32.91±0.33
5	55	191	17	2.05±0.02	12.74±0.13
6	35	163	10	4.14±0.04	19.15±0.19
7	55	122	29	5.71±0.06	33.24±0.33
8	75	81	10	6.22±0.06	20.36±0.20
9	55	191	17	2.29±0.02	11.49±0.12
10	55	122	17	5.59±0.06	32.18±0.32
11	55	122	29	5.81±0.06	29.58±0.30
12	55	122	17	5.68±0.06	30.59±0.31
13	55	53	17	5.51±0.06	19.10±0.19
14	55	53	17	5.39±0.05	19.38±0.19
15	55	122	17	6.07±0.06	28.64±0.29
16	35	81	10	5.84±0.05	18.35±0.18
17	55	122	17	5.86±0.05	26.60±0.27
18	89	122	17	7.24±0.06	24.70±0.25
19	55	122	17	5.73±0.05	23.95±0.24
20	75	163	24	5.25±0.05	29.96±0.30
21	55	53	17	5.32±0.05	20.98±0.21
22	89	122	17	7.09±0.06	26.46±0.27
23	55	122	17	6.48±0.07	28.96±0.29
24	55	122	17	6.13±0.06	29.42±0.30
25	55	122	29	5.53±0.06	22.95±0.23
26	75	163	10	5.45±0.05	27.86±0.28
27	75	81	24	6.75±0.07	24.56±0.25
28	35	81	10	5.80±0.05	18.42±0.19
29	55	122	17	5.97±0.07	33.51±0.34
30	55	122	17	6.48±0.07	29.77±0.30
31	75	81	10	5.98±0.06	26.48±0.27
32	35	81	24	5.25±0.05	20.11±0.20
33	35	163	24	2.58±0.03	13.51±0.14
34	55	122	5	5.63±0.06	25.01±0.25
35	75	81	24	7.00±0.07	25.04±0.25
36	75	163	24	5.13±0.05	25.23±0.25
37	21	122	17	5.42±0.05	25.69±0.26
38	35	81	24	5.93±0.07	22.74±0.23
39	75	163	10	5.20±0.00	9.44±0.09
40	35	81	10	6.38±0.06	20.79±0.21
41	55	122	17	5.86±0.06	30.56±0.31
42	35	163	10	3.68±0.04	16.47±0.17
43	75	81	24	6.09±0.06	24.60±0.25
44	55	122	17	5.34±0.05	29.45±0.30
45	55	122	17	6.13±0.06	30.96±0.31
46	89	122	17	7.44±0.07	38.76±0.39
47	55	122	17	5.56±0.06	30.82±0.31
48	55	122	17	5.56±0.06	28.53±0.29
49	55	191	17	2.29±0.02	11.98±0.12
50	35	163	24	2.14±0.02	11.38±0.11
51	35	163	24	2.19±0.02	13.98±0.14
52	55	122	5	6.11±0.06	31.24±0.31
53	35	163	10	3.44±0.03	15.37±0.15
54	55	122	5	5.98±0.06	28.42±0.29
55	75	81	10	6.40±0.06	27.59±0.28
56	35	81	24	5.32±0.05	21.29±0.21
57	55	122	17	6.15±0.06	33.11±0.33
58	21	122	17	5.26±0.05	25.45±0.26
59	55	122	17	5.89±0.06	32.65±0.33
60	75	163	10	5.66±0.06	27.11±0.27

Data are presented as number only or mean±SD.
[1)]The number of experimental conditions by central composite design.
[2)]Factors are as described in Table 1.
[3)]Sum of individual ginsenoside content (Rb1 + Rg1 + Re + Rf + Rh1 + Rc + Rb2 + Rd + Rg6 + F4 + Rk3 + Rh4 + Rg3 + Rk1 + Rg5).

tive linear effects of the three independent variables on all the response variables were significant (X_1, X_2, $P<0.001$ or X_3, $P<0.05$). All three extraction parameters (X_1, X_2, $P<0.001$ or X_3, $P<0.05$) significantly affected the sum of ginsenosides Rg1, Rb1, and Rg3. In addition, all quadratic parameters (X_1^2, X_2^2, and X_3^2) were significant at the level of $P<0.0001$, whereas the interaction quadratic parameters (X_1X_2) were significant at the level of $P<0.0001$, but X_1X_3 and X_2X_3 were insignificant ($P>0.05$).

Analysis of the surface plots

The interaction of two out of the three variables and their effect on the sum of ginsenosides (Rg1, Rb1, and Rg3) and the total ginsenoside content recovery, keeping the remaining three variables constant, is illustrated in the three-dimensional RSM plot (Fig. 1 and Fig. 2). The interactive effect of ethanol concentration and extraction temperature while fixing the extraction time (17 min) at a constant level was investigated (Fig. 1A). The sum of the ginsenosides Rg1, Rb1, and Rg3 content increased as the ethanol concentration increased from 21% to 89% with the extraction temperature up to 116℃. It remained constant or decreased slightly as the extraction temperature increased with a fixed extraction concentration; it also peaked at 7.39 mg/g at an ethanol concentration of 89% and an extraction temperature of 116℃.

Fig. 1B shows the ethanol concentration-extraction time relationship. A similar linear effect was observed with respect to ethanol concentration, which caused an increase in the sum of ginsenosides Rg1, Rb1, and Rg3. The sum of ginsenosides Rg1, Rb1, and Rg3 content increased until the ethanol concentration reached 89%. However, for ethanol concentrations between 21% and 45%, the content decreased as the extraction time increased, but at ethanol concentrations exceeding 46%, the content increased with increasing extraction time at a fixed extraction temperature. The optimum sum of ginsenosides Rg1, Rb1, and Rg3 peaked at 7.68 mg/g at an

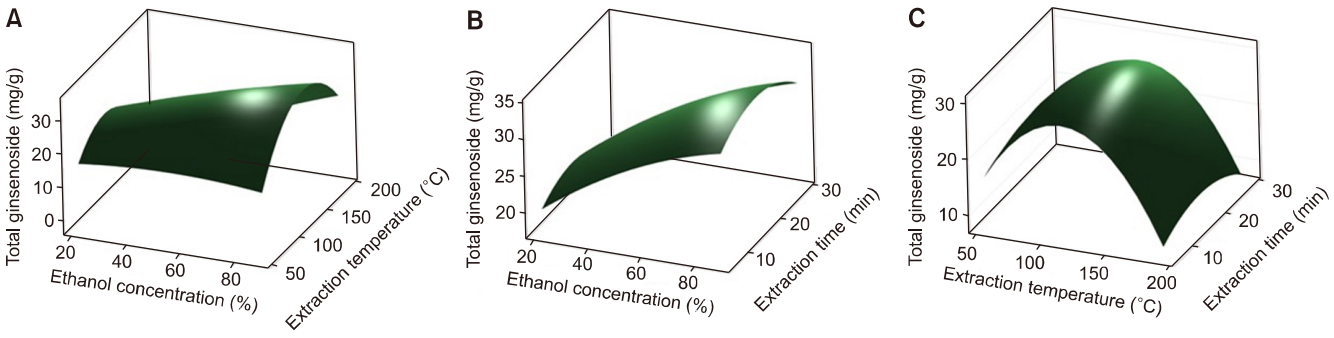

Fig. 2. Response surface plot analysis of ethanol concentration, extraction temperature, and extraction time on the total ginsenoside. The fixed variables were set to coded value 0 as 17 min (A), 122°C (B), and 55% ethanol (C).

ethanol concentration of 89% and an extraction time of 29 min.

Fig. 1C (3D plots) shows the interactive effect between extraction temperature and time. The sum of ginsenoside Rg1, Rb1, and Rg3 content increased and decreased as the ethanol concentration varied from 21% to 89%, and there was no clear correspondence with the extraction time. The highest recovery of 6.18 mg/g of the sum of ginsenosides Rg1, Rb1, and Rg3 content was recorded at an extraction temperature of 100°C and an extraction time of 13.6 min.

The effect of ethanol concentration and extraction temperature on the total ginsenoside content is illustrated in the response surface 3D plot in Fig. 2A. The total ginsenoside content increased as the ethanol concentration varied from 21% to 89% with the extraction temperature up to 130°C. Subsequently, the total ginsenoside content increased and decreased slightly with the increase in the extraction temperature at a fixed extraction concentration, with the maximum total ginsenoside content peaking at 34.56 mg/g at an ethanol concentration of 89% and extraction temperature of 130°C. Because molecular mobility accelerates with increasing temperatures, greater extraction efficiency is achieved at higher temperatures. Furthermore, at higher temperatures, the dissolution capability of the solvent is enhanced, and the surface tension and solvent viscosity decrease, which improves the mass transfer rate and, thereby, the availability of bioactive chemicals for extraction (Chen et al., 2007). This finding corroborates earlier research demonstrating that saponin is a thermolabile substance and that high temperatures can reduce saponin extraction efficiency (Shi et al., 2004).

Fig. 2B illustrates the effect of the ethanol concentration-extraction time interaction on total ginsenoside content. The increase in ethanol concentration caused a similar increase in the total ginsenoside content. The total ginsenoside content remained constant or decreased slightly with increasing extraction time at a fixed extraction temperature. When the extraction temperature exceeded approximately 122°C, the total ginsenoside content began to decrease slightly. At an ethanol concentration of 89% and extraction time of 16 min, the total ginsenoside content was 34.43 mg/g.

Fig. 2C shows the three-dimensional response surface plot of the extraction temperature-extraction time interaction. The negative square effect of this interaction on the total ginsenoside content is illustrated. The total ginsenoside content increased and decreased with the increase in the extraction temperature and extraction time at a fixed extraction concentration, with the total ginsenoside content peaking at 30.04 mg/g at an extraction temperature of 114°C and extraction time of 15 min. Tan et al. (2013) reported that excessive extraction time is not required because the solvent and sample are in full equilibrium after a given time, based on Fick's second diffusion law. By the time full equilibrium is attained, the extraction procedure slows down.

Table 4. Predicted and observed values of the optimum cultivated wild ginseng extract treatment conditions for the maximized ginsenosides of cultivated wild ginseng by the ridge analysis of their response surfaces

Responses	Cultivated wild ginseng extract treatment condition			Maximum yield (mg/g)		Matching ratio[1)]
	Ethanol concentration (%)	Extraction temperature (°C)	Extraction time (min)	Predicted	Actual	
Sum of ginsenosides Rg1, Rb1, and Rg3	88.64	105.98	28.77	7.80	7.45	95.51
Total ginsenosides[2)]	88.64	129.66	15.92	33.58	32.82	97.74

[1)]Actual content/predicted content × 100 (%).
[2)]Sum of individual ginsenoside content (Rb1 + Rg1 + Re + Rf + Rh1 + Rc + Rb2 + Rd + Rg6 + F4 + Rk3 + Rh4 + Rg3 + Rk1 + Rg5).

Determination of optimal conditions and validation of the model

Experiments were performed using optimal extraction conditions to verify the model accuracy for optimal yield prediction. The values were very close to the predicted values, indicating the reliability of the optimization accomplished in this study. The quadratic polynomial regression model generated optimal extraction conditions with a desirability of 1.000. The desirability functional value was 0～1. A value of 0 implies an undesirable response from the variables, whereas 1 implies the optimal functioning of the studied variable (Jeong and Kim, 2009). The optimal extraction conditions for the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside were as follows: 88.64% and 88.64% ethanol of extraction solvent, extraction temperature of 105.98℃ and 129.66℃, extraction time of 28.77 and 15.92 min, and one extraction cycle. Under these optimal conditions, the sum of ginsenosides (Rg1, Rb1, and Rg3) and the total ginsenoside content were 7.80 and 33.58 mg/g, respectively (Table 4). To verify the model's capability to accurately predict the actual value, five replicates of verification experiments were undertaken, and the outcomes were 7.45 and 32.82 mg/g, respectively, which were very close to the predicted value. The efficacy of ASE in saponin extraction has been studied and compared with other extraction methods. A higher saponin yield was obtained from cow cockle seeds using ASE compared to ultrasonic-assisted extraction in pure and aqueous solvents of ethanol and methanol (Güçlü-Üstündağ et al., 2007). Similarly, the pressurized hot water system extracted a greater yield of ginsenosides (11.2 mg/g) compared to the ultrasound-assisted method (7.2 mg/g) from *Panax quinquefolium* (Engelberth et al., 2010). Pressurized liquid extraction showed distinctive advantages in yielding a total amount of saponins of 7.36% over other green extraction methods of ultrasound of 5.77%, and conventional extractions of Soxhlet of 6.99% and maceration of 6.00%, in the extraction of saponins from *Panax notoginseng* (Wan et al., 2006).

In conclusion, RSM was used to model and optimize the ASE technique to extract the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside from cultivated wild ginseng. This approach could effectively estimate the effect of three main independent variables (ethanol concentration, extraction temperature, and extraction time) using the contour and surface plots in RSM. In addition, a second-order polynomial model was used to optimize ginsenoside extraction from cultivated wild ginseng using ASE technology. The optimal extraction conditions for the sum of ginsenosides (Rg1, Rb1, and Rg3) and total ginsenoside were as follows: 88.64% and 88.64% ethanol of extraction solvent, extraction temperature of 105.98℃ and 129.66℃, extraction time of 28.77 and 15.92 min, and one extraction cycle. Under the optimum conditions, the experimental extraction yields of the sum of ginsenosides (Rg1, Rb1, and Rg3) and the total ginsenoside content agreed closely with the predicted yields of 7.45 and 32.82 mg/g, respectively. Overall, the present study provides a novel and efficient method for extracting cultivated wild ginseng.

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AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

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