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Optimization of Microwave-Assisted Extraction (MAP)† for Ginseng Components by Response Surface Methodology

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Response surface methodology (RSM) was applied to predict optimum conditions for microwave-assisted extraction—a MAP technology—of saponin components from ginseng roots. A central composite design was used to monitor the effect of ethanol concentration (30−90%, X_1) and extraction time (30−270 s, X_2) on dependent variables, such as total extract yield (Y_1), crude saponin content (Y_2), and saponin ratio (Y_3), under atmospheric pressure conditions when focused microwaves were applied at an emission frequency of 2450 MHz. In MAP under pre-established conditions, correlation coefficients (R^2) of the models for total extract yield and crude saponin were 0.9841 ($p < 0.001$) and 0.9704 ($p < 0.01$). Optimum extraction conditions were predicted for each variable as 52.6% ethanol and 224.7 s in extract yield and as 77.3% ethanol and 295.1 s in crude saponins, respectively. Estimated maximum values at predicted optimum conditions were in good agreement with experimental values.

KEYWORDS: Microwave-assisted processes; response surface factor; ginseng; saponins; optimization of extraction parameters

INTRODUCTION

Recently, scientific demonstrations of the value of ginseng as a herbal medicine and health-food material have resulted in an apparent increase in its consumption (*1*, *2*). Saponins are known to be the main effective components of ginseng (*3*, *4*). Their chemical structures have been extensively described in the literature (*5*, *6*).

The *Korean Food Standard Code* (*3*) prescribes the methods used in preparing ginseng extracts in terms of raw materials, solvent concentration (ethanol), maximum temperature (90 °C) for the extraction and evaporation, and saponin amounts (>80 mg/g) in the extracts. However, limitations exist in the current extraction methods, and the raw material is often subjected to extraction at lower temperature (80 °C) for relatively long time periods (as many as five periods of 8 h each) using various percentages of ethanol (*7*, *8*). Accordingly, there is a need to improve such a conventional process with the aim of reducing the extraction time and the energy consumption.

The Microwave-Assisted Processes (MAP) family of technologies was originally developed and patented by Canada's Federal Department of the Environment as methods for the extraction of target compounds from various materials (*9*−*12*). The process allows for the direct extraction of dried as well as fresh materials. It is based on the use of extraction solvents that are transparent to microwaves relative to the materials to be extracted, thereby serving as a coolant as well as a means to solubilize the target compounds. The use of microwave energy in the field of foods and natural products is not new, but until the advent of MAP, there had been few reports on the application of microwave energy to selectively heat the sample materials to enhance the extraction of soluble components contained therein. The advantages of this process over the conventional methods include shorter process time and reduced energy consumption, solvent use, and waste generation (*9*−*12*).

Response surface methodology (RSM) was originally described by Box and Wilson (*13*) as being effective for responses that are influenced by many factors and their interactions. This study was designed to determine the efficacy of microwave-assisted extraction for the effective removal of components from ginseng and to optimize the extraction conditions, such as solvent concentration and extraction time for total extract yield and crude saponin content using RSM.

MATERIALS AND METHODS

Materials. The lateral and fine roots of *Panax ginseng* C. A. Meyer were used. The dried roots were milled to >250 μm, packed in a polyethylene (PE) pail (moisture content = 9.26%), and stored at room temperature (18 ± 3 °C) until used.

Reagents and Apparatus. The reagents used for this experiment included methanol, *n*-butanol, diethyl ether, acetonitrile, and chloroform, all of first grade and/or HPLC grade. For the filtration of extracts, Whatman 41 and quantitative Q2 filter papers (porosity, fine; flow rate, slow; Fisher Scientific) were used along with membrane filters (0.45 μm, MFS). The MAP extractor was a Soxwave-100 (Prolabo) capable of emitting 30−300 W in 15 W increments. Further operational details can be found in ref *14*.

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Table 1. Central Composite Design for the Prediction and Optimization of Microwave-Assisted Extraction (MAP) Conditions for Ginseng Components[a]

	independent variables		response variables[b]		
design point	ethanol concn (X_1, %)	extraction time (s) (X_2)	extract yield (Y_1, %)	saponin extracted (Y_2, %)	saponin ratio (Y_2/Y_1, Y_3, %)
1	75 (1)[c]	210 (1)	21.8	5.16	23.6
2	75 1)	90 (–1)	20.8	4.94	23.8
3	45 (–1)	210 (1)	26.3	4.56	17.3
4	45 (–1)	90 (–1)	25.2	4.33	17.2
5	60 (0)	150 (0)	26.8	4.94	18.4
6	60 (0)	150 (0)	26.8	4.91	18.3
7	90 (2)	150 (0)	14.6	5.13	35.0
8	30 (–2)	150 (0)	22.2	3.41	15.4
9	60 (0)	270 (2)	27.1	5.24	19.3
10	60 (0)	30 (–2)	23.6	4.31	18.3

[a] Nonrandomized. [b] Mean values of triplicate determinations. [c] Numbers in parentheses are coded symbols for levels of independent parameters as per the text.

Experimental Design. RSM was applied to monitor the extraction characteristics of ginseng components in MAP and to determine the optimum conditions. A central composite design (*15*, *16*) was used to investigate effects of two independent variables (extraction conditions), ethanol concentration (X_1, varying between 30 and 90%), and extraction time (X_2, varying between 30 and 270 s) on dependent variables (Y_n) of the extracts. The independent variables at constant power (105 W) were coded at five levels (−2, −1, 0, 1, and 2), and their values were selected on the basis of preliminary experiments for extract yield (Y_1), saponin extracted (Y_2), and saponin yield (Y_3) (**Table 1**). The complete design consisted of 10 experimental points including two replications of the center points.

Extraction. Preliminary MAP work was performed at full power (300 W) for 30 s on the following amounts of ginseng powder: 2.5, 5.0, and 10.0 g and 50 mL of 60% ethanol. This showed that the optimal sample-to-solvent ratio was 5 g/50 mL (*17*). On the basis of these pre-established conditions, powdered samples of 5.00 ± 0.05 g were used for the MAP extractions with 50 mL of ethanol solutions at different concentrations. Irradiation was done at 105 W for various time periods. The extracts were then filtered under vacuum and collected in a volumetric flask. All glassware was washed with ~15 mL of proper solvent, and the washings were collected and combined with the extracts. The combined extracts were diluted to 250 mL and used in the determination of total extract yield, crude saponin, and saponin components. A gas thermometer (Megal) was used to follow the temperature curve of the extraction process.

The MAP extraction was validated against a conventional extraction method as follows. A conventional reflux (*18*, *19*) was carried out at 75 ± 1 °C for 3 h on a mixture consisting of 5 g of sample powder and 50 mL of 80% methanol. The extraction residue was taken back and re-extracted three more times using fresh solvent each time with the same conditions as above. The extraction steps were equally adapted to MAP for saponin analysis.

Determination of Total Extract Yield. Fifty milliliters of the combined extracts obtained from 10 extraction conditions was transferred to the tared round-bottom flask and evaporated. Upon completion of the evaporation, the flask was dried at 105 °C, cooled in a desiccator, and weighed to the constant weight according to the *Korean Food Standard Code* method (*3*). The difference in weight corresponds to the soluble solid (total extract yield) of the sample.

Determination of Crude Saponin Content. On the basis of the methods of Ando et al. (*18*), Hong et al. (*20*), and the Korean Ginseng and Tobacco Research Institute (*21*), 100 mL of the extracts obtained from 10 extraction conditions were evaporated using a rotary evaporator under vacuum at 55 °C. The evaporated residue was dissolved with 50 mL of distilled water and washed twice with 50 mL of diethyl ether in a separatory funnel to remove the fat. The aqueous layer was extracted four times with 50 mL of water-saturated *n*-butanol. The butanol solution collected was washed twice with 30 mL of distilled water to remove the impurities, thereby obtaining crude saponins. The remaining butanol solution was transferred to the tared round-bottom flask for evaporation. The flask with the evaporated residue was dried at 105 °C to constant weight. The difference in weight corresponds to crude saponin content of the sample.

Analysis of Regression. Triplicate extractions were performed at all design points in randomized order. The corresponding extracts were subjected to analysis for dependent variables (responses), such as total extract yield (Y_1), crude saponin content (Y_2), and saponin ratio (Y_3). Mean values of triplicate determinations were analyzed to fit the following second-order polynomial models to all dependent Y variables. The model proposed for each response of Y is

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \qquad (1)$$

where X_1 and X_2 correspond to independent variables, namely, ethanol concentration and extraction time. The b_n values represent corresponding regression coefficients (*15*). A statistical analysis system from SAS Institute, Inc. (*22*), was used to predict models through regression analysis and analysis of variance (ANOVA). In this specific case the terms $b_{12}X_1X_2$ and $b_{11}X_1^2$ can be neglected.

Response surfaces and contour plots were developed using fitted polynomial equations. When the results showed a saddle point in response surfaces, optimal conditions were determined using analysis of ridge. Optimal independent variables (extraction conditions) for maximized responses of Y_1, Y_2, and Y_3 were pre-established by superimposing the corresponding contour plots, by which subsequent confirmatory experiments were carried out to validate their equations.

RESULTS AND DISCUSSION

Total extract yield (Y_1), saponin content (Y_2), and saponin ratio (Y_3, Y_1/Y_2) for each set of variable combinations were obtained (**Table 1**). Multiple regression coefficients were calculated by employing a least squares technique to predict quadratic polynomial models for Y_1, Y_2, and Y_3. The regression analysis for the three responses indicated that the results were highly significant ($p < 0.01$) under the models, respectively, which suggested that they adequately explained the responses observed. The positive coefficients for X_1 and X_2 indicated linear effects to increase Y_1 and Y_2. The results suggest that the linear and quadratic effects of ethanol concentration were the primary determining factors of the responses, but no significant interaction existed between both independent variables.

Total Extract Yield. The analysis variance of total extract yield (Y_1) showed that the regression model had low dispersion ($R^2 = 0.9841$), and the linear and quadratic effects of extraction time were significant ($p < 0.01$) (**Table 1** and **Figure 1**). Using the coefficients determined, the predicted model for Y_1 was

$$Y_1 = -2.29 + 0.958X_1 + 0.0394X_2 - 0.0000864X_2^2$$

The model indicated that the ethanol concentration had the most linear effect on total extract yield as it showed the largest positive linear coefficient. The response surface plot developed for total extract yield (**Figure 1**) showed that the maximum extract yield was predicted as 27.3% (db) at the extraction conditions of 52.6% and 224 s. The ethanol concentration was the most important factor, whereas extraction time had no significant effect on total extract yield. These results reconfirmed the previous findings that the ethanol concentration plays a critical role in the extraction of soluble solids from different natural products (*19*, *23*).

Crude Saponin Content. To examine conditions that might affect the MAP extraction of crude saponin content (Y_2), its regression model was predicted as follows:

$$Y_2 = -0.00980 + 0.114X_1 + 0.00664X_2 - 0.0000109X_2^2$$

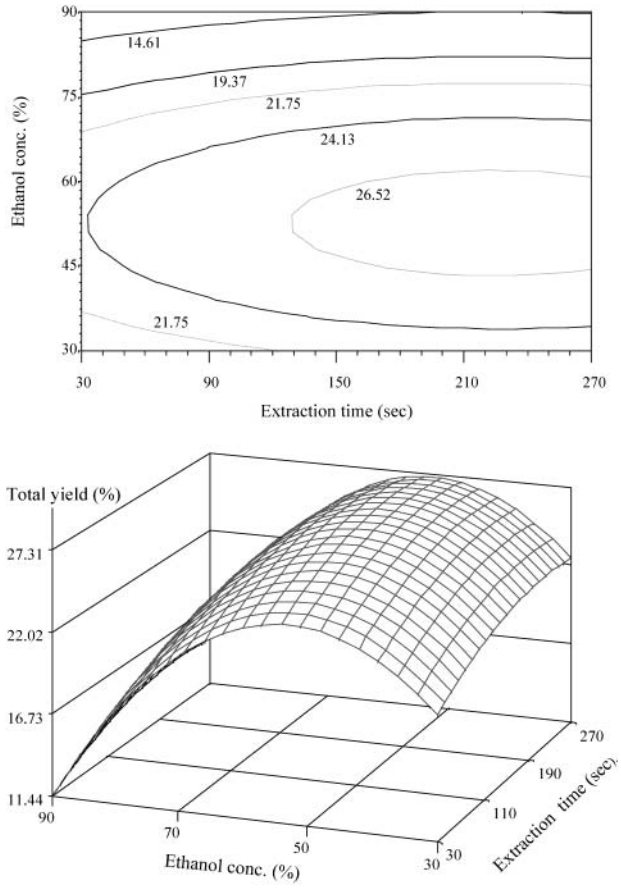

Figure 1. Contour map (top) and response surface (bottom) for the effects of extraction conditions on total yield of ethanol extracts from ginseng powder by microwave-assisted process (MAP).

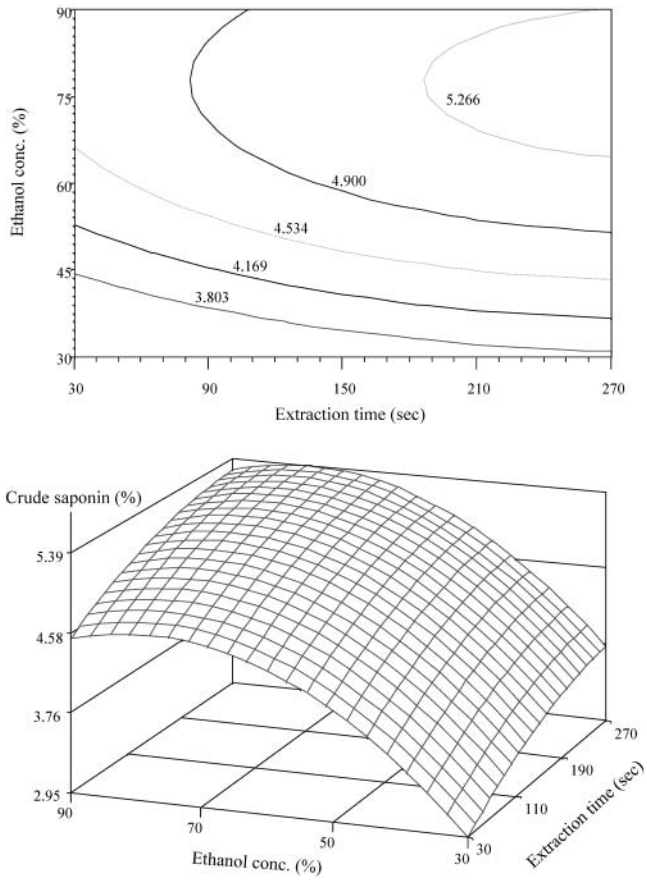

Figure 2. Contour map (top) and response surface (bottom) for the effects of extraction conditions on crude saponin of ethanol extracts from ginseng powder by microwave-assisted process (MAP).

The regression equation was highly significant ($p < 0.01$) with a satisfactory coefficient of determination, $R^2 = 0.9704$. This model had characteristics similar to those of Y_1, showing that the ethanol concentration had significant linear and quadratic effects on percent crude saponin content, with no interaction effect. The relationship between independent and dependent variables is illustrated in three-dimensional representation of the response surface (**Figure 2**) generated for the model developed for the crude saponin contents. The response surface indicated that the saponin contents of the extracts showed noticeable increases depending upon the ethanol concentration, whereas no significant effect was observed in the extraction time within the periods studied. The maximum saponin content was predicted as 5.40% (db) at the extraction conditions of 77.3% and 295 s. In a study of the effect of ethanol concentration on the extract yield and saponin content of red ginseng, Ko et al. (*19*) reported that contrary to the extract yield, saponin yield linearly increased with the increase of ethanol concentration, showing the highest content at 80%, which is well in agreement with our findings.

Percent Saponin Ratio. To monitor the saponin extractability within the range of designed independent variables of MAP and predict its conditions for the maximized and/or minimized percent saponin ratio (Y_3, % Y_2/Y_1), regression coefficients were obtained for the polynomial model of Y_3. On the basis of the coefficients determined, the predicted model was as follows:

$$Y_3 = 27.5 - 0.597X_1 + 0.000442X_2 + 0.0000244X_2^2$$

The regression model was highly significant ($p < 0.01$) with a satisfactory coefficient of determination, $R^2 = 0.9732$. This model indicated that the ethanol concentration had a significant quadratic effect on the percent saponin ratio. On the basis of coded data, canonical analysis of response surface for the percent saponin ratio showed the stationary point as a minimum, showing the predicted value, 15.5%, at the critical conditions of 40.1% (EtOH) and 57 s (extraction time) (**Figure 3**). However, the estimated ridge of maximum response for Y_3 showed 34.0% at 90.0% and 150 s. The response was affected by the ethanol concentration but not by the extraction time. These results could be attributed to the relative extraction selectivity of water- and ethanol-soluble components in ginseng matrices as previously reported for conventional reflux methods (*8*, *19*, *24*).

Optimization. The responses considered to be most important with respect to the quality of ginseng extracts were total extract yield (Y_1) and crude saponin content (Y_2). According to the canonical analysis described by Myers (*16*), the stationary points were located for the corresponding responses. The search criteria were to find the extraction conditions that would give the greatest increase in crude saponin content while maintaining a large total extract yield. The maximum extraction conditions predicted for each corresponding response variable of the ethanol extract were 52.6% and 224 s for total extract yield and 77.3% and 295 s for crude saponin content. Verification experiments, carried out at the predicted conditions, showed values reasonably close to those predicted and further confirmed the adequacy of predicted models.

Judging from the regression coefficients for independent variables and dependent responses, the most important factor influencing the extraction yields of total solids and crude saponins in MAP was the ethanol concentration. However, the

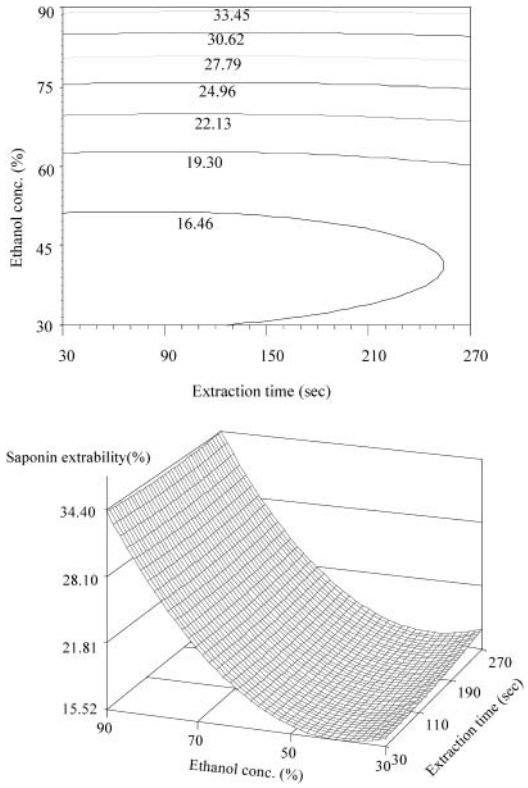

Figure 3. Contour map (top) and response surface (bottom) for the effects of extraction conditions on saponin extractability of ethanol extracts from ginseng powder by microwave-assisted process (MAP).

extraction time in MAP was very short and thus highly competitive with other conventional extraction methods (*8*, *18*, *24*, *25*). As well, our results support the ones of Carro et al. (*26*) showing the crucial dependence of solvent concentration. The three-dimensional response surfaces generated by RSM were effectively applied not only for monitoring and interpreting the combined effects of solvent concentration and extraction time but also for optimizing the extraction conditions.

To further increase the extraction yields of total extracts and saponin components in this design, other variables such as microwave power (watts) and time of extraction may be studied. Central composite design with RSM was found to be very effective for reducing the number of experimental trials without compromising the validity of the results.

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