Extraction Conditions for Phenolic Compounds with Antioxidant Activities from White Rose Petals

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Received: 21 December 2014 / Accepted: February 5 2015 / Published Online: 30 June 2015
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Abstract The extract of white rose petals has an antioxidant effect and can be used to treat allergic disease. The purpose of this study was to identify optimal conditions for extracting antioxidative compounds from white rose petals with 2,2-diphenyl-1-picrylhydrazyl scavenging activities. A response surface methodology based on a central composite design was used to investigate the effects of three independent variables: ethanol concentration (X_1), extraction temperature (X_2), and extraction time (X_3). The estimated optimal conditions for obtaining phenolic compounds with antioxidant activities were as follows: ethanol concentration of 42% (X_1), extraction temperature of 75°C (X_2), and extraction time of 80 min (X_3). The estimated optimal conditions for obtaining flavonoid compounds with antioxidant effects were an ethanol concentration of 41% (X_1), extraction time of 119 min (X_3), and an extraction temperature of 75°C (X_2). Under these conditions, predicted response values for the phenolic and flavonoid contents were 243.5 mg gallic acid equivalent/g dry mass and 19.93 mg catechin equivalent (CE)/g dry mass, respectively.

Keywords anti-oxidative compounds · response surface methodology · total flavanoid contents · total phenolic contents · white rose petals

Introduction Phenolic compounds are naturally occurring bioactive phytochemicals found in plants (Cowan, 1999; Kris-Etherton et al., 2002). The chemical structures of these reagents consist of a hydroxyl group bonded directly to one or more aromatic benzene rings (Cai et al., 2006). Many types of phenolic compounds including flavonoids, anthocyanins, and carotenoids are found in a wide range of foods such as herbs, vegetables, and legumes (Rauha et al., 2000; Zheng and Shiow, 2001; López-Amorós et al., 2006; Huang et al., 2007). Phenolic compounds possess antioxidant properties that help protect against free radical damage by donating a hydrogen atom, and reduce the risk of various diseases associated with oxidative stress (Rice-Evans et al., 1996; Kähkönen et al., 1999). Therefore, consumption of natural antioxidants containing phenolic compounds may help protect the human body against various disorders such as cancer, diabetes, and cardiovascular disease (Rao et al., 1995; Stephens et al., 1996; Padayatty et al., 2003; Bajpai et al., 2005; Al-Mustafa and Al-Thunibat, 2008). Roses are ornamental plants grown in gardens or parks. Many species of roses have long been used in herbal and folk medicines to alleviate menstruation problems, treat blood circulation disorders, and control cancer growth (Kwon et al., 2006; Shafei et al., 2010;
Rezaie-Tavirani et al., 2013) A recent study demonstrated that flowers of *Rosa spp.* contain a high level of phenolic compounds with a significant antioxidant capacity (VanderJagt et al., 2002). Many investigations have also revealed that roses contain a wide diversity of phenolic compounds such as gallic acid, kaempferol, rutin, myricetin, and quercetin that not only possess antioxidant activities but also exert anti-inflammatory, anti-oxidative, antibacterial, antiviral, antifungal, antidepressant, and anti-stress effects (Jeon et al., 2009; Ulusoy et al., 2009; Talib and Mahasneh, 2010; Boskabady et al., 2011).

In the food industry, organic cultured roses are known as edible flowers and phenolic compounds extracted from this plant have been used to make tea and functional beverages that exert beneficial effects on human health (Vinokur et al., 2006). In addition, the essential oil extracted from rose flowers are ingredients for perfumes and cosmetics. Essential rose oil is rich in polyunsaturated fatty acids derived from α-linolenic acid, linoleic acid, ascorbic acid, and α-tocopherol that have a high antioxidant capacity and can regulate numerous bodily functions.

Due to the great interest in the use of natural and functional ingredients derived from rose flowers, it is important to identify optimum conditions for extracting phenolic compounds from roses with high antioxidant capacities more effectively and economically. Until now, solvent extraction has been a widely utilized technique designed to recover soluble phenolic compounds from roses using solvents such as water, ethanol, methanol, or acetone. Baydar and Baydar (2013) reported that the organic solvent extracts from white or red rose flowers may contain high concentrations of antioxidants. In fact, extraction with 85% ethanol produced a significantly higher yield and the antimicrobial effect of the resulting extract was more potent than that of other ethanol extracts. However, research regarding white roses is lacking and the extraction of phenolic compounds from white rose petals using a response surface methodology (RSM) has not been previously reported. RSM is a mathematical and statistical technique for exploring the interactions between several variables and one or more response variables. The objective of the present study was to identify and optimize highly efficient processes for extracting phenolic compounds from white rose petals using an RSM. A central composite design (CCD) for three variables including ethanol concentration, reaction temperature, and reaction time was employed.

**Materials and Methods**

**Plant material and chemicals.** White rose (*Rosa spp.*) petals harvested in May 2013 were purchased from Roserangs farm (Korea), then dried and milled using a cutter mill (Tefal Ltd., China). The milled petals were passed through a sieve (Chung Gye Sang Gong S.A., Korea) and a fraction that was sieved was then passed through a 14-mesh (1.41 mm) sieve and retained on a 20-mesh (0.84 mm) sieve before being collected. Ethanol was purchased from Merck (Whitehouse Station, USA). Folin-Ciocalteu phenol reagent (2 N), 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium nitrite, aluminum chloride, catechin hydrate, and gallic acid monohydrate reagent were purchased from Sigma-Aldrich Chemical Co. (USA). All chemicals were of analytical grade.

**Extraction procedure.** Milled dry petals (10 g) were dispersed in 250 mL of solvents with various ethanol-to-water ratios (1:99-99:1) in 1 L glass bottles (Schott UK Ltd., UK) to prevent solvent loss during extraction of the anti-oxidative compounds. The bottles were placed in a shaking water bath (Jeio Tech. Co., Ltd., Korea) set at a speed of 120 r/min and the desired temperature (25–75°C). After extraction, the petals were filtered through Whatman filter paper no. 4 (Whatman Inc., USA) using a vacuum pump (BOC International Ltd., London, UK). The extracts were vacuum evaporated on a rotary evaporator (BÜCHI, Switzerland) with water bath at 40°C until the ethanol was removed and then the extracts were freeze-dried at −60°C for 24 h using a freeze-dryer (Ishinbiobase Co. Ltd., Korea). The dried extracts were used as experimental samples to measure phenolic extraction yield, phenolic compound contents, and antioxidant activity.

**Measurement of total phenolic contents (TPC) and total flavonoid contents (TFC).** The TPC of the rose petal extracts were determined according to a colorimetric Folin-Ciocalteu method (Fernandes de Oliveira et al., 2012). For this, 100 µL of the diluted extract (1:20) in distilled water were mixed with 50 µL of 2 N Folin-Ciocalteu reagent and incubated for 5 min at room temperature in the dark. Next, 300 µL of a 20% sodium carbonate solution was added to the reaction and the solution was incubated for 15 min at room temperature. After centrifugation at 1,250 rpm for 5 min, absorbance of the supernatants was read at 725 nm using a UV-Vis Max 384 spectrophotometer (Molecular Devices, USA). The results are expressed as mg gallic acid equivalents (GAE)/g (dry weight) of sample. The TFC of the rose petal extracts were determined using a modified aluminum chloride method based on the formation of a flavonoid-aluminum complex (Zhishen Jia et al., 1999). A 5-mL aliquot of the extracts in 0.5 mL deionized water was mixed with 0.3 mL of 5% sodium nitrite. After 5 min, 0.3 mL of 10 % aluminum chloride was added. After 6 min, 2 mL of 1 M sodium hydroxide was added and the total volume was brought up to 10 mL with deionized water. Absorbance was read at 510 nm. The results are expressed as mg of catechin equivalents (CE)/g (dry weight) of sample.

**Determination of total antioxidant activity (TAA).** Antioxidant activity of the extract was evaluated with a DPPH assay as previously described (Brand-Williams et al., 1995). Briefly, 3 mL of 81 µM DPPH solution was mixed with 200 µL of the rose petal extract solution and allowed to incubate in the dark at room temperature for 1 h. The absorbance was then measured at 517 nm using a UV-Vis Max 384 spectrophotometer. Ascorbic acid was used as the positive control and all measurements were performed in triplicate. The DPPH radical scavenging capability was calculated by comparing the absorbance values of the control and samples using the following formula: total antioxidant activity (%)=[(1− absorbance of sample/absorbance of control)×100.

**Statistical analysis.** All experiments were performed in triplicate.
Data are expressed as the mean ± standard deviation (SD). Differences were analyzed with a one-way analysis of variance (ANOVA), Tukey’s multiple comparison test, and Dunnett’s multiple comparison test using GraphPad Prism version 5.03 (USA). P-values less than 5% (p < 0.05) were considered statistically significant. The CCD was established using MINITAB Release 14 software (Minitab Inc., State College, USA).

**CCD.** A CCD consisting of 20 experimental runs was used to characterize the relationships between the dependent variables (extraction yield, TPC, TFC, and TAA) with three independent variables (ethanol concentration, extraction temperature, and extraction time) at five levels (-1.68, -1, 0, +1, and +1.68). The coded levels and center point values of the three independent variables are presented in Table 1. The center point values were determined based on preliminary experimental data (Figs. 1, 2, and 3). All experiments were conducted in triplicate. To evaluate suitability of the extraction conditions, a quadratic regression model was used. The equation for the model was as follows:

\[ Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j \]

where Y is the predicted response, \( \beta_0 \) is a constant, \( \beta_i \) is the linear coefficient, \( \beta_{ij} \) is the quadratic coefficient, \( \beta_{ij} \) is the interaction coefficient, and \( X_i \) and \( X_j \) are independent variables. Adequacy of the model was determined by the coefficient of determination \( R^2 \) while significance of the model was determined based on the F-test and p-values.

**Results and Discussion**

**Determination of the experimental ranges.** The appropriate experimental ranges of ethanol concentration and extraction time were examined. Differences in polarity according to the ethanol concentration may affect phenolic compound recovery and profiles. Liquid-to-solid ratio and particle size of rose petal were also important factors. Polyphenol extraction from white rose petals and anti-oxidative properties of the recovered compounds have been rarely studied, so we performed experiments to determine the appropriate experimental conditions and ranges for the extraction procedure. Extraction time for the phenolic contents varied the most among the independent variables. Time had a significant effect (p < 0.05) on phenolic content extraction. The phenolic contents after 5, 10, 20, 60, 120, and 180 min of extraction were 88.33, 106.91, 201.76, 222.77, 208.00 and 194.78 mg GAE/g dry mass, respectively. When the extraction time was 60 min, the phenolic contents had a maximum of 222.77 mg GAE/g dry mass.

**Table 1** The coded levels of 3 independent variables in the central composite design for optimization of extraction conditions

| Factor   | Independent variables     | Coded levels           |
|----------|---------------------------|------------------------|
| X1       | Ethanol Concentration (%) | -1.68  -1  0  1  1.68  |
| X2       | Extraction temperature (°C) | 25  35  50  65  75   |
| X3       | Extraction time (min)     | 1  25  60  95  119    |

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**Fig. 1** Effect of time on the extraction of phenolic contents from white rose petals with 50% ethanol solution at 50°C, at the liquid-to-solid ratio of 25:1. Different letters indicate significant differences between groups (p < 0.05).

**Fig. 2** Effect of the liquid-to-solid ratio on the extraction of phenolic contents from white rose petals with 50% ethanol solution at 50°C for 2 h. Different letters indicate significant differences between groups (p < 0.05).

**Fig. 3** Effect of ethanol concentration on the extraction of phenolic contents from white rose petals at fixed extraction conditions (50°C, 2 h, liquid-to-solid ratio of 25:1). *p < 0.05 in comparison to the 0% ethanol solution, ***p < 0.001 in comparison to the 0% ethanol solution.
Kinetics of the extraction procedure could be divided into two phases: a first phase from 5 to 60 min, and second phase from 60 to 180 min (Fig. 1). During the first phase, the phenolic contents were quickly extracted. During the second phase, the extracted phenolic contents were slowly decreased. Prolonged extraction time could lead to degradation of some flavonoid compounds due to exposure to factors such as light, polyphenol oxidase and heating (Juntachote et al., 2006; Chirinos et al., 2007; Suganya Devi et al., 2012). Results of Tukey’s multiple comparison test showed that significant differences existed among all experimental groups (p < 0.05). Therefore, a ratio of 25:1 was needed to collect the most phenolic compounds existed among all experimental groups (p <0.05). Results of Tukey’s multiple comparison test showed that significant differences existed at concentrations of 30 and 50 % compared to the control (p <0.05), but no significant difference was observed with concentrations of 10 or 80%. Therefore, an ethanol concentration of 50% was found to be the most suitable for extracting phenolic compounds.

Statistical analysis and model fitting. The dependent variables of 20 runs using the CCD are presented in Table 2. A response surface analysis of dependent variables was performed to assess the quality of fit to the quadratic models. Results showing the quadratic model fit based on the coefficient of determination R² are presented in Table 3. Statistical significance of the models was determined by the F and p-values. Results of the ANOVA for the independent variables are presented in Table 4. The lack-of-fit (p >0.05) indicated the suitability between models and experimental data.

Extraction yields. Unpublished data showed that the antioxidant activity of the phenolic compounds extracted using ethanol was the highest. Therefore, extraction performed in the current study was carried out using ethanol. Extraction yield expressed as the percentage of weight of the crude extract to that of the dried white rose petals (10 g) is presented in Table 2. The maximum yield (40.9%) was obtained with X₁ =50%, X₂=75°C, and X₃=60 min.

Table 2 Central composite design of the independent variables (X₁, X₂ and X₃) and experimental results for the response variables, extraction yield (Y₁), total phenolic contents (Y₂), total flavonoid contents (Y₃) and total antioxidant activities (Y₄)

| Run Order | X₁ EtOH (%) | X₂ temp (°C) | X₃ time (min) | Y₁ (%) | Y₂ (mg GAE/g dry mass) | Y₃ (mg CE/g dry mass) | Y₄ (%) |
|-----------|-------------|--------------|---------------|--------|------------------------|----------------------|--------|
| 1         | 99          | 50           | 60            | 18.64  | 120.68±5.54            | 11.31±0.14           | 40.00±5.41       |
| 2         | 50          | 75           | 60            | 40.96  | 240.12±1.80            | 16.87±0.55           | 83.78±6.08       |
| 3         | 79          | 65           | 95            | 34.83  | 207.53±3.84            | 16.00±0.23           | 66.49±5.32       |
| 4         | 50          | 50           | 60            | 36.29  | 210.54±4.95            | 16.14±0.41           | 75.14±0.00       |
| 5         | 50          | 50           | 119           | 39.00  | 194.62±2.01            | 17.34±0.15           | 74.59±6.22       |
| 6         | 79          | 35           | 25            | 20.74  | 179.70±4.93            | 14.41±0.29           | 51.35±2.48       |
| 7         | 50          | 50           | 60            | 36.03  | 210.00±2.61            | 15.91±0.14           | 74.59±1.43       |
| 8         | 50          | 25           | 60            | 31.53  | 191.25±18.38           | 13.92±0.41           | 65.95±3.29       |
| 9         | 21          | 35           | 95            | 33.96  | 201.28±13.52           | 15.48±0.03           | 68.65±1.43       |
| 10        | 50          | 50           | 60            | 36.63  | 215.12±9.28            | 16.35±0.32           | 78.92±0.54       |
| 11        | 21          | 65           | 95            | 33.86  | 213.71±4.95            | 18.07±0.41           | 71.89±4.42       |
| 12        | 50          | 50           | 60            | 33.23  | 224.93±1.49            | 16.93±0.23           | 67.57±3.90       |
| 13        | 50          | 50           | 1              | 15.21  | 183.96±6.51            | 12.87±0.12           | 50.81±1.62       |
| 14        | 79          | 35           | 95            | 28.26  | 197.31±4.19            | 13.94±0.11           | 48.65±0.54       |
| 15        | 50          | 50           | 60            | 34.76  | 212.52±1.11            | 16.52±0.09           | 70.27±0.00       |
| 16        | 50          | 50           | 60            | 37.98  | 214.52±2.22            | 16.07±0.15           | 74.89±2.81       |
| 17        | 21          | 65           | 25            | 38.68  | 206.99±0.88            | 16.84±0.15           | 65.41±2.16       |
| 18        | 79          | 65           | 25            | 33.64  | 182.81±3.20            | 15.69±1.03           | 59.46±1.87       |
The minimum yield (15.2%) was obtained with $X_1 = 50\%$, $X_2 = 50\degree C$, and $X_3 = 1\text{ min}$. The model for extraction yield had an adequate coefficient of determination $R^2 (0.813)$. Results of the ANOVA for the model were significant ($p$-value = 0.011). The linear and quadratic coefficients ($p < 0.05$) were significant while the cross product not significant. The influence of $X_3$ (extraction...
time) on $Y_1$ was most important ($p < 0.01$, coefficient of regression $= 4.243$). The response surface plots for optimizing the extraction conditions are presented in Fig. 4. Extraction yield increased with an increase in extraction temperature and reached the maximum at a ethanol concentration of 43%, extraction time of 60 min. Kim and Hong. (2012) previously reported that ethanol extraction yields for *Lespedeza cuneata* were affected by extraction time and ethanol concentration. The response surface plots were similar to the results from our study.

**TPC.** As shown in Table 2, the maximum content (240.12 mg GAE/g dry mass) was obtained with $X_1 = 50\%$, $X_2 = 75$, and $X_3 = 60$ min. The minimum content (120.68 mg GAE/g dry mass) was obtained with $X_1 = 99\%$, $X_2 = 50^\circ C$, and $X_3 = 60$ min. The model had an adequate coefficient of determination $R^2$ (0.866) and the result of the ANOVA for the model was significant ($p$-value = 0.002). The quadratic coefficient ($p < 0.05$) was significant while the linear and cross product were not. The influence of $X_1$ (ethanol concentration) on $Y_2$ was the most important ($p < 0.01$, coefficient of regression $= -21.083$). Therefore, the TPC were more influenced by ethanol concentration than the other variables. The response surface plots for optimizing the extraction conditions are presented in Fig. 4. Min et al. (2010) previously reported on the TPC and TFC from *Zizyphus jujuba Mill.* Leaves were the most affected by ethanol concentration. These results were similar to ones from our study.

**TFC.** TFC ranged from 11.315 mg CE/g dry mass to 18.086 mg CE/g dry mass. The experimental conditions to recover the maximum contents were $X_1 = 21\%$, $X_2 = 65^\circ C$, and $X_3 = 95$ min. The model had an adequate coefficient of determination $R^2$ (0.740) and the result of the ANOVA for the model was significant ($p$-value =

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**Fig. 4** Response surface plots for extraction yield, TPC, TFC, and antioxidant activities (DPPH radical scavenging). (A) Interaction of ethanol concentration and extraction temperature; (B) Interaction of ethanol concentration and extraction time; (C) Interaction of extraction temperature and extraction time.
The linear and quadratic coefficients (p < 0.05) were significant but the cross product was not. The influence of Xu (ethanol concentration) on Y3 was the most important (p < 0.01, coefficient of regression = 1.036). The response surface plots for optimizing the extraction conditions are presented in Fig. 4. TAA. According to the DPPH assay, experimental conditions that produced extracts with a maximum antioxidant activity were X1 = 50%, X2 = 75°C, and X3 = 60 min. Conditions that resulted in extracts with the minimum antioxidant activity were X1 = 99%, X2 = 50°C, and X3 = 60 min. The model had an adequate coefficient of determination R2 (0.933) and results of the ANOVA for the model were significant (p < 0.001). The linear and quadratic coefficients (p < 0.01) were significant while the cross product and lack of fit were not. The influence of all independent variables on Y3 was important (p < 0.01). Our results indicated that the total antioxidant activity was more influenced by ethanol concentration than the other variables. The response surface plots for optimizing the extraction conditions are presented in Fig. 4. Koh et al. (2008) previously reported that antioxidant activity of extracts from dandelion leaves is affected by extraction time and extraction temperature. The response surface plots were similar to results from our.

**Optimization and verification.** The optimal conditions were identified based on combinations of two or more variables. Table 5 shows predicted values of response variables in optimal conditions. The estimated optimal conditions for recovering phenolic contents with antioxidant activities were an ethanol concentration of 42% (X1), extraction time of 80 min (X2), and extraction temperature of 75°C (X3). The estimated optimal conditions for collecting flavonoid contents with antioxidant properties were an ethanol concentration of 41% (X1), extraction time of 119 min (X2), and extraction temperature of 75°C (X3). The estimated optimal conditions for collecting flavonoid contents with antioxidant properties were an ethanol concentration of 41% (X1), extraction time of 119 min (X2), and extraction temperature of 75°C (X3). Under these conditions, the predicted responses values for the phenolic and flavonoid contents were 243.5 mg GAE/g dry mass and 19.93 mg CE/g dry mass, respectively. All models for the responses values of extraction yield had an adequate coefficient of determination R2 and great significance (p < 0.05). This results of analysis verified that the response model was adequate for reflecting the expected optimization. Therefore, the models can be successfully employed to optimize the conditions for extracting anti-oxidative compounds from white rose petals.

### Acknowledgment
This research was supported by High Value-added Food Technology Development Program, Ministry of Agriculture, Food and Rural Affairs (MAFRA; grant number 113034-3).

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### Table 5 Predicted values and experimental values of response variables in optimal conditions of white rose petals extract

| Responses | Independent variables | Optimal conditions | Predicted values | Experimental values |
|-----------|-----------------------|--------------------|-----------------|---------------------|
| Y1 (%)    | EtOH conc (%)         | 42.02              | 42.92           | 40.17               |
|           | Extraction temperature (°C) | 75.00         | 243.54          | 246.30              |
|           | Extraction time (min)  | 60.72              |                 |                     |
| Y2 (mg GAE/g dry mass) | EtOH conc (%)         | 42.02              | 19.93           | 23.69               |
|           | Extraction temperature (°C) | 75.00         |                 |                     |
|           | Extraction time (min)  | 80.89              |                 |                     |
| Y3 (mg CE/g dry mass) | EtOH conc (%)         | 41.03              | 19.30           | 23.69               |
|           | Extraction temperature (°C) | 75.00         |                 |                     |
|           | Extraction time (min)  | 119.00             |                 |                     |
| Y4 (%)    | EtOH conc (%)         | 41.03              | 85.74           | 82.37               |
|           | Extraction temperature (°C) | 75.00         |                 |                     |
|           | Extraction time (min)  | 89.41              |                 |                     |
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