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Effect of Heat Treatment, Ethanol Content, Extraction Time and Ratio of Solvent on the Efficiency of Polyphenol Extraction from Licorice Root (*Glycyrrhizauralensis*)

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감초폴리페놀 추출효율에 있어 열처리, 에탄올 농도, 추출시간 및 용매비율이 미치는 영향 탐색

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Effects of pretreatment and extraction conditions on total polyphenol yield from licorice root were investigated using statistical method. For pretreatment, heat treatment at 121°C for 10 min was applied. Licorice root content in solvent (10, 20, and 30%) ethanol concentration (20, 40, and 60%) and reaction time (1, 2, and 3 h) were used as variables for extraction conditions. Two experiments, with heat treated and no treated licorice, were prepared with same experimental design. Box behnken design was employed and produced a total of 15 trials. Total polyphenol yield from licorice root was not affected by heat treatment. Among variables, licorice content in solvent showed most significant effect regardless of other variables ($p<0.05$). Finally, optimum conditions for the extraction of total polyphenol from licorice root was detected as following: 10% of licorice in solvent, 52%

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ethanol as solvent, 2 h of reaction time and non-heat treatment and the extraction yield from optimized condition was 17.6 mg/g licorice root.

Key words : *licorice root, total polyphenol, aqueous ethanol, box behnken design, optimization*

Ⅰ. Introduction

Food contamination cause by use of antibiotics has been issued in the concern of public health and the restriction of the use of these antibiotics has been enforced particularly in animal farming. Therefore, the development of natural products that can substitute used antibiotics is requested for organic farming. Plant extract is regarded as a promising antibiotics replacer because its various health benefit features such as antioxidant, antibacterial and anti-inflammatory activities (Chang et al., 2010).

Licorice (*Glycyrrhizauralensis* Fisch) is a perennial leguminous plant and it contains 6~14% of glycyrrhizin, a compound of sweet taste and flavor (Lee et al., 2006). Licorice is used as flavoring and sweeting agents for tobaccos, chewing gum, candies, toothpaste and beverages (Wang et al., 2001). Generally root of licorice has been used and it does not contain poisonous compounds so it has been widely utilized as a primary ingredient for oriental medicine (Kang et al., 2001). Health beneficial features of licorice root such as antibacterial activity, antioxidant activity andanti-inflammatory effect giverise to its broad application in various industrial fields (Lee et al., 2006; Lim et al., 2010). Particularly, polyphenols in licorice root have resulted in greater interest on its industrial application (Kim et al., 2006).

Phenolic compounds found in plant and vegetables have been showing health benefit through their protective effects such as antioxidant and antibacterial activity (Rice-Evans et al., 1997). Simple phenol, phenolic acid, phenyl propanoid and phenolic quinone are included in phenolic compounds in plant and vegetables (Kim et al., 2001). Generally these phenolic compounds are water soluble, hence, organic solvents such as methanol, ethanol, ethyl acetate, hexane and acetone have also been applied in their extraction (Kim et al., 2006; Mussatto et al., 2011). Particularly, in the study of Kim et al. (2006) the antioxidant activity derived by the extracted polyphenol from licorice using ethanol as a solvent was higher compared to methanol and water extracts. Different extraction techniques such as organic solvents, extraction condition, ratio of solvent to solute are reported to be crucial in the improvement of polyphenol extraction efficiency and the economics of the extraction process (Chirinos et al., 2007).

To optimize a process, various factors that can influence the performance of process should be considered. Traditionally, a conventional method that investigates one factor at a time, while the others are fixed in constant has been applied. However, this traditional technique has a drawback, missing the interactions among investigated factors and requiring a lot of experiments and is time consuming (Cohran et al., 2002). Fractional factorial design and response surface methodology have been developed to achieve the effective optimization of various factors with less number of experimental trials and more accuracy (Box et al., 1960; Cho et al., 2010; Soni et al., 2007).

The present study was conducted to investigate the effect of heat treatment, licorice root content in solvent, ethanol concentrations and extraction time on polyphenol extraction from licorice using response surface methodology.

Ⅱ. Materials and methods

1. Chemicals

General chemicals were purchased from Sigma-Aldrich (St. Louis, Mo, USA), unless otherwise stated.

2. Preparation of licorice root

Licorice root produced at Uzbekistan was purchased from Home Nature Co. (Gyeonggi, Gimpo, Korea) and it was finely ground using cutter miller (Philips HR2860, Netherlands) and used for experiment. Autoclaving procedure (121℃, 15 min) was employed for heat treatment of licorice root powder.

3. Variables for extraction condition and experimental design

According to heat treatment, two experiments were prepared using same experimental design. As variables for extraction conditions, licorice root content in solvents, ethanol concentration and reaction time were used and their three levels were assigned to 15 trials according to Box Behnken Design (Box et al., 1960). The configuration of variables and their used levels were summarized in Table 1. Coded and uncoded values in Table 1 mean experimental point and real value of variables, respectively. All experiment was performed in triplicates.

Table 1. Box Behnken design, observed and predicted total polyphenol extraction yield from each trials

Trials	Variables						Responses, total polyphenol yield (mg/g licorice root)			
	Licorice root powder		Ethanol,%		Time, h		Non heat treated		Heat treated	
	Coded	Uncoded, %	Coded	Uncoded, %	Coded	Uncoded, %	Observed	Predicted	Observed	Predicted
1	-1	10	-1	20	0	2	16.29	16.42	17.57	17.17
2	1	30	-1	20	0	2	7.50	7.85	9.08	9.19
3	-1	10	1	60	0	2	17.76	17.41	17.90	17.80
4	1	30	1	60	0	2	8.89	8.76	9.11	9.51
5	-1	10	0	40	-1	1	17.65	17.77	16.10	16.38
6	1	30	0	40	-1	1	8.51	8.42	8.69	8.46
7	-1	10	0	40	1	3	16.98	17.07	17.17	17.40
8	1	30	0	40	1	3	9.33	9.20	9.33	9.05
9	0	20	-1	20	-1	1	10.87	10.61	10.59	10.71
10	0	20	1	60	-1	1	11.51	11.73	11.73	11.56
11	0	20	-1	20	1	3	11.03	10.81	11.71	11.89
12	0	20	1	60	1	3	11.34	11.60	12.12	11.99
13	0	20	0	40	0	2	11.77	12.01	12.85	12.14
14	0	20	0	40	0	2	12.26	12.01	11.86	12.14
15	0	20	0	40	0	2	11.99	12.01	11.69	12.14

4. Extraction procedures

Licorice root powder was submerged into an erlenmeyer flask containing aqueous ethanol and placed in 60℃ water bath without agitation. After the extraction, the mixture was filtered through filter papers (Whatman No 1.). Then, the filtrate was centrifuged (10,000 rpm, 5 min) and used for total polyphenol assay.

5. Determination of total polyphenol

Total polyphenol concentration in the filtrate was determined according to (Juan et al., 2010) using spectrophotometer (Option 2120 UV, Korea) and total polyphenol content was estimated as gallic acid mg equivalent. Finally, total polyphenol yield was calculated with used amount of licorice root.

6. Statistical analysis

Construction of Box Behnken design composed of 15 trials with three variables, analysis of variance, calculation of optimal condition using response surface model were performed by MINITAB® software (version 14, Minitab Inc., USA).

Ⅲ. Results

Effects of heat treatment, licorice root content in solvent, ethanol concentrations and reaction time on polyphenol extraction yield from licorice root were investigated. Total polyphenol yields (TPYs) fromeach trial in experiment design and the predicted polyphenol extraction yield were summarized in Table 1. Predicted TPYs were calculated by response surface model derived from observed TPYs. The TPYs were varied from 7 mg/g licorice to 17 mg/g licorice according to extraction conditions. The significant differences between non-heat and heat treatment were found only at trial number of 1, 2 and 5 in Table 1. However, the manner of effect of heat treatment was not constant. In trial 1 and 2, TPYs in heat treated licorice root were higher than those of non-heat treated. However, TPY in trial 5 was vice versa. So heat treatment may not be significantly beneficial for polyphenol extraction from licorice. Observed total polyphenol yields and predicted polyphenol yields were plotted in Figure 1. In two experiments, all results showed good linearity. And two clusters, one is low yields and the other is high yields were founds. Large differences in observed TPYs and occurrence of clusters indicate that selected variables are intensively influencing on the responses. Analysis of variances for two experiments were determined (Table 2). The significant effects were found in regression, linear effect and square effect at both experiments (p<0.05). However, overall interactions among variables were not significant in both experiments (p>0.05). The probabilities in lack of fit at two experiments were not significant (p>0.05) and this meansthat the regressions calculated from observed

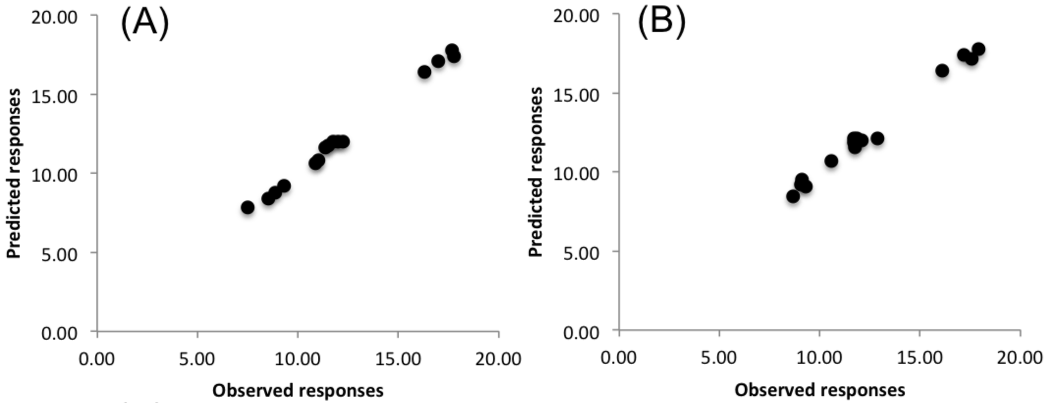

Figure 1. Observed and predicted total polyphenol yields from experimental trials. (A) and (B) are non heat treated and heat treated experiment.

Table 2. Analysis of variance1 for 15 trial of Box Behnken experiments

Sources	DF	SS	MS	F value	P value
Non treated					
Regression	9	158.94	17.66	131.52	<0.01
Linear	3	150.15	6.89	51.35	<0.01
Square	3	8.21	2.74	20.38	<0.01
Interaction	3	0.58	0.19	1.44	0.34
Residual error	5	0.67	0.13		
Lack of fit	3	0.55	0.18	2.94	0.26
Pure error	2	0.12	0.06		
Total	14	159.61			
Heat treated					
Regression	9	142.14	15.79	53.12	<0.01
Linear	3	134.05	5.64	18.98	<0.01
Square	3	7.89	2.63	8.84	0.02
Interaction	3	0.21	0.07	0.23	0.88
Residual error	5	1.49	0.3		
Lack of fit	3	0.7	0.23	0.59	0.68
Pure error	2	0.79	0.39		
Total	14	43.63			

[1] DF, degree of freedom; SS, sum of square; MS, mean of square.

Table 3. Regression coefficients for response surface regression and their probabilities

Terms	Coefficient	Standard error of coefficient	T value	P value
Non heat treated				
Constant	22.4771	1.82603	12.309	<0.001
X1	-1.0065	0.09312	-10.809	<0.001
X2	0.1671	0.04656	3.588	0.016
X3	0.0628	0.93122	0.067	0.949
X12	0.0127	0.00191	6.634	0.001
X22	-0.0017	0.00048	-3.482	0.018
X32	-0.1549	0.1907	-0.812	0.454
X1X2	-0.0001	0.00092	-0.117	0.911
X1X3	0.0371	0.01832	2.025	0.099
X2X3	-0.0041	0.00916	-0.451	0.670
Heat treated				
Constant	20.2598	2.71718	7.456	0.001
X1	-0.8833	0.13857	-6.374	0.001
X2	0.0383	0.06928	0.552	0.605
X3	3.3705	1.38568	2.432	0.059
X12	0.0128	0.00284	4.523	0.006
X22	-0.0001	0.00071	-0.005	0.996
X32	-0.5965	0.28376	-2.102	0.090
X1X2	-0.0004	0.00136	-0.281	0.790
X1X3	-0.0107	0.02726	-0.394	0.710
X2X3	-0.0092	0.01363	-0.675	0.530

X1, X2 and X3 mean variables of licorice, ethanol and solvent amount, respectively.

responses were properly fitted. Regression coefficients for response surface models of two experiments were summarized in Table 3. In non-heat treatment, licorice content in solvent (X1) and ethanol concentration (X2) showed significant effects ($p<0.05$). However, variable X1showed negative effect that means increasing licorice content in solvent could have caused reduction in total polyphenol yield. The probabilities in square effects of those two variables (X1, X2) were also significant ($p<0.05$). And other coefficients were not significant ($p>0.05$). In the experiment with heat treated licorice, the coefficients for linear and square effects of X1 showed significant (p<0.05). The linear effect of X1 showed also negative similar with non-heat treated licorice.

Contour plots with calculated response surface models were represented at Figure 2. Licorice content in solvent was crucial in total polyphenol yield regardless of reaction time (Figure 2A and 2D), ethanol contents (Figure 2B and 2E) and heat treatment. The relationships between reaction time and ethanol contents showed somewhat different depending on heat treatment. In the experiment with non-heat treated licorice, about 50% of ethanol and 2 h of reaction time showed the highest total polyphenol yield and in the experiment with heat treated licorice, about 60% of ethanol and 2 h of reaction time showed the highest yield of total polyphenol (Figure 2C and 2F). Finally, optimum condition for total polyphenol extraction from licorice using aqueous ethanol was calculated via optimization tool in MINITAB program and the result was summarized in Figure 3. As a result, 10% of licorice in solvent, 52% ethanol and 2 h of reaction time were achieved and TYP of 17.6 mg/g licorice was expected as total polyphenol yield.

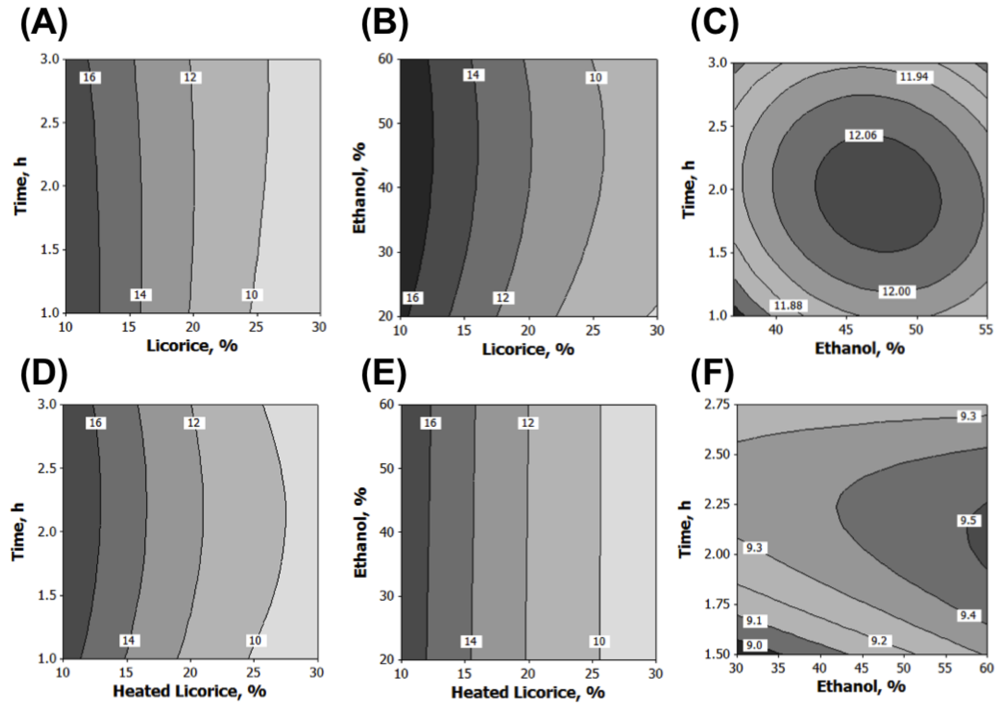

Figure 2. Contour plots for the relationships among variables. When two variables were compared, remaining variable was fixed to its center point. Plots (A), (B) and (C) are from non-heat treated experiment and plots (D), (E) and (F) are from heat treated experiment.

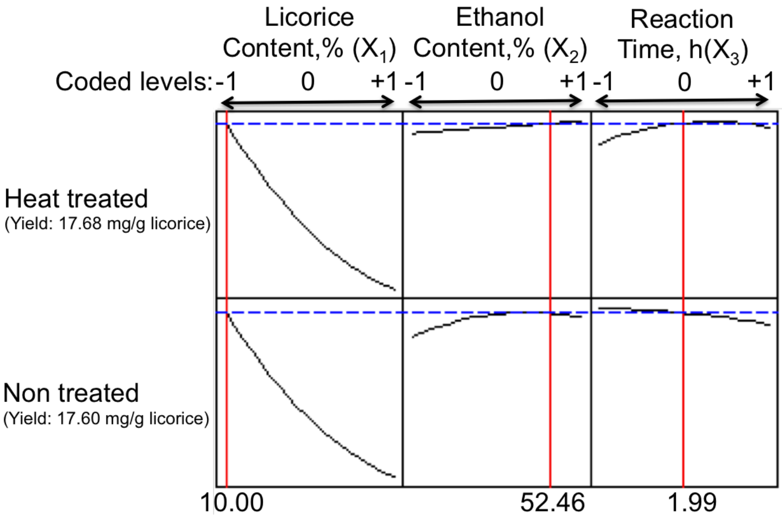

Figure 3. Optimization results.

Ⅳ. Discussion

For the extraction of phytochemicals from plant materials, different organic solvents have been used and the efficiency of extraction can be considerably influenced by used solvent relevant (Kaushik et al., 2006). It was reported that phenolic compound can beextracted by aqueous organic solvents effectively and aqueous ethanol is regarded as an economical solvent (Mussatto et al., 2011). Licorice root has been frequently used in broad range of industrial fields such as oriental medicine, food and feed industries because of its various physiological functions such as antioxidant activity, antibacterial activity and anti-inflammatory effect (Lee et al., 2006; Lim et al., 2010). Particularly, polyphenol in licorice root has been interested (Kim et al., 2006). Therefore many researches have been conducted to extract total polyphenol from licorice root, efficiently and economically. In the study of Woo et al. (2007), it was found that heat treatment of licorice at 130℃ for 3 h improved the extraction efficiency and the yield of extraction was about 8 mg/g licorice. However, in this study the improving effect of heat treatment in total polyphenol yield was not found. In the study of Kim et al. (2006), they investigated the effects of various concentration of ethanol on total polyphenol extraction from licorice root and 50% ethanol showed the highest efficiency. It was similar to the result of present study. The present study provides primary information for the development of polyphenol extraction procedure using licorice root.

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