OPTIMIZATION OF PHYTOESTROGENS EXTRACTION FROM SOY GERM USING RESPONSE SURFACE METHODOLOGY

Le Minh Chau[1*], Do Thi Hoa Vien[2], Ho Phu Ha[2]

Abstract: *Phytoestrogens are phytochemicals with antioxidant activities and potential health benefits. Their contents in soy germ is the highest compared to that in parts of soy seed. Response surface methodology (RSM) using Box Behnken Design of four factors was employed to optimize the extraction conditions for phytoestrogens. The Box Behnken Design with five replicates at central point was applied. The four independent variables investigated in this experiment were extraction time (X_1) 60-120 minutes, solvent/solid ratio (X_2) 8-12, extraction pH (X_3) 8-10, ethanol concentration (X_4) 50-70%. The high coefficient values indicated that the variables were fitted to the regression for the total phytoestrogens (R^2 = 0.9887). Optimum conditions for maximizing total crude phytoestrogen content were 90 minutes for the extraction time, 12/1 for the extraction ratio, 9 for the extraction pH, and 65% for the ethanol concentration.*

Keywords: Soy germ; Phytoestrogens; RSM.

1. INTRODUCTION

Soybean (*Glycine max (L.) Merrill* is one of the most popular crops in many Asian countries. The interests in soybeans and soy products have been significantly grown due to many health benefits. Soybean is the richest natural source of isoflavones, belonging to a class of compounds called phytoestrogens that interact with human estrogen. Soybean seed consists of three parts: coat, cotyledon and embryo. The embryo, known as germ, weights only 2% of the whole soybean seed, but contains the highest concentration of phytoestrogens compared to other parts of soy seed [1]. There are four kinds of isoflavones in soy germ, including aglycone isoflavones (genistein, daidzein, glycitein), glycoside isoflavones (genistin, daidzin, glycitin), acetyl glycoside isoflavones (acetyl genistin, acetyl daidzin, acetyl glycitin), and malonyl glycoside isoflavones (malonyl genistin, malonyl daidzin, malonyl glycitin) [2]. Currently, soy germ is not commonly used for human consumption. It is mainly used as an ingredient in animal feeds. However, the antioxidant activities and other beneficial characteristics of soy germ oil and phytoestrogens have changed this circumstance, leading intensive studies and applications of them [3].

Phytoestrogen, therefore, have been extracted and the efficiency of this process plays important role for the studies and applications. There are many factors that affect phytoestrogens extraction. High extraction temperatures will reduce the extraction time, due to faster particle diffusion rates. However, if the temperature is too high (greater than 80 ^oC), it will cause changes in protein structure that can reduce phytoestrogens' extraction and quality. Sang-Moon Bae *et al* performed extraction at different temperatures from 20 °C – 50 °C. The results showed that the total phytoestrogens was highest at 30 °C for 2 hours, and the ethanol concentration was 80% [4]. Therefore, 30 °C was chosen for all of our experiments. The extraction time can range from 1 to 72 hours depending on the extraction conditions such as temperature, stirring speed and solvent, the ratio of solvent and raw material. According to Mercedes (2002), the conditions for effective extraction of isoflavones were one hour, at room temperature, 70% ethanol concentration and 0,1% acid acetic, using stirring at 250 rpm to save time when using large amounts of material [5]. The higher the ratio of the solvent/ solid, the faster the extraction rate is. The high extraction yield (94.34%) of isoflavones was achieved under the optimal extraction conditions of pH 9.0, 70 ^oC, 60 minutes, ethanol concentration of 65%, and 1:15 for the solid to liquid ratio [6]. Adjustment of pH during extraction was proceeded by several previous studies

and conclusions reached using alkalis to increase pH showed higher extraction efficiency. The extraction solvent popularly used to extract isoflavones from soybean is methanol, ethanol or acetonitrile. Ethanol was chosen for our experiment because it gives high efficiency and non-toxic solvent. The ethanol concentration could also affect the extraction amount. The optimal concentration of ethanol ranged from 50 to 80% [7]. In the past, in order to optimize extraction condition, the factors were surveyed in turn. However, the influencing factors were concurrent; so that the aim of this study was to optimize the extraction factors using Response surface methodology (RSM) Design Expert which was powerful tool to evaluate RSM design. The advantages offered by the RSM can be summarized as determining the interaction between the independent variables, modeling the system mathematically, and saving time and cost by reducing the number of trials. Box Behnken model has been shown as advantageous for optimization of phytoestrogens extraction with high efficiency and accuracy. A comparison between this design and other RSM designs has demonstrated that the Box Behnken design and Doehlert matrix are slightly more efficient than the composite central design but much more efficient than the three-level full factorial design [8].

2. MATERIALS AND METHODS

This study used soy germ with size of 0.1 mm from Vinanusoy Co., Vietnam. It is commercially available and contains a high level of total isoflavones. Soy germ was defatted at 40 ^{o}C, 180 rpm, solid/liquid ratio 1:5; n-Hexane 95%, and extraction time 5 hours. Defatted soy germ with moisture of 5,79 ± 0,09% (contained 1646.80 ± 7.77 mg isoflavones/100 g equivalent to 1748.01 ± 8.25 mg isoflavones/ 100 g absolutely dried defatted soy germ) was packed in dark glass grinder and stored at – 4 ^{o}C for further analysis.

Experimental design

Extraction of phytoestrogens from 2 g defatted soy germ flour was performed in a 100 mL round-bottom flask equipped with a magnetic stir at 180 rpm. The four independent variables including extraction time, (X_1) 60-120 minutes, solvent / solid ratio (X_2) 8-12, pH extraction (X_3) 8-10, and ethanol concentration (X_4) 50-70% were optimized for the extraction of crude total phytoestrogens. The extracted liquid was separated from insoluble fractions by filtration after extraction, and then dried to constant volume at 105 ^{o}C to quantify the crude total phytoestrogens (Y). Each experiment was repeated 3 times to obtain a mean result; the difference between the experiments was less than 5%. Dependent variable was highly fitted to the regression equation for total amount crude phytoestrogens (Y- mg/g). Each factor was conducted at three levels (-1, 0, +1) according to the table 1.

Table 1. *Variables and their coded levels.*

Variables	Symbol	Coded level of variable		
		-1	0	+1
Extraction time (minutes)	X_1	60	90	120
Solvent/solid ratio	X_2	8	10	12
pH	X_3	8	9	10
Ethanol concentration (%)	X_4	50	60	70

The total number of experiments was 29 (5 experiments at the center) with the objective function of the crude total phytoestrogens content. The influencing factors chosen for the experimental planning corresponding to the variables X_1, X_2, X_3, X_4 are not only influenced directly, but also interact with each other to the objective function Y.The full quadratic model for total phytoestrogen was established by using Design Expert 12.

$$Y = ao + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{14}X_1X_4 + a_{23}X_2X_3 + a_{24}X_2X_4 + a_{34}X_3X_4 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{44}X_4^2$$

Where Y is a response variables of total phytoestrogen content, the a_i are regression coefficients for linear effect; a_{ik} are regression coefficients for quadratic effects; and X_i are coded experimental levels of the variables. Design Expert software (Stat Ease Inc, Minneapolis) version 11 were used to design experiments.

Extraction of crude phytoestrogens from soygerm at optimal conditions: Extraction of phytoestrogens from 5 g defatted soy germ flour was performed in a 100 ml round-bottom flask equipped with magnestic stir at 180 rmp with optimal conditions. The extracted liquid was seperated from insoluble fractions by filtration after extraction, and then was analyzed by HPLC to quantify the phytoestrogens. The extraction yield (%) was defined as follows:

Extraction yield (%) = [total content of phytoestrogens after extraction (mg)/total content of phytoestrogens in initial soy germ flour (mg)]×100%

HPLC analysis: Phytoestrogens in crude total phytoestrogens extract were analysed by Alliance System, Waters, USA equipped with a Zorbax SB-C18 (5 µm x 4.6 mm x 150 mm). The HPLC conditions were set at column temperature of 35 ^{0}C, detection wavelength of 260 nm, mobile phases were A - acetic acid 0.1%; B - acetic acid/acetonitrile 20/80, flow rate of 1.0 ml /min. The detection was carried out under the linear gradient elution with percentage of mobile phase changing from A 88%, B 12% to A 60%, B 40% and finishing at A 88%, B 12%. The quantification of each phytoestrogens was calculated by integrating chromatographic peak areas into calibration curves.

Statistical analysis: All measurements were conducted in triplicates and statistically analysed by analysis of variance (ANOVA) followed by Duncan's multiple range test and the relation between them was performed using the SPSS software programme version 25 (SPSS Inc., Chicago, IL, USA). Significance of difference was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

The experimental design was established by Box Behnken model with 29 experiments including 5 central points. The result of crude total phytoestrogens extract was shown in table 2.

Table 2. *Total crude phytoestrogens.*

Experiment number	X_1 (min)	X_2	X_3 (pH)	X_4 (%)	Y (mg/g)
1	60	8	9	60	242.110 ± 1.826
2	120	8	9	60	239.113 ± 3.653
3	60	12	9	60	267.461 ± 4.472
4	120	12	9	60	280.120 ± 1.201
5	90	10	8	50	264.072 ± 2.035
6	90	10	10	50	255.182 ± 2.021
7	90	10	8	70	251.559 ± 1.971
8	90	10	10	70	258.155 ± 1.289
9	60	10	9	50	248.587 ± 1.163
10	120	10	9	50	261.860 ± 4.493
11	60	10	9	70	255.183 ± 2.168
12	120	10	9	70	254.437 ± 3.966
13	90	8	8	60	237,629 ± 1,028
14	90	12	8	60	277.995 ± 5.752
15	90	8	10	60	243.598 ± 1.583
16	90	12	10	60	269.729 ± 4.806
17	60	10	8	60	253.352 ± 3.654

18	120	10	8	60	262.182 ± 3.127
19	60	10	10	60	256.131 ± 3.898
20	120	10	10	60	257.083 ± 3.739
21	90	8	9	50	245.660 ± 3.690
22	90	12	9	50	273.540 ± 3.067
23	90	8	9	70	235.638 ± 2.569
24	90	12	9	70	274.065 ± 3.366
25	90	10	9	60	266.874 ± 3.213
26	90	10	9	60	266.027 ± 3.145
27	90	10	9	60	262.279 ± 3.198
28	90	10	9	60	264.439 ± 3.245
29	90	10	9	60	261.007 ± 3.151

The content of crude total phytoestrogens extract from 29 experiments from 235.638 to 280.12 mg/g. The model summary statistics showed that Quadratic Model was the most suitable for the crude total phytoestrogens extraction. Test of significance of coefficients and model compatibility was conducted by regression analysis was shown in table 3.

Table 3. *ANOVA for RSM quadratic Model.*

Source	Sum of squares	df	Mean of square	F Value	p-value (Prob >F)
Model significant	3920.63	14	280.05	87.93	< 0.0001
a_1	85.18	1	85.18	26.73	0.0001
a_2	3305.46	1	3305.46	1037.34	< 0.0001
a_3	3.98	1	3.98	1.25	0.2825
a_4	32.88	1	32.88	10.32	0.0063
a_{12}	61.28	1	61.28	19.23	0.0006
a_{13}	15.52	1	15.52	4.87	0.0445
a_{14}	49.13	1	49.13	15.42	0.0015
a_{23}	50.66	1	50.66	15.90	0.0013
a_{24}	27.81	1	27.81	8.73	0.0105
a_{34}	59.95	1	59.95	18.82	0.0007
a_{11}	115.07	1	115.07	36.11	<0.0001
a_{22}	61.62	1	61.62	19.34	0.0006
a_{33}	61.57	1	61.57	19.32	0.0006
a_{44}	112.90	1	112.90	35.43	<0.0001
Residual	44.61	14	3.19		
LOF	20.21	10	2.02	0.33	0.9288
Pure error	24.40	4	6.10		
Cor Total	3965.24	28			

The Model F-value of 87.93 implied the model was significant. The "Lack of Fit F-value" of 0.33 implied the Lack of Fit was not significant relative to the pure error.

Table 4. *Analysis of R value of the model.*

Std.Dev	1.79	R^2	0.9887
Mean	258.11	Adj R^2	0.9775
C.V%	0.69	Pred R^2	0.9610
Press	154.53	Adeq Precision	34.292

The quadratic model in this study satisfied all criteria for a model with P <0.0001; AP = 34.292; LOF = 0.9288 was not statistically significant; R^2 = 0.9887 indicated that there was high consistency of the experimental value and the predicted value.

Table 5. *Coefficient estimate of each factor.*

Factor	Coefficient estimate/ Terms of coded factors	Terms of actual factors
Intercept	264.13	-255.977
a_1	2.66	1.57066
a_2	16.6	25.94183
a_3	-0.58	55.35413
a_4	-1.66	1.08964
a_{12}	3.91	0.065233
a_{13}	-1.97	-0.06565
a_{14}	-3.5	-0.01168
a_{23}	-3.56	-1.77938
a_{24}	2.64	0.13184
a_{34}	3.87	0.38715
a_{11}	-4.21	-4.68E-03
a_{22}	-3.08	-0.77051
a_{33}	-3.08	-3.08093
a_{44}	-4.17	-0.04172

The crude total phytoestrogens (Y) of coded factor

$Y = 264.13 + 2.66X_1 + 16.6X_2 – 0.58X_3 – 1.66X_4 + 3.91X_1X_2 – 1.97X_1X_3 – 3.5X_1X_4 – 3.56X_2X_3 + 2.64X_2X_4 + 3.87X_3X_4 – 4.21X_1^2 – 3.08X_2^2 – 3.08X_3^2 – 4.17X_4^2$

The crude total phytoestrogens (Y) of actual factor

$Y = - 255.977 + 1.57066X_1 + 25.94183X_2 + 55.35413X_3 + 1.08964X_4 + 0.065233X_1X_2 – 0.06565X_1X_3 – 0.01168X_1X_4 –1.77938X_2X_3 + 0.13184X_2X_4 + 0.38715X_3X_4 – 4.68E\text{-}0.3X_1^2 – 0.77051X_2^2 – 3.08093X_3^2 – 0.04172X_4^2$

In the survey area, the regression equation showed that crude total phytoestrogens content was affected by the first and second order of all four factors and simultaneously by the pair of factors: extraction time – solvent /solid ratio, extraction time – pH, extraction time – ethanol concentration, solvent /solid ratio– pH, solvent /solid ratio – ethanol concentration, pH - ethanol concentration.

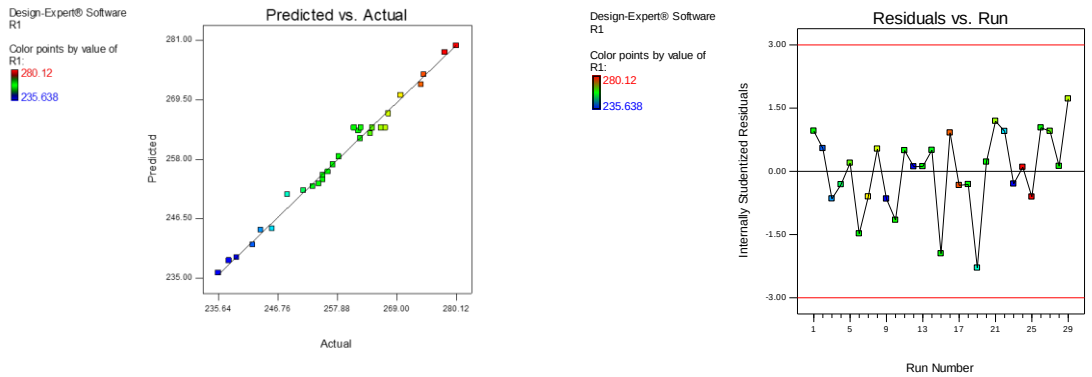

Figure 1. *Graph of experimental values - prediction and random distribution of 29 experiments.*

In addition, a number of other factors are also used to evaluate the model is completely compatible with the experimental results and predicted and actual value plots and charts of the random distribution of the experiments (residuals versus runs models). The data in the figures also show that the model is well correlated when the points are centered on a straight line and the distribution of the experimental points is random.

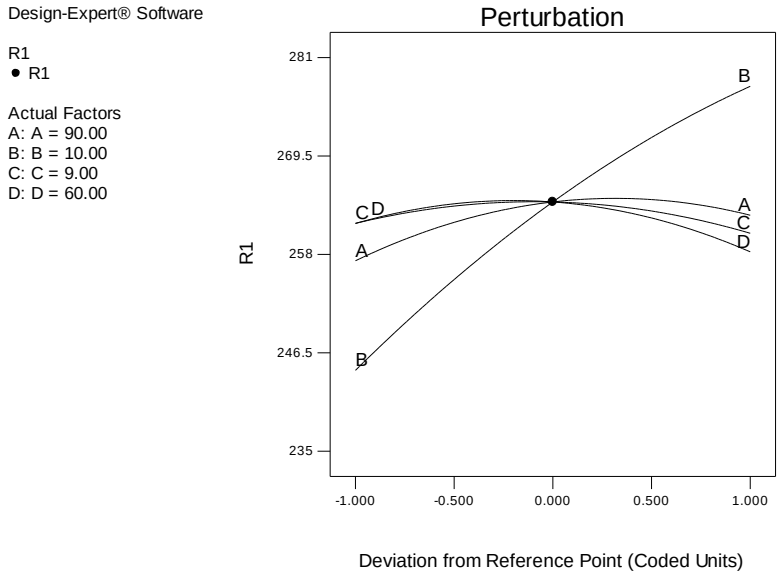

Figure 2. *Variation of extraction factors in the survey area.*

The 2D graph showed that there was a relationship between effects of the experimental elements on the target crude total phytoestrogens content: solvent: solid ratio, extraction time, pH, solvent concentration. In the area (-1, + 1), when the solvent / solid ratio increased, the total phytoestrogens increased significantly. In the area (-1, 0) when the extraction time, pH and solvent concentration increased, the total phytoestrogens also increased; in the area (0, +1), when the extraction time, pH and solvent concentration increased, the total phytoestrogens decreased. At the center (0), the crude total phytoestrogens reached maximum volume.

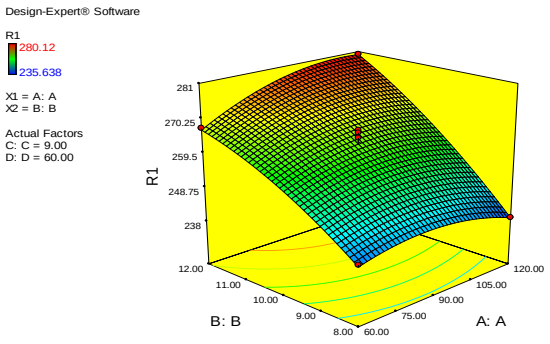

Figure 3A. *Effects of extraction time and solvent/solid ratio on crude total phytoestrogens.*

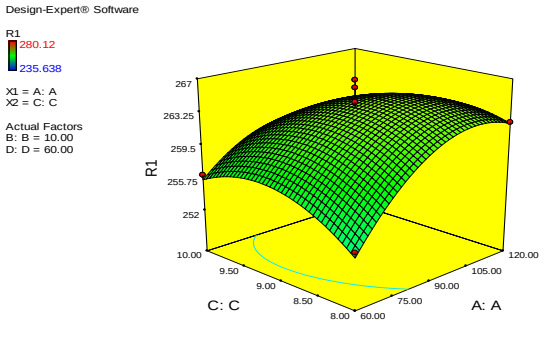

Figure 3B. *Effects of extraction time and pH on crude total phytoestrogens.*

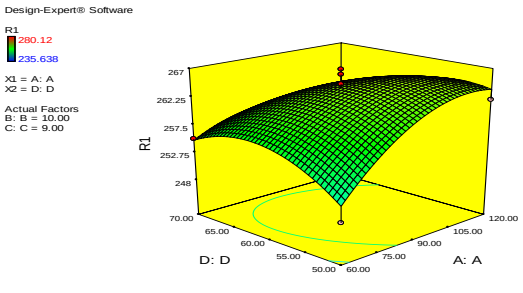

Figure 3C. *Effects of extraction time and ethanol concentration on crude total phytoestrogens.*

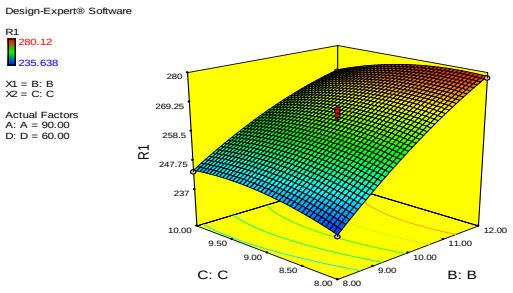

Figure 3D. *Effects of solvent/solid ratio and pH on crude total phytoestrogens.*

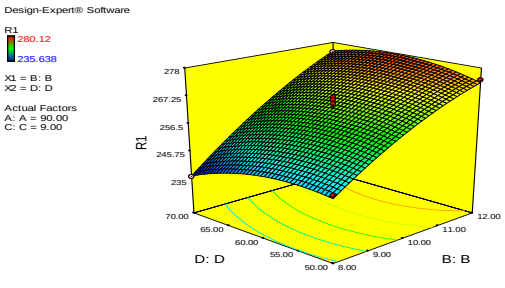

Figure 3E. *Effects of solvent/solid ratio and ethanol concentration on crude total phytoestrogens.*

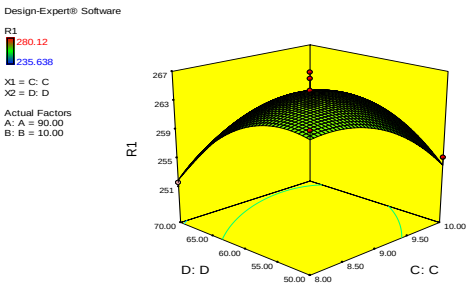

Figure 3F. *Effects of pH and ethanol concentration on crude total phytoestrogens.*

Figure 3A to 3F showed the effects by the pair of factor: extraction time – solvent /solid ratio, extraction time – pH, extraction time – ethanol concentration, solvent /solid ratio– pH, solvent /solid ratio – ethanol concentration, pH - ethanol concentration on content of crude total phytoestrogens extract. From 30 solutions given by Design Expert, the theoretical solution was chosen is extraction time, solvent/solid ratio, pH, ethanol concentration which were determined as follows: 98.71 mins, 11.26/1, 8.17, and 62.71%, respectively with the prediction crude total phytoestrogens of 273.712 mg/g. The optimal factors chosen including extraction time, solvent/solid ratio, pH, ethanol concentration were determined as follows: 90 mins, 12:1, 9.0, and 65%, respectively. At these conditions, the practical crude total phytoestrogens extraction reached 271.139 mg/g (yield practical versus theoretical crude total phytoestrogens reached 99.08%). These results confirm that the model for the extraction of phytoestrogens was able to predict the experimental conditions. The extraction time of 90 minutes is in agreement with studies of Sang-Moon Bae, Mengfan Wang, Mercedes C.Carao-Panizzi [4-6]. All the results showed that the optimal extraction time for phytoestrogens was from 1 to 2 hours at 30^{o}C. The shorter extraction time was important when a large number of samples were analyzed and facilitated the subsequent analysis process.According to Mengfan Wang *et al.*, the highest solvent /solid ratio was 25 [6]. However, solvent should not be used with large amount in extraction process because of their cost and intensive energy consumption. At the solvent/solid ratio of 12, the total phytoestrogens did not increase significantly so the value of 12 was chosen as the optimal ratio.It can be seen that glycoside isoflavones (daidzin and genistin) were the main components of isoflavone and the highest extraction yield was achieved at pH 9.0. The alkaline

condition could promote the conversion of conjugated forms (acetyl glycoside isoflavones and malonyl glycoside isoflavones) into their non-conjugate forms (glycoside isoflavones) since the ester bond in acetyl and malonyl forms was weak to alkaline and high temperature. Furthermore, the solubility of isoflavones in aqueous ethanol solution reached the highest value at pH 9.0 [6]. Ethanol concentration of 65% was the optimal point for extraction phytoestrogens from soy germ. Aqueous ethanol seemed to be a good condition to solubilize both aglycones and glycosides isoflavones.

Quantitative analysis of isoflavones in the crude total phytoestrogens

Acording to D. T. H. Vien, the optimal conditions were 90 minutes, 10:1, 9.0, 60%, and 40 ^{o}C for extraction time, solvent/solid ratio, pH, ethanol concentration, and temperature, respectively [9]. These numbers for optimal conditions by RSM were: 90 minutes; 12:1; pH 9.0; 65%, 30 ^{o}C, respectively. The D. T. H. Vien method was repeated and compared with RSM method. All total phytoestrogens from crude extracts at the optimal conditions were quantitatively analyzed by HPLC and the results were shown in table 6. Under these conditions, comparing the optimization results by D. T. H. Vien method and by RSM, the total phytoestrogens and total aglycones (daidzein, glycitein, genistein) according to RSM was higher than that of D.T. H. Vien method. The efficiency of extraction has increased 14.48%, from 81.14% to 95.62%.

Table 6. *Comparison of phytoestrogen yield between Do Thi Hoa Vien method and RSM.*

Ingredient	**Phytoestrogen yield (mg/100g)**	
	Do Thi Hoa Vien method	**Optimization by RSM in this study**
Daidzin	288.95 ± 2.27	281.68 ± 1.16
Glycitin	171.15 ± 1.70	167.42 ± 0.69
Genistin	114.55 ± 1.44	169.19 ± 0.69
Malonyldaidzin	42.95 ± 1.23	38.10 ± 0.16
Malonylglycitin	20.80 ± 0.44	17.38 ± 0.07
Acetyldaidzin	248.81 ± 0.91	327.47 ± 1.34
Acetylglycitin	12.23 ± 0.09	8.63 ± 0.04
Malonylgenistin	266.04 ± 0.48	295.70 ± 1.21
Daidzein	24.88 ± 0.20	42.09 ± 0.17
Glycitein	28.15 ± 0.22	66.45 ± 0.27
Acetylgenistin	188.80 ± 0.82	219.77 ± 0.90
Genistein	10.95 ± 0.08	37.65 ± 0.15
Total	**1418.27 ± 10.23**	**1671.54 ± 6.86**
Yield (%)	81.14	95.62

4. CONCLUSIONS

From this study, the model gained by the experimental Box Behnken design was found to be effective in optimizing the extraction conditions for maximizing the crude total phytoestrogens. The obtained regression coefficients of the predicted quadratic model for the coded variables were high and this indicated that the variables were fitted to the regression for the total phytoestrogens (R^2 = 0.9887). Optimum conditions for extraction of phytoestrogens were extraction time (X_1) of 90 minutes, solvent: solid ratio (X_2) of 12, pH (X_3) 9, ethanol concentration (X_4) of 65%. Under these conditions, extraction yield obtained 95.62 % which is 14.48% higher than that of D. T. H. Vien method. Therefore, RSM using Box Behnken design is a useful method to optimize different factors for an efficient extraction of phytoestrogens.

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REFERENCES

[1]. J.-A. Kim, S.-B. Hong, W.-S. Jung, C.-Y. Yu, K.-H. Ma, J.-G. Gwag, and I.-M. Chung, "*Comparison of isoflavones composition in seed, embryo, cotyledon and seed coat of cooked-with-rice and vegetable soybean (Glycine max L.) varieties"*. Food Chemistry. **Vol. 102**. pp 738-744, (2007).

[2]. H. Wang and P. A. Murphy, "*Isoflavone Content in Commercial Soybean Foods"*. Journal of Agricultural and Food Chemistry. **Vol. 42**. pp 1666-1673, (1994)

[3]. J. Chen, G. Tang, J. Zhou, W. Liu, and Y. Bi, "*The characterization of soybean germ oil and the antioxidative activity of its phytosterols"*. The Royal Society of Chemistry Advances. **Vol. 9**. pp 40109-40117, (2019).

[4]. S. M. Bae, C. H. Jang, J. H. Kim, H. A. Lim, J. R. Kim, J. H. Kim, and J. S. Kim, "*Optimization of Isoflavone Extraction from Soy Germ"*. Journal of Food Science and Nutrition. **Vol. 10**. pp 290-293, (2005).

[5]. M. C. Carrão-Panizzi, S. P. de Goes Favoni, and A. Kikuchi, "*Extraction Time for Soybean Isoflavone Determination"*. Brazilian Archives of Biology and Technology. **Vol. 45**. pp 515-518, (2002).

[6]. M. Wang, "*An Effective and Green Method for the Extraction and Purification of Aglycone Isoflavones From Soybean"*. Food Science and Biotechnology. **Vol. 22**. pp 705-712, (2013).

[7]. S. Y. Cho, Y. N. Lee, and H. J. Park, "*Optimization of ethanol extraction and further purification of isoflavones from soybean sprout cotyledon"*. Food Chemistry. **Vol. 117**. pp 312-317, (2009).

[8]. X.-L. Yu and Y. He, "*Application of Box-Behnken designs in parameters optimization of differential pulse anodic stripping voltammetry for lead(II) determination in two electrolytes"*. Scientific Reports. **Vol. 7**. pp 2789, (2017).

[9]. D. T. H. Vien and D. T. Trang, "*Research on extraction of isoflavones from soybean germ"*. Vietnam Journal of Science and Technology. **Vol. 54**. pp 61-68, (2018).

TÓM TẮT

TỐI ƯU HÓA QUÁ TRÌNH CHIẾT XUẤT PHYTOESTROGEN TỪ PHÔI ĐẬU TƯƠNG SỬ DỤNG PHƯƠNG PHÁP ĐÁP ỨNG BỀ MẶT

Phytoestrogen là một estrogen thực vật có hoạt tính chống oxy hóa và mang lại nhiều lợi ích cho sức khỏe. Hàm lượng phytoestrogen trong phôi đậu tương là lớn nhất so với các bộ phận khác của hạt. Phương pháp đáp ứng bề mặt sử dụng mô hình Box Behnken với 4 yếu tố để tối ưu hóa quá trình chiết xuất phytoestrogen từ phôi đậu tương với 29 thí nghiệm, trong đó, 5 thí nghiệm lặp lại ở điểm trung tâm. 4 yếu tố được khảo sát là thời gian chiết xuất (X_1) từ 60-120 phút, tỉ lệ dung môi : nguyên liệu (X_2)= 8-12; pH chiết xuất (X_3)= 8-10; nồng độ ethanol (X_4) = 50-70%. Biến phụ thuộc của mô hình là tổng lượng cao chiết phytoestrogen Y (mg/g). Điều kiện tối ưu được xác định với R^2=0.9887; X_1 = 90 phút; X_2 = 12; X_3 = 9; X_4 = 65%. Hiệu quả của quá trình chiết xuất tại điểm tối ưu đạt 95.62%.

Từ khóa: Phôi đậu tương; Phytoestrogen; RSM.

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Author affiliations:
[1] University of Economics - Technology for Industries;
[2] Hanoi University of Science and Technology.
*Corresponding author: lmchau.uneti@moet.edu.vn.