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Optimization of isoflavones extraction from soybeans using full factorial design

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Abstract

This study focuses on the optimization of isoflavones extraction from soybeans cultivated in Quangnam province, Vietnam, using aqueous ethanol solvent extraction. The total isoflavone concentration was determined with six isoflavone standards including daidzin, glycitin, genistin, daidzein, glycitein, and genistein by Reversed Phase HPLC method. The effects of temperature, extraction time, and ratio of solvent to material on the extracted isoflavone content were investigated for use in the experimental planning method with full element 2^3 and optimization using the Box-Wilson model. The results of the optimization showed that the optimal conditions of isoflavones extraction from soybeans using ethanol 80% was in temperature of 72.5°C, extraction time of 67.5 min, and solvent to dry soybean ratio of 26.5/1 (ml/g). The maximum amount of isoflavone content obtained from this extraction condition was 1,932.44 μg/g dry matter.

Practical applications

The extraction of isoflavones from soybeans involves the interaction between various factors including solvent concentration, temperature, extraction time, and ratio of solvent to material. There is a need to study the combined effect of these factors to optimize the extraction conditions within a minimum number of experiments. Hence, this work applies the full factorial design to study the effects of several factors on one or more responses to achieve the optimum extraction condition. This research work is important to obtain the most efficient extraction conditions for isoflavones production without consuming too much efforts on the experimentation. The results attain from this work can contribute to the extension of efficient isoflavones extraction in industrial scale.

1 | INTRODUCTION

Isoflavones, occurring in a large quantity in Fabaceace families, especially in soybeans, are secondary plant metabolites (polyphenols) which belong to a subclass of flavonoids. The structure of isoflavones is similar to the endogenous steroid hormone 17β-estradiol (E2), hence they exhibit weak estrogenic activity described as phytoestrogens. Some of positive effects of isoflavones have been reported on cardiovascular diseases, osteoporosis, cancers, improvement of bone health, and antioxidant activity (Hati, Patel, Patel, & Prajapati, 2017). Soy isoflavones includes genistein, daidzein, glycitein, and their respective acetyl, malonyl, and aglycone forms (Murphy, Barua, & Hauck, 2002; Wally et al., 2019; Wang, Guo, Qi, Su, & He, 2013). Aglycones have high bioavailability, where β-glycosides form a large proportion of the total isoflavones content and are capable of being converted into aglycone, in which they both generate interest for extraction and application (Cho, Lee, & Park, 2009; Lakshmi, Rao, Ravi, & Raghavarao, 2013; Nemitz et al., 2017; Rostagno et al., 2010).

The extraction of isoflavones from soybeans is generally carried out using conventional and non-conventional methods. Conventional techniques used are maceration, stirring, shaking, hot, and reflux extraction based on solvents with heating and stirring assistance to increase the extraction yield of isoflavones. Although these techniques often requires large amounts of solvents, are time consuming and cause degradation of some isoflavones, they have low processing cost and are easy to operate (Blicharski & Oniszczuk, 2017; Easmin et al., 2015; Rostagno et al., 2010). Among these methods, mechanical stirring extraction is the conventional process having the highest extraction efficiency due to strong mixing with heating process, ease of handling, low equipment costs as well as capable for industrial scale production. Although solvents such as acetonitrile, ethanol, methanol, and water are often used for these extraction methods, the binary mixture of ethanol and water is the best extraction solvent in terms of environment, health, and safety (Cho, Nam, & Park, 2009; Jankowiak, Trifunovic, Boom, & van der Goot, 2014; Lakshmi et al., 2013; Wang et al., 2013).

A mechanic stirring extraction not only influences various single factors such as solvent concentration, temperature, extraction time, and ratio of solvent and material, but it also creates interactions among these factors. Previous works have been carried out by optimizing three parameters such as the ethanol concentration, extraction time, and reaction temperature using response surface methodology (Cho et al., 2009; Lakshmi et al., 2013; Wang et al., 2014; Zhang, Ng, & Luo, 2007). Although the optimization of these studies focused on these major parameters, but the results carried out showed significant difference. According to Cho et al. (2009), a solid phase extraction method was used, the optimization results indicated that the extract temperature was over 90°C and the process time reached 100 min. This high temperature and longer time could lead to loss in the amount of the six major isoflavone components, in which the work done have just illustrated on two isoflavones which are daidzin and genistin in their study (Cho et al., 2009). Similarly, Wang et al., (2014) showed that the extraction time has gone beyond two hours in the analysis of puerarin, daidzein, and total isoflavone (Wang et al., 2014). On the contrary, the application of a full factorial design is an effective method for studying the combined effects of several factors on one or more responses so that optimizing the extraction conditions can be achieved within the minimum number of experiments (Lazic, 2006; Loumouamou et al., 2017; Montgomery, 2017). Hence, the optimization study of isoflavone extraction from soybean using mechanical stirring extraction can be performed to evaluate the effectiveness of this technique on isoflavone extraction.

This study aims to investigate the effect of the factors on the extraction efficiency of isoflavones and to find out the optimal extraction conditions for the mechanic stirring extraction of isoflavones using the solvent mixture of ethanol and water through the implementation of the first-order regression extraction model. The effects of significant factors determined by 2^3 full factorial design were screened to locate the optimum conditions by applying steepest ascent experiments with Box-Wilson model, thereby extending the possibilities of isoflavone extraction to industry scale.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Soybeans collected from five locals in Dailoc district, Quangnam province (Vietnam) were mixed and dried at 60°C to the moisture content of dry matter (9.716%). The raw sample was grounded, then stored in a dark glass jar, at −20°C. Ethanol of 99.7% was obtained from Ducgiang company, Vietnam. HPLC grade acetonitrile and analytical purity grade phosphoric acid, solid NaOH, hydrochloric acid of 37% were purchased from Merck, Germany. The isoflavone standards such as genistein, daidzein, glycitein, genistin, daidzin, and glycitin were provided by United States Pharmacopeia and all the standards were stored a −20°C for further experimental purposes. Working standard solutions were prepared in 40% (v/v) acetonitrile and stored at −20°C (Collison, 2008).

2.2 | Experimental set-up

2.2.1 | Extraction of isoflavones from soybean

Soy flour was accurately weighed according to the expected ratio of solvent to solid matter and adjusted to a 500-ml round bottom flask containing 250 ml of the solvent of ethanol/water. The samples were placed in a thermostat water bath (model HH-2, China) to maintain a specific temperature, while an electric laboratory stirrer (model: JJ-1, China) was used for stirring. The mixture was mechanically stirred with a specific condition of stirring rate for all experiments. After the extraction process, the samples were cooled to room temperature, centrifuged and vacuum filtrated. Ethanol in the filtrate was removed using a rotary evaporator (DAIHAN Scientific, model DHWEV01001V) operating at 48–52°C with vacuum pressure of 700 mmHg and rotation speed of 70 rpm. The ethanol was recovered up to an appropriate concentration and was diluted to make 50 ml. The extract was kept at −20°C and adequately prepared prior to analysis by HPLC. The purity of the recovered ethanol was tested using an alcoholmeter and the amount of fresh ethanol needed was adjusted accordingly for reuse in subsequent extractions.

2.2.2 | Quantification of isoflavone content in soy samples by HPLC

Analytical sample by HPLC was arranged as followed: Soybean extracts were modified based on the method AOAC 2008.03 for determining total soy isoflavones in the soy food (Collison, 2008). About 3.75 ml of soybean extracts was added into a 100 ml Erlenmeyer flask and 4.25 ml of acetonitrile along with 2 ml of water were then added. After mixing at room temperature

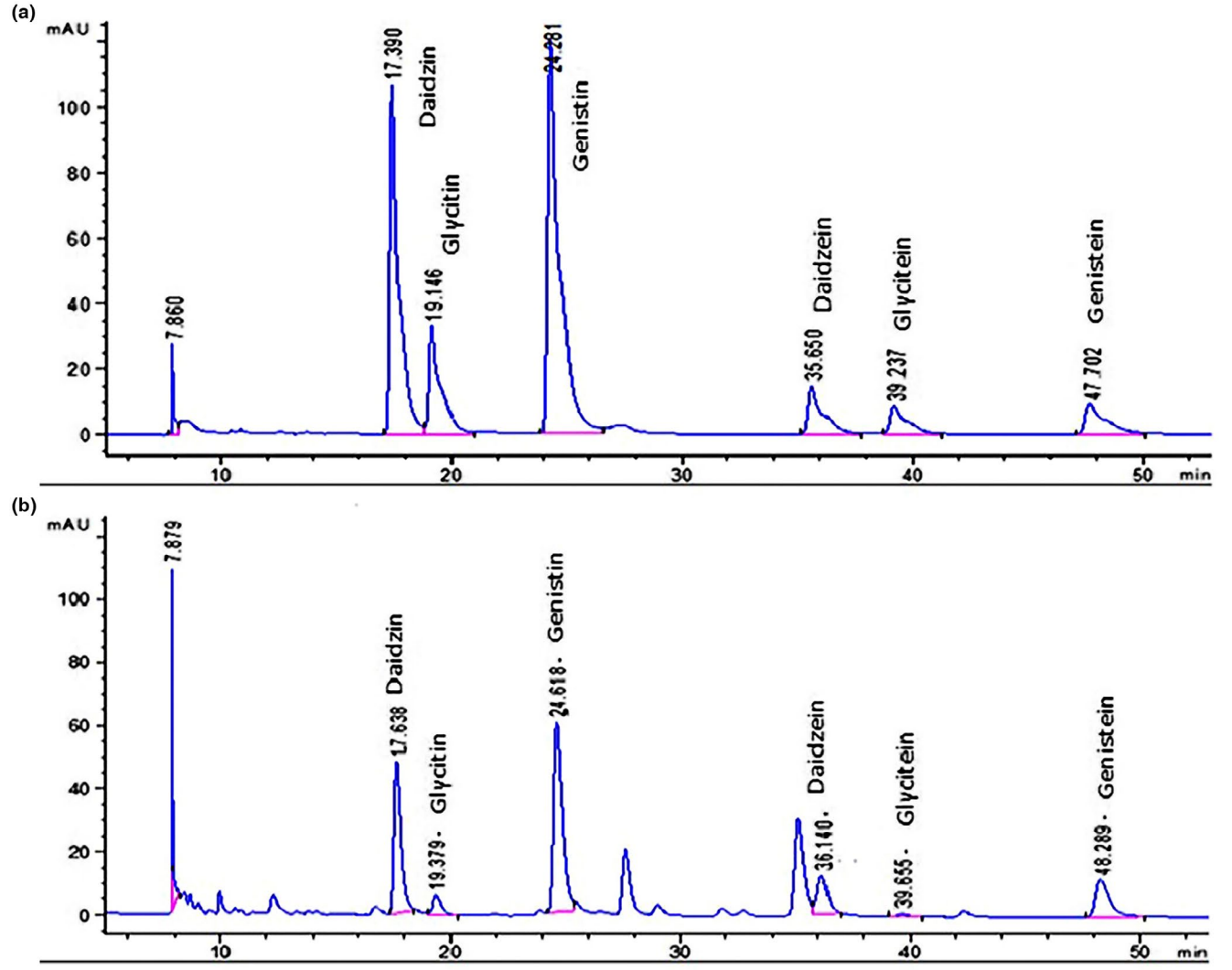

FIGURE 1 A typical HPLC chromatogram of the isoflavone standards (a) and of an isoflavone soybean sample (b)

for 60 min at a speed of 150 rpm, the extract was transferred into a 15 ml Falcon Conical Centrifuge Tube and centrifuged at 5,000 rpm for 5 min. The supernatant was filtrated through a filter membrane (PTFE, 0.2 µm, Sartorius, code 17575K) before injected into the HPLC instrument. HPLC analysis of isoflavones: Isoflavones were analyzed using Agilent 1200 System (Agilent Technologies, CA, USA) equipped with a Lichrosphere 100 RP column (C18, 5 µm × 4.6 mm × 250 mm, USA). Mobile phase A was 0.05% acid phosphoric acid in water and mobile phase B was acetonitrile. A diode array UV detector was set up at 260 nm. The flow rate was 1.5 ml/min. The sample was eluted by gradient elution as follows: 100% of A in 5 min initial, change from 10% to 30% of B (linear) over 60 min, 5 min wash 90% of B, 10 min equilibration at 10% B. The quantification of each isoflavone was based on integrating chromatographic peak areas using external standard method. Figure 1 demonstrates two typical HPLC chromatographs of standard mixture and the soybean extract. From the relationship between the peak area and the concentrations (µg/ml) of each isoflavone standard, a calibration curve of this isoflavone was established and used to calculate the Limit of Detection (LOD) and Limit of Quantification (LOQ) as presented in Table 1. The content of each isoflavone compound (X_i(µg/g)) in the dry material was calculated using the following Equation (1):

$$X_i = A_i \times f \times V/m \quad (1)$$

where A_i (g/ml) is the concentration of each isoflavone compound in the injected solution calculated from the calibration curve; f is the dilution factor of the solution injected into the HPLC; V (ml) is the volume of extract of isoflavones from the soybean sample; m (g) is the mass of the soybean sample (calculated as the dry matter content). The total isoflavones is given as the sum of all the isoflavone compound (Equation (2)):

$$\text{The total isoflavones: } X\ (\mu g/g) = \sum X_i\ (\mu g/g) \quad (2)$$

TABLE 1 Calibration equation, regression correlation coefficient R^2, Limit of Detection (LOD) and Limit of Quantification (LOQ) for each isoflavone compound in this study

Isoflavone	Calibration equation[a]	R^2	LOD (μg/ml)	LOQ(μg/ml)
Daidzin	$Y = 21.138x - 2.087$	0.993	14.337	47.792
Glycitin	$Y = 20.41x + 4.005$	0.995	4.288	14.294
Genistin	$Y = 30.426x + 2.718$	0.994	13.337	44.457
Daidzein	$Y = 31.897x - 0.344$	0.993	2.016	6.720
Glycitein	$Y = 20.828x + 11.823$	0.997	0.779	2.597
Genistein	$Y = 37.35x + 0.103$	0.993	1.415	4.715

[a]Y, peak area; x, concentration of isoflavone compound (μg/L).

2.2.3 | Full factorial designs of isoflavones extraction conditions

The experimental design was carried out in three steps. Firstly, a single variable experimental method was used to investigate the effect of each factor on the amount of isoflavones extracted from soybeans as follows: three chosen pH media were pH 2.5, neutral pH and pH 9; the concentration of ethanol was altered from 60% to 100% (v/v) with intervals of 10%; the ratio of solvent to material was set as 15/1, 20/1, 25/1, 30/1; the extraction temperature was selected from 40 to 70°C with gap of 10°C; the extraction time was altered from 30 to 90 min with time intervals of 15 min. Secondly, in order to study the interaction of the extraction factors and calculate the regression model, a 2^3 full factorial design with 3 factors and 3 center points was launched (Mäkelä, 2017; Montgomery, 2017). Three factors selected for the optimization were the ratio of solvent to material (X_1), the extraction temperature (X_2) and the extraction time (X_3). The regression equation for the design was as follows (Equation (3)):

$$\tilde{Y} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{23}x_2x_3 + b_{13}x_1x_3 + b_{123}x_1x_2x_3 \quad (3)$$

where: $\tilde{Y}$ is the predicted extraction yield; x_1, x_2, x_3 are the coded values of the independent variables including temperature, time and ratio of ethanol to soybean, respectively; b_0 is the global mean and b_i represents the other regression coefficients.

Finally, to maximize the amount of the total isoflavones extracted from soybeans, the direction of steepest ascent was used with the Box-Wilson model. The path of steepest ascent was taken as the line through the center of the region of interest and normal to the fitted surface (Figure 2). Thus, the steps size Δ_i along the path are proportional to the regression coefficients b_i and interval λ_i.

2.2.4 | Statistical analysis

All of experiments were done in triplicate. Data were analyzed using analysis of variance (ANOVA). Differences within the group were determined at a p-value < 0.05. After conducting 11 experiments, the regression coefficients b_i, of the Equation (3) was calculated and the main effects and interactions between different factors were determined. The statistical significance was checked using an F-test. The Minitab 18 was used to analyses variance (ANOVA) and the experimental design.

3 | RESULTS AND DISCUSSION

3.1 | Effect of factors on total extracted isoflavones amount

3.1.1 | Effect of pH

The total amounts of the isoflavones in ethanol extracts with the pH values of 2.50 ± 0.08, neutral pH of 7.12 ± 0.04 and 9.00 ± 0.05 at extraction temperature of 40°C in 60 min at the ratio of solvent to material of 20/1 were presented in Table 2. The results showed that total amount of isoflavones from the extracts obtained at the varying pH values did not significantly differ. This result is in agreement with the study by Murphy et al. (2002) which used ethanol in water with and without HCl to extract isoflavones from soybeans (Murphy et al., 2002). Wang et al. (2013) illustrated that at a pH in the range of 7–11 along with other extraction conditions such as ethanol concentration of 65%, extraction temperature of 70°C, extraction time of 60 min, and 15/1 solvent to material ratio increased glycoside content because in alkaline media, the malonyl forms were hydrolysed to release glycoside (Wang et al., 2013). Mathias, Ismail, Corvalan, and Hayes (2006) illustrated that malonyl and acetyl forms in pure solution were also converted to β-glycoside. This conversion increased with increment of temperature, the highest at 100°C (Mathias et al., 2006). Meanwhile, when isoflavones and other compounds in the extract are at the lower extraction temperature (40°C), the malonyl forms were not transformed into acetyl forms, glucosides or even to free aglycones. Hence, isoflavones in alkaline and acidic media will be easily degraded by increasing the extraction temperature (Klump, Allred, MacDonald, & Ballam, 2001; Luthria & Natarajan, 2009). In our experimental conditions, the pH factor did not affect the total isoflavones amounts in the extracts, a neutral pH medium for the extraction was selected to carry out further studies.

3.1.2 | Effect of ethanol concentration

The total isoflavone amounts extracted from the soybean through changing the concentration of ethanol in water from 60% to 100% (v/v) were presented in Figure 3a. The results showed that the amount of aglycone and glycoside increased gradually when the ethanol concentration was increased from 60% to 80%. However, the amount of

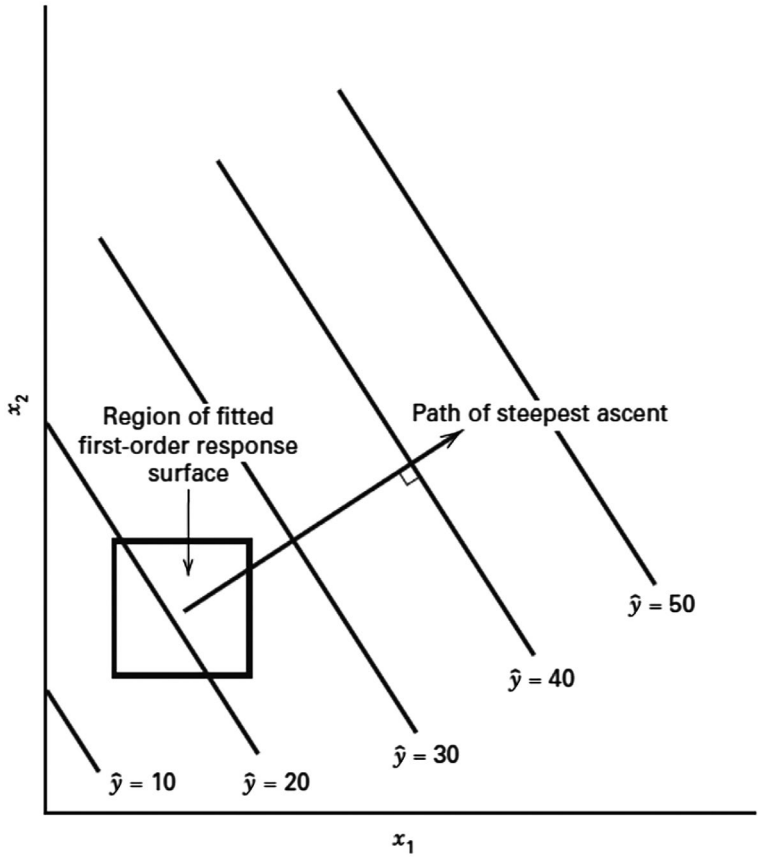

FIGURE 2 First-order responses surface and path of steepest ascent (Montgomery, 2017)

TABLE 2 Content of isoflavones compounds ethanol extracts

Isoflavone compounds		Content of isoflavones (μg/g)		
		IsF neutral pH	IsF pH 2.5	IsF pH 9
Aglycones	Daidzein	129 ± 4.2	126.7 ± 2.8	124.7 ± 4.7
	Glycitein	9.7 ± 1.2	9.0 ± 0.9	9.3 ± 1.2
	Genistein	133.3 ± 3.8	130.3 ± 1.4	128.3 ± 2.8
	Total	272.0 ± 9.4	266.0 ± 5.2	262.3 ± 9.0
Glycosides	Daidzin	515.0 ± 4.2	515.3 ± 4.7	504.3 ± 4.2
	Glycitin	67.7 ± 1.9	67.0 ± 2.8	66.3 ± 1.3
	Genistin	578.0 ± 2.8	574.3 ± 3.9	567.0 ± 3.8
	Total	1,160.7 ± 5.2	1,156.6 ± 11.3	1,137.6 ± 9.3
Total of isoflavones		1,432.7 ± 14.6[a]	1,422.6 ± 16.5[a]	1,399.9 ± 18.9[a]

Note: IsF neutral pH, IsF pH 2.5, IsF pH 9: Content of isoflavones in ethanol extract at neutral pH, pH 2.5, and pH 9, respectively. The same letter is not significantly different ($p > 0.05$).

glycoside significantly decreased with further increase of ethanol concentration from 80% to 100%, where an especially sharp drop was observed at 100% (Jankowiak et al., 2014; Lakshmi et al., 2013; Wang et al., 2013). The extracted amounts of aglycones were not substantially different, this difference may be explained by the solubility of both aglycones and glycosides being better in ethanol than in water. Apart from that, the polarity of aglycones is weaker than that of glycosides so that aglycones will dissolve more in ethanol than glycosides in water (Lin & Giusti, 2005; Yang et al., 2013). The increase in ethanol concentration, i.e. reducing the content of water, leaded to the decrement in the polarity of solvent and decreased the swelling of plant cells, in which the solvent will meet difficulties in penetrating to dissolve the isoflavones (Jankowiak et al., 2014). As a result, the maximum amount of total isoflavones per gram dry matter was achieved at the ethanol

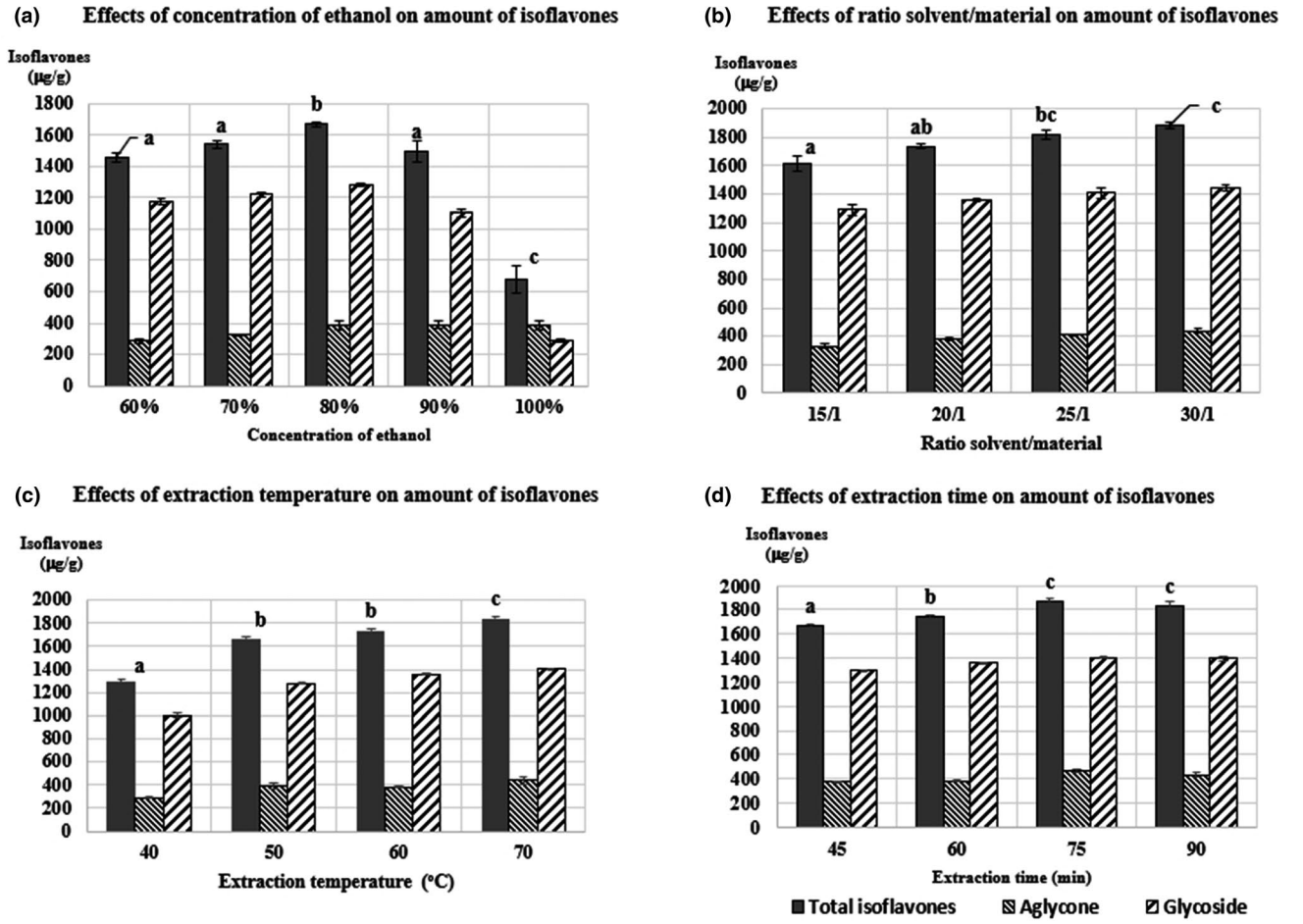

FIGURE 3 Effect of the extraction process on the total amount of isoflavones per gram of solid: (a) Effect of ethanol concentration; (b) Effect of ratio of the solvent to material; (c) Effect of extraction temperature; (d) Effect of extraction time. Error bars indicate standard deviations. Bars with the same letter are not significantly different ($p > 0.05$)

concentration of 80% (v/v), and this concentration was chosen for the next experiments.

3.1.3 | Effect of the ratio of solvent to material

Earlier studies have been carried out for the ratios of solvent to material, which was low at 3/1 (Zhang et al., 2007) or at 5/1 (Cho et al., 2009) in order to upgrade the efficiency of aglycones extraction due to their insignificant composition in soybean. This has led to the selection of higher ratio to obtain more efficient isoflavone content. The results from Figure 3b showed that larger the ratio of solvent to material obtained, the more isoflavones that were extracted from soybeans due to the increase in mass transfer. However, there was no significant difference between the total isoflavones amounts for two adjacent ratios of solvent to material observed at $p \leq 0.05$. This trend can be explained by the fact that the chosen ratios of solvent to material from 15/1 to 30/1 were rather high so the amount of isoflavones in the extracts for each case was nearly the same when the liquid-solid equilibrium was established according to Wang et al. (2013). As a result, the ratios of solvent to material from 15/1 to 25/1 were selected for the experimental design.

3.1.4 | Effect of extraction temperature

The effect of extraction temperature on the total extracted amount of isoflavones from soybeans was illustrated in Figure 3c. It can be seen that more isoflavones were extracted as the extraction temperature increased from 50 to 70°C. This increase in extraction temperature will promote the diffusion coefficient of the solvent and the solubility of isoflavones in the solvent. However, the extraction temperature in this study was not higher than 70°C due the restriction of the boiling point of ethanol, which is 78.8°C. Higher temperature may lead to solvent lost due to the vaporization. In addition, the high temperature will cause the degradation of the compounds (Wang et al., 2013), especially malonyl and acetyl forms (Mathias et al., 2006). Hence, the extraction temperature selected for further experiments in this study was from 50 to 70°C.

3.1.5 | Effect of extraction time

Based on the results illustrated in Figure 3d, the effect of extraction time on the total extracted amounts of isoflavones was significant

($p < 0.050$). The amount of isoflavones extracted increased gradually along with the increase in extraction time and achieved the maximum at 75 min, this was followed by a slight decrease, which shows similar trend to previous reports (Lakshmi et al., 2013; Wang et al., 2013). It takes a specific time for the solvent to penetrate the plant cells due to osmosis, whereby sufficient time is need for the isoflavones to diffuse from plant cells into the solvent. In the initial stage, isoflavones diffuse rapidly through the cell membrane due to the large difference in molecular concentration inside and outside of the cell across the cell membrane. When the time elapses, the rate of diffusion decreases gradually with decreasing the isoflavones concentration inside the cells until an equilibrium is established between two phases of the liquid and the solid according to the mass transfer law. In addition, lengthening extraction time leads to the possibility of isoflavones oxidation to increase due to the reaction with oxygen in the air. As a result of this investigation, the extraction time was selected from 45 to 75 min for the experiment design.

3.2 | Optimization of isoflavones extraction from soybeans

3.2.1 | The first-order polynomial model of orthogonal experimental design

The mechanisms of the isoflavone extraction process involves a mechanical stirring extraction method, which is a conventional extraction method using a solvent to directly extract from solid to liquid. This method utilizes only the stirring heating without relying on any other energy source such as ultrasonic energy or microwave energy. Therefore, the content of isoflavones in the extract increased by six times the initial isoflavone components in the extraction process. This method also shows no reciprocal transformation or composition changes. The optimization of the isoflavones extraction was then performed to increase the extraction efficiency by evaluating three factors of impact including temperature, extraction time, and solvent to material ratio. Based on the above single variable experiments, the optimization of isoflavones from the soy flour was conducted by the application of 2^3 full factorial design. The experimental design was presented in Table 3. All experiments were conducted according to a random order derived from Minitab 18 and experimental results derived from the first order of orthogonal matrix model with 2^3 full factorial design are presented in Table 4.

The results of analyzing regression and variance by Minitab version 18 showed the *F*-value of the model was high at 82.05 and had the *p*-value of 0.012 (<0.05), which indicated that the model result shows significant difference between the coefficients. The regression coefficients of x_1, x_2, x_3 variables and x_1x_3 interaction variable represented at 0.005, 0.03, 0.004, and 0.009, respectively. These values had the p-value smaller than the significant level ($\alpha = 0.05$) where the variables of temperature, time, the ratio of solvent to material were in a proportional effect on the total

TABLE 3 Levels of experimental design of factors

	Levels of factor			
Factor	Low level (−)	Center level (0)	High level (+)	Interval (λ)
Temperature, X_1 (°C)	50	60	70	10
Time, X_2 (min)	45	60	75	15
Ratio of solvent to material, X_3	15:1	20:1	25:1	5

TABLE 4 The first-order orthogonal experimental matrix and experimental results

		Coded Variables			Natural variables			Response
Run	x_0	x_1	x_2	x_3	X_1(°C)	X_2(min)	X_3(solvent/material)	$\tilde{Y}$ (ug/g)
1	+	+	+	+	70	75	25/1	1,919.45
2	+	−	+	+	50	75	25/1	1,870.35
3	+	+	−	+	70	45	25/1	1,865.85
4	+	−	−	+	50	45	25/1	1,834.85
5	+	+	+	−	70	75	15/1	1,863.24
6	+	−	+	−	50	75	15/1	1,661.34
7	+	+	−	−	70	45	15/1	1,826.01
8	+	−	−	−	50	45	15/1	1,587.96
C1	0	0	0	0	60	60	20/1	1,750.08
C2	0	0	0	0	60	60	20/1	1,744.32
C3	0	0	0	0	60	60	20/1	1,726.16

Note: x_0 is the point without variable; *C* is the center point and *Y* is the target function (response).

extracted amount of isoflavones. Meanwhile the interaction between temperature and the ratio of solvent to material had a reversed effect on the target function $\tilde{Y}$. The analyzing of the Pareto plot, Main Effects Plot, Interaction Plot, and Normal Plot of the Standardized Effects was done according to Montgomery (2017) (Montgomery, 2017).

After removing non-affecting factors, we constructed the following regression Equation (4):

$$\tilde{Y} = 1\,803.6\,3 + 6\,5.00x_1 + 2\,4.9\,6x_2 + 6\,8.9\,9x_3 - 4\,4.9\,8x_1x_3 \quad (4)$$

Using ANOVA to test the fitting of chosen model showed the "Lack-of-fit," the value used to indicate the lack of fit of estimated model compared with experimental model, was equal to 0.536, which was much higher than $\alpha = 0.05$, exhibiting the lack of model fit was not significant in probability. The value R^2_{Adj} of 0.9848 showed a tight corresponding relationship between the theoretical results and practical results. Both Pareto plot and Normal Probability Plot also represent the fit degree of regression equation $\tilde{Y}$. However, the value of CtPt had the *p*-value smaller than $\alpha = 0.05$ so that there was a significant difference between experiments at the center and the coefficient of both due to experimental error. Consequently, these mentioned results showed that the first-order regression model chosen was adequate. Pareto plot and normal probability plot in Figure 4 showed that the distribution of residuals is very close to the standard distribution of the regression equation (Montgomery, 2017).

Figure 5 presents factors affecting mainly the regression equation in three-dimensional surface plots. Figure 5a and c showed linear on the surfaces, meanwhile the surface in Figure 5b was curved due to the interaction between two factors of temperature and ratio of solvent to material. This concludes that the analyzing model is linear and there is a need to conduct the steepest ascent experiments to find out the maximum value of target function according to Box-Wilson model.

3.3 | Optimization of the extraction by steepest ascent

An experimental design based on the direction of steepest ascent with Box-Wilson model from center point C1 to find out the maximum of

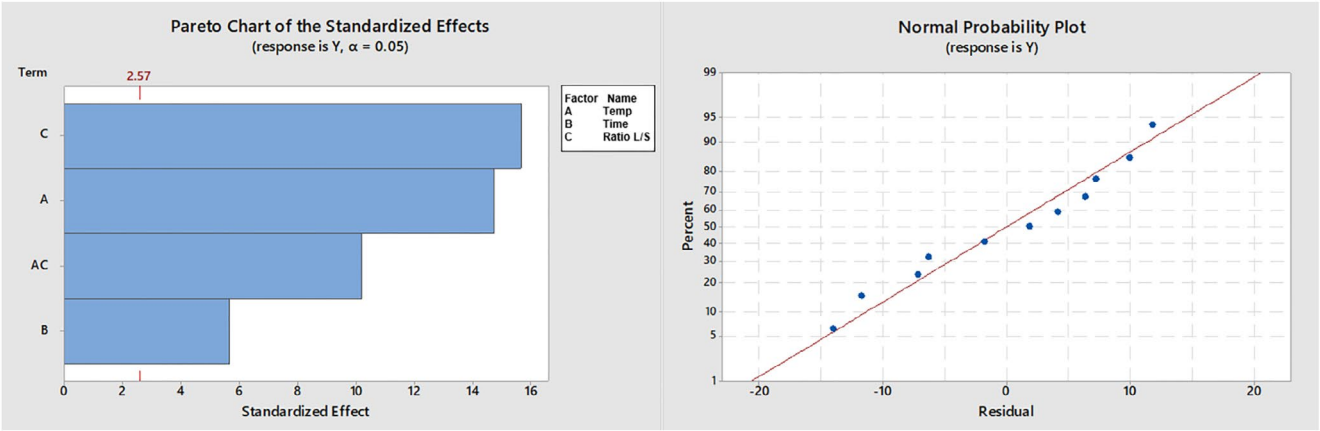

FIGURE 4 Pareto chart and normal probability plot

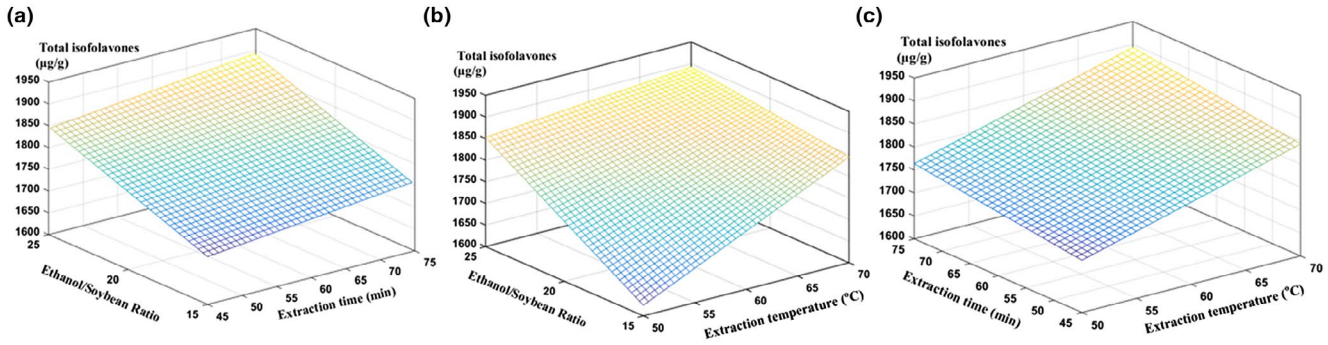

FIGURE 5 Three-dimensional plot of the regression equation: (a) Effect of ratio of solvent to material and extraction time on total isoflavones; (b) Effect of ratio of solvent to material and extraction temperature on total isoflavones; (c) Effect of extraction time and extraction temperature on total isoflavones

TABLE 5 Content of isoflavones versus steps along the path of steepest ascent

	Coded Variables			Natural variables			Response
Step	x_1	x_2	x_3	Z_1 (°C)	Z_2 (min)	Z_3 (solvent/material)	Y (μg/g)
Δ_i	1.000	0.576	0.531	2.5	1.5	1.3	
C1	0	0	0	60.0	60.0	20.0	1,739.74
C1 + Δ_i	1	0.576	0.531	62.5	61.5	21.3	1,769.544
C1 + $2\Delta_i$	2	1.152	1.062	65.0	63.0	22.6	1,839.74
C1 + $3\Delta_i$	3	1.728	1.593	67.5	64.5	23.9	1,857.67
C1 + $4\Delta_i$	4	2.304	2.124	70.0	66.0	25.2	1,887.53
C1 + $5\Delta_i$	**5**	2.880	2.655	**72.5**	**67.5**	**26.5**	**1,932.44**
C1 + $6\Delta_i$	6	3.456	3.186	75.0	69.0	27.8	1,857.60
C1 + $7\Delta_i$	7	4.032	3.717	77.5	70.5	29.1	1,646.34

Note: *Z* is the notation to distinguish the natural variable of the calculation for the steepest ascent with the orthogonal experimental design. The row at the fifth step presented as the maximum of target function Y is set in bold type.

the target function along with corresponding results was presented in Table 5. As can be seen from the results, the optimal extraction conditions were chosen as follows: temperature of 72.5°C, extraction time of 67.5 min, the ratio of solvent to material of 26.5/1 at which the total amount of isoflavones achieved the maximum of 1,932.44 (μg/g). Previously, reports about the isoflavone extraction from soybeans often used high temperature, long extraction time along with the low ratio of solvent to material. For instance, Zhang et al., (2007) reported the extraction condition with the temperature of 80°C in 8 hr, using the pure solvent of ethanol (Zhang et al., 2007), and Cho et al., (2009) indicated the conditions to be ethanol concentration of 80%–90% in 95 min at the temperature of 85°C, and ratio of solvent to material of 5/1 (Cho et al., 2009). The conditions for extraction of isoflavones from the root of *P. lobate* were obtained: ethanol concentration = 90%, extraction temperature = 90°C, extraction time = 2 hr, ratio of solid to liquid = 1/6 (g/ml), and three time the extraction cycles (Wang et al., 2014). However, Wang et al. (2013) the extraction condition was as follows: ethanol 65% at pH 9, temperature of 70°C, extraction time of 60 min, ratio of solvent to material of 15/1 (Wang et al., 2013), meanwhile Lakshmi et al., (2013) selected the condition of 44°C, 105 min, 78% ethanol, ratio of solvent to material of 15/1 for magnetic stirring extraction method with heating, decreasing extraction of time, and temperature to avoid the degradation of isoflavone compounds (Lakshmi et al., 2013). Thus, the extraction condition in this study has the same trend of extracting isoflavone in a short time as compared to traditional extraction methods.

4 | CONCLUSION

This study investigated the effect of factors such as pH, extraction temperature, extraction time, solvent concentration, and ratio of solvent to material on the isoflavone extraction process from soybeans using the solvent extraction. The first-order orthogonal experimental model was successfully applied in the isoflavone extraction conditions using mechanic stirring extraction method. The model considered the main effects of temperature, time and ratio of solvent to material and optimized the temperature, time and ratio of solvent to material on the total extracted amount of isoflavones. The optimal extraction conditions chosen from the model were 72.5°C for temperature, 67.5 min extraction time, ratio of solvent to material of 26.5/1 by which the maximum amount of the total extracted isoflavones was 1,932.44 (μg/g) using stirring extraction method with the solvent of 80% (v/v) ethanol.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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