ISSN: 2277-9655
(I2OR), Publication Impact Factor: 3.785

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

OPTIMIZATION THE EXTRACTION PROCESS FOR DETERMNATION OF FLAVONOIDS AND ANTIOXIDANT CAPACITY FROM SOYBEAN SEEDS

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ABSTRACT

Optimization the extraction process of flavonoids from soybean seeds (*Glycine max L.)* by response surface methodology (RSM) in conjunction with central composite design (CCD) was performed. The maximum yield of total flavonoids content (TFC) and the 2,2-diphenyl-1-pierylhydrazyl (DPPH) free-radical scavenging potential were used as quality indicators. The extraction was carried out three cycles by using acetone as solvent. The range of the independent variables, namely acetone concentration (60–80%, v/v), the ratio of solvent and material (6:1–10:1, v/w), the extraction temperature (30–50°C) and time for each cycle (180–240 minutes) were identified by a first set of single and two factor experiments. The optimum conditions for extraction were found to be at acetone concentration 69%, the ratio of solvent and material 8:1 (v/w), extraction temperature 42°C and 184 minutes for each cycle extraction. Under these optimized conditions, the experimental yields of TFC and DPPH free-radical scavenging potential were respectively 2.2 ± 0.01 mg QE/g DW and 79.49 ± 0.16% which were in close agreement with predicted values, This indicates the suitability of the models developed and the success of RSM in optimizing the extraction conditions.

KEYWORDS: Response surface methodology, extraction, total flavonoids content, antioxidant capacity, soybean seeds.

INTRODUCTION

Soybean *(Glycine max L.)* is one of the most commonly consumed legumes worldwide [1]. Soybean intake has received increasing interest not only by its high nutritional contents, but also because of health benefits. These health benefits have been ascribed substantially to the bioactivity of soy flavonoids, including their antioxidant effects [2]. Therefore, the soybean has been the subject of several studies around the world, especially in respect to the determination of their antioxidant capacities.

Several methods have been employed to evaluate the antioxidant capacity of plant materials. The antioxidant capacity assays are performed after an initial antioxidant compounds extraction step. The chemical composition of material plays an important role in its biological activity, therefore the extraction process should be stabilize the bioactive compounds, especially for flavonoids and other phenolics [3]. The extraction step is very important because the yield, composition, and purity of antioxidant substances are dependent on the applied extraction method [4], as well as its variables such as: solvent type, solvent concentration, temperature, time and solvent to solid ratio [5-7]. With the aim to determine accurately the antioxidant capacity of materials, the parameters affecting the extraction process should be investigated in order to optimize the recovery of the bioactive compounds.

The optimization of extraction processes may be achieved by statistical and empirical models. Response surface methodology (RSM) can be described as a technique that involves complex calculation for optimization process [8, 9]. This method is based on the adjustment of a polynomial equation and symmetrical models to the experiment data to describe the behavior of the independent variables [10]. The main objective of the present work is the optimization of the extraction procedure to determine flavonoid compounds and antioxidant capacity of soybean seeds using response surface methodology.

MATERIALS AND METHODS

Preparation of samples

Soybeans (*Glycine max L.*, MTĐ 760 variety) were supplied from Department of Agricultural Genetic, College of Agricultural and Applied Biology, Cantho University, Vietnam. The cleaned soybeans were ground, defatted in a Soxhlet extractor for 10h with petroleum ether [11] and then were stored at 5^{o}C after removal of petroleum ether. For each treatment, a mass of 0.5g of defatted soybean powder was extracted using aqueous acetone as solvent with shaking for three cycles. The triplicates extracts were combined for determination the TFC and antioxidant potential [12].

Determination of the TFC

The TFC was determined by the Dowd method with slight modification [13] and using a standard curve of quercetin. Thus, the results were expressed as milligrams of quercetin equivalents (QE) per g of dry matter sample (mg QE/g).

Antioxidant capacity

Antioxidant activity of the phytochemicals extracted from soybean was assessed by measuring their radical scavenging activity that was measured by the bleaching of the purple-coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses stable DPPH radical as a reagent. The DPPH radical scavenging activity was evaluated from the difference in peak area decrease of the DPPH radical detected at 517nm between a blank and a sample [14].

Experimental design

The single factor experimental was employed to guide the preliminary range of variables including X_1 (acetone concentration), X_2 (acetone solvent to material ratio), X_3 (extraction temperature), and X_4 (extraction time). A central composite design (CCD) consisted of 32 experimental points, including eight replications of the centre points was used to investigate the effects of four independent variables, X_1, X_2, X_3 and X_4 on the yield of TFC (Y_1) and DPPH free-radical scavenging potential (Y_2). In detail, X_1 (60, 70, 80; %, v/v), X_2 (6, 8, 10; v/w), X_3 (30, 40, 50; ^{o}C) and X_4 (180, 210, 240; min) were investigated, respectively.

Statistical analysis

The response variables were fitted to a general form of quadratic polynomial model that was showed in equation (1):

$$Y = \beta_0 + \sum\beta_i x_i + \sum\beta_{ii} x_i^2 + \sum\beta_{ij} x_i x_j \quad (1)$$

Where Y represents the predicted responses; β_0, β_i, β_{ii} and β_{ij} are the coefficients of intercept, linear, squared and interaction; x_i and x_j are the independent variables. Portable Statgraphics Centurion software was used to estimate the response of each set of experimental design and optimized conditions. The fit of the models was inspected by the regression coefficient R^2.

RESULTS AND DISCUSSION

Fitting the models

The response surface methodology was conducted with the aim to determine the experimental conditions for optimal total flavonoids concentration and free radical scavenging activity. The employed experimental design and the data of TFC and the DPPH radical scavenging activity from the experiment at different experimental combinations for the independent variables are presented in Table 1. Experimental results from Table 1 were processed with multiple linear regressions using the second-order polynomial model. The regression equations (2) and (3) expressed the relation between the response variables and independent variables with significant coefficients. All response values from equation (2) and (3) were shown to fit best the second order polynomial model, due to the values (R^2) were 96.3% and 96.5% for the response models of TFC and DPPH radical scavenging capacity, respectively. The closeness to 1 of these R^2 values indicates a high degree of correlation between the observed and predicted values. The accuracy of the response surface equations can be proved by comparing experimental value and predicted data. As can be seen from Figure 1, the agreement between predicted values and experimental values was proved because both the slopes of regression equations and R^2 values were in close to 1. Therefore, it was suggested that quadratic model fitted well with the experimental data.

Table 1. Central composite design and results of TFC, DPPH radical scavenging

Run	Variables levels				Y_1	Y_2	Run	Variables levels				Y_1	Y_2
	X_1	X_2	X_3	X_4				X_1	X_2	X_3	X_4		
1	60	6	30	180	1.823	74.813	17	70	8	40	150	2.064	79.366
2	90	8	40	210	1.261	67.456	18	70	8	40	210	2.164	79.392
3	60	10	50	180	1.787	75.832	19	70	8	20	210	1.163	69.075
4	60	6	30	240	1.535	74.583	20	60	6	50	240	1.737	74.984
5	70	8	40	210	2.202	79.589	21	70	8	40	210	2.202	79.613
6	70	8	40	210	2.180	79.574	22	80	6	30	180	1.740	72.817
7	60	10	50	240	1.776	75.279	23	70	8	40	210	2.182	79.544
8	70	4	40	210	1.769	76.330	24	80	6	50	180	1.887	76.412
9	60	10	30	180	1.845	74.710	25	70	8	40	210	2.171	79.538
10	60	10	30	240	1.627	74.189	26	80	6	30	240	1.629	72.182
11	70	8	60	210	1.480	73.887	27	80	10	50	180	1.720	76.895
12	80	6	50	240	1.698	74.897	28	80	10	30	180	1.516	72.774
13	70	8	40	210	2.240	79.524	29	70	8	40	210	2.231	79.559
14	60	6	50	180	1.880	74.672	30	80	10	50	240	1.654	75.883
15	50	8	40	210	1.414	74.615	31	80	10	30	240	1.465	70.876
16	70	8	40	270	1.967	78.124	32	70	12	40	210	1.959	77.007

$$TFC = -12.20 + 0.28^{*} \times X_1 + 0.34^{*} \times X_2 + 0.15^{***} \times X_3 + 0.005^{**} \times X_4 - 0.002^{***} \times X_1^2 - 0.0177^{***} \times X_2^2 + 0.0004^{*} \times X_2 \times X_4 - 0.002^{***} \times X_3^2 - 0.000037^{*} \times X_4^2 \quad (R^2 = 0.963) \quad (2)$$

$$DPPH = -63.44 + 2.77^{***} \times X_1 + 1.02^{***} \times X_3 + 0.152^{*} \times X_4 - 0.02^{***} \times X_1^2 + 0.008^{***} \times X_1 \times X_3 - 0.179^{***} \times X_2^2 - 0.02^{***} \times X_3^2 \quad (R^2 = 0.965) \quad (3)$$

Where,

X_1: acetone concentration (%); X_2: acetone solvent to material ratio (v/w); X_3: extraction temperature (°C) and X_4: extraction time (min).

* Significant at $p < 0.05$; ** Significant at $p < 0.01$; *** Significant at $p < 0.001$.

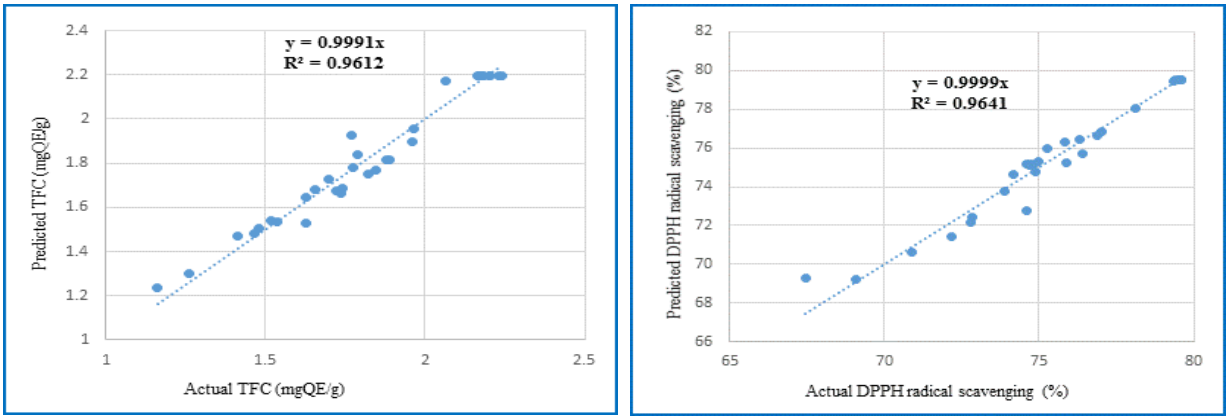

Figure 1. Comparison of predicted and actual values of the study

Influence of extraction parameters on the model responses

The response surface plots of TFC and DPPH radical scavenging are presented in Figure 2. Figure 2 showed the relationship between TFC and DPPH radical scavenging to extraction temperature and time, with acetone 69% as solvent and solvent to soybean ratio of 8:1 (v/w). Figure 2 showed that, the extraction temperature and time play an important role in determining flavonoid yields and DPPH radical scavenging capacity. These responses reached to maximum values of around 2.25mg QE/g and 80.5% respectively, while extraction temperature and time were approximate 40–43ºC and 180 min. Extraction temperature impacts the solubility, mass-transfer rate, and stability of flavonoid compounds [15]. Below a certain limit, higher temperatures improve the efficiency of extraction due to the increased solubility and diffusion coefficients of flavonoids; decreased solvent viscosity; enhanced mass transfer and penetration of solvent into the material matrix [16, 17]. Elevating the temperatures up to a certain level might be followed by their possible decomposition and degradation of flavonoid compounds due to the hydrolysis, internal redox reactions and polymerizations [18, 19].

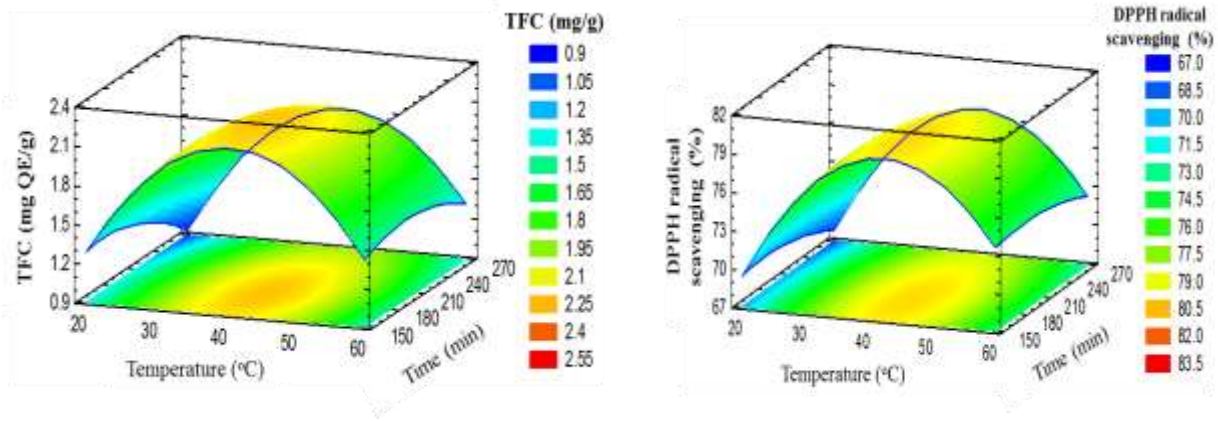

Figure 2. Response surface plots showing the effect extraction temperature and time on the TFC and DPPH radical scavenging response (solvent of acetone 69%, solvent to soybean ratio of 8:1, v/w)

The optimum extraction time is considered the time required for final equilibrium between the solute concentrations in the solid matrix and in the solvent being reached [20]. Prolonged extraction time increases the chance of decomposition and oxidation of flavonoids due to their long exposure to unfavorable environmental factors like temperature, light and oxygen [4] leading to deceleration in the extraction yield.

Optimization procedure

Numerical optimization section and desirability function were used to determine the values of parameters that accommodate the optimum conditions. The optimum parameters obtained were showed in Table 2. To apply the experimental method, the extraction conditions were adjusted according to actual production condition. These values are as following: acetone concentration of 69%, solvent-to-material ratio of 8:1 (v/w), extraction temperature 42°C and time 184 min (Table 2).

Table 2. Optimum conditions and the value of responses

Variables	Acetone (%)	Solvent: soybean ratio (v/w)	Extraction temperature (ºC)	Extraction time (min)	Optimum conditions	Verified conditions	Experiment(1)
TFC (mg QE/g)	68.9	7.7	41.2	184.5	2.23	2.21[a]	2.20[a] ± 0.01
DPPH radical scavenging (%)	69.0	8.4	42.7	183.9	79.91	79.73[a]	79.49[a] ± 0.16
Verified Values	69.0	8.0	42.0	184.0			

(1): Data are presented as mean from triplicate of experimental run ± SD
(The same letters within row indicate not significant difference by t' test from hypothesis for mean at 5 % level)

A triplicate experiment was set up to validate the optimized conditions. As shown in Table 2, the experimental data were in good agreement with the predicted values due to the verification values for TFC and DPPH radical scavenging obtained are not significant different with predicted values. This clearly showed that the model fitted very well to the experimental data and therefore the optimization of the flavonoids and antioxidant extraction was efficient within the specified range of process parameters.

CONCLUSION

Base on the experiment results, the experimental values agreed with the predicted values, using hypothesis testing for mean, indicating an excellent fit of the model used and the success of response surface methodology for modeling extraction of soybean seed. The optimal values of variables were determined at acetone concentration 69%, solvent to soybean ratio 8:1 (v/w), extraction temperature 42°C and time at 184 minutes. Under these extraction conditions, the experimental yields of TFC and DPPH free-radical scavenging potential were respectively 2.2 ± 0.01 mg QE/g DW and 79.49 ± 0.16% which were in close agreement with predicted values.

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