Optimization of supercritical fluid extraction of daidzein from soybean after extrusion

Zhou Quancheng, Li Lei, Shen Dechao

(*School of Light Industry and Agricultural Engineering*, *Shandong University of Technology*, *Zibo* 255091, *China*)

Abstract: A set of optimal conditions for supercritical fluid extraction (SFE) of daidzein in soybean previously subjected to extrusion at the rotate speed of screw 4.5×*g* and the temperature of 80℃ was developed. The quadratic orthogonal rotation design with five factors (extracting temperature, extracting time, ethanlol content, cosolvent quantity and flow rate) was used and a mathematical model was developed based the experimental results. Optimal conditions were verified and compared with control experiments conducted in parallel. The results from SFE showed that all five experiment factors had significant effects on the yield of daidzein. The mathematical model agreed well with the experimental results. It was found that there were 27 optimal extract conditions under which daidzein yield was higher than 0.092%. The optimal extracting conditions were stable and reproducible. Results from the control experiments showed that extrusion could significantly enhance the extraction of daidzein. The extraction of daidzein from extruded soybean and its products with supercritical CO_2 is a potential technique for practical applications in the future.

Key words: extraction, daidzein, supercritical, extrusion, cosolvent, mathematical model

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1 Introduction

Interest in soybeans and soy based products grew significantly in the last decade due to their reported nutritional and health-promoting benefits. Researchers have credited phytochemicals in soybean, especially isoflavone, for some of these beneficial health effects. Soy isoflavone was reported playing a role in the prevention of osteoporosis, and several hormonally influenced cancers[1,2] and acting as phytoes trogens in humans[3,4]. Isoflavone as antioxidants may also prevent oxidative damage in living tissue[5]. Daidzein, one of the major isoflavones in soybeans, is associated with a broad variety of benefits to human health[6], such as prevention of cancer and angiocardiopathy.

Extrusion is an important and popular food processing technique classified as a high temperature, high pressure/short time process to promotes both cell wall disruption and oilseed microstructural changes that seem to favor extraction of oil and bioactive ingredients[7,8]. Cell wall disruption, which accelerates mass transfer and extraction kinetics, can be obtained either by mechanical or enzymatic treatment, or a combination of both.

Traditional methods for the extraction of plant materials include steam distillation and organic solvent extraction using percolation, maceration or Soxhlet techniques[9,10]. These methods, however, have distinct drawbacks such as being time-consuming and labor-intensive operations, involving large volumes of hazardous solvents and extensive concentration steps,

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Biographies: Zhou Quancheng, Ph.D, Professor, majored in Agricultural Product Processing. **Li Lei**, graduate student. Email: loilei@163.com. **Shen Dechao,** Professor, research in Agricultural Product Processing.

Corresponding Author: Zhou Quancheng, Ph.D, professor, Member of CSAE. School of Light Industry and Agriculture Engineering, Shandong University of Technology, Zibo 255049, China. Tel: 15964483027. Email: zhouquancheng@126.com

which can result in the loss or degradation of target compounds. Moreover, there is an increasing interest for alternative extraction technologies that consume less organic solvents, because of the rising solvent acquisition, disposal cost and regulatory restrictions.

Supercritical fluid extraction (SFE) offers several advantages over conventional solvent extraction methods. SFE can penetrate into the pores of solid materials more effectively than techniques based upon liquid solvents, and therefore it enables a much faster mass transfer, resulting in faster extractions. For instance, the extraction time can be reduced from hours or days for a liquid—solid extraction to a few minutes for SFE, with comparable or better recoveries. Also, in SFE, fresh fluid is continuously pumped through the samples. It can provide quantitative or complete extraction. The solvation power of the fluid can be manipulated by adjusting the pressure and/or temperature, facilitating a remarkable high selectivity[10-15]. The most important advantage of utilizing supercritical CO_2 is easy separation of the solvent from the extracted material and operation at an ambient temperature that does not affect the heat sensitive compounds. Further, supercritical CO_2 provides lower mass transfer resistance than solvents used in conventional separation processes. These advantages have attracted an increasing interest from researchers and especially from the food, pharmaceutical and environmental-engineering industries.

However, due to the limited solubility of polar organic compounds in supercritical CO_2 or to their interaction with the matrix, quantitative extraction of these compounds with pure supercritical CO_2 is impossible. The addition of a polar modifier (e.g, methanol) to supercritical CO_2 is the simplest and most effective way to obtain the desired polarity of CO_2-based fluids. Modifiers can also overcome interactions between the analyte and the matrix, increasing the extraction efficiency of polar organic compounds[11,15].

In the present study, SFE was used to extract isoflavone aglycones (daidzein) from soybean after extrusion, and the extracts were analyzed using colorimetric method without any further treatment. Extraction conditions were adjusted in order to obtain the highest yield of daidzein, and the influence of the extraction conditions of the method was examined. The results obtained were compared with the results from the corresponding control experiments.

2 Materials and methods

2.1 Materials

Carbon dioxide (99.99% pure) supplied in a cylinder with a dip tube was purchased from Haifeng Gas Co. Ltd. (Zibo, Shandong, China). Daidzein was obtained from the National Institute for the Control of Pharmaceutical and Biological Product, Beijing, China, and other solvents were of analytical grade (99%). Daidzein content in soybean purchased from supermarket was (0.21±0.02)%.

2.2 Sample preparation

The soybean was pulverized and then sieved to obtain particles with diameters ranging from 1.5 to 2.0 mm. Pulverizer was made by Kunsan Qiangwei Fenti Shebei Co. Ltd. (Kunshan, Hebei, China). Single-screw extrusion machine was designed and made by professor Shen Dechao. The unit was equipped with a die with four openings at 10 mm in diameter and 12 mm long. The length and diameter of screw were 600 mm and 80 mm, respectively. The barrel consisted of three independently controlled heating barrels. The barrel temperatures, measured via Fe-CuNi thermo elements inserted in the bottom of each barrel, during the process were 85℃ maintained by electric heater. Soybean flakes contained 10% moisture content were fed into the unit and processed via the intermeshing rotating screw at a constant 2.8×*g*. Processed material was fed through until equilibrium conditions were reached before material was collected for experimental use. Extruded soy pellets were collected directly and dried to moisture content of 3% at 25℃ in the hot air drier made by Hangzhou Dingsheng Co. Ltd. (Hangzhou, Zhejiang, China). Extrusion conditions under which oil yield was highest were selected according to research results of Xu Honghua[20] and results of preliminary tests.

2.3 Photospectrometry method for quantification analysis of daidzein

2.3.1 Standard curve

The standard curve of daidzein which was used as the benchmark for yield determination was obtained as follows. A solution consisting of daidzein (0.8 mg/mL) was prepared through 200 mg daidzein solved in 250 mL double distillated water. Different volumes of 0, 0.1, 0.2, 0.4, 0.8, 1.2 and 1.6 mL were transferred into 10 mL test tubes, respectively. Then methanol was added to make the total volume being 10 mL. After being cooled to room temperature, with a blank solution as the reference, the absorbance was scanned using a double beam UV/Vis spectrophotometer in the range of 200−700 nm. Scanning results of daidzein showed that the maximum adsorption was at 249 nm, so the absorbance A at Vis 249 nm was determined with a glass cell of 10 mm.

The concentrations of daidzein isoflavone in the samples were calculated from standard curves calibrated using the daidzein standard. The calibration curve (correlation coefficients) for daidzein was Y=6.51X－0.1007, R^2=0.998, where Y is the adsorption value of daidzein, X is the content of daidzein, r is coefficient.

2.3.2 Determination of extracted compounds

The extracted compounds were dissolved in methanol to a designed concentration and 0.05 mL extract solutions were added to a tube. The absorbency of the sample was determined by the colorimetric method as described in Section 2.3.1. The contents of daidzein in extracted compounds were determined by reading the values from the standard curve.

2.4 Supercritical fluid extraction

2.4.1 Supercritical fluid extraction method

A supercritical-fluid extractor with a 1000 mL stainless steel thimble (Huali SFE Instrument Company, Nantong, Jiangsu, China) was used. The extractor employed a variable restrictor to allow an instant depressurization of the supercritical fluid and the decoupling of flow and pressure in order to control the pressure independently of the supercritical fluid flow rate. An amount of 100 g of extruded soybean was weighed and packed into the thimble. The SFE extraction at 70℃ and 25 MPa which was selected according to preliminary results was performed for 3 h in order to extract soybean oil. Then the static extraction was performed for 0.5 h under the conditions of 20−40 MPa pressure, 40−80℃ and 9−13 kg/h of CO_2 flow rate after a co-solvent was pumped into the SFE extractor. The dynamic SFE extraction was carried out for 3 h, which was set when there was no matrix extracted from extract vessel by supercritical CO_2 after trial and error. The SFE extract was collected on a solid-phase trap held at 40−80℃ and 20−40 MPa.

2.4.2 Experimental design

The quadratic orthogonal rotation design with five factors was used to study the model equations and determine the optimal combination of variables. The effects of independent variables X_1 (extracting temperature, T), X_2 (extracting pressure, P), X_3 (ethanol content, E), X_4 (cosolvent quantity, Q) and X_5 (flow rate, F), at five variation levels (Table 1) in the extraction process on the yield of daidzein from soybean, are shown in Table 2. Ten replicates (treatments 27−36) at the centre of the design were used for the estimation of a sum of squares pure error. Experiments were randomized in order to maximize the effects of unexplained variability in the observed responses due to extraneous factors. The co-solvent in this study consisted of ethanol and methanol in 3:7−7:3 ratio. Ethanol is innoxious and has very high solvency and methanol is defined as polar matrix. Ethanol content is the ratio of ethanol volume to the volume of ethanol and methanol.

The variables were coded according to the following equation

$$x_i=(X_i-\overline{X}_i)/\Delta X_i \quad (1)$$

where x_i is the dimensionless value of an independent variable; X_i is the real value of an independent variable; $\overline{X}_i$ is the real value of an independent variable at the center point; ΔX_i is the step change. The specific codes are:

$$x_1=(T-60)/10 \quad (2)$$

where x_1 is the specific code of X_1 (extracting temperature, T) and

$$x_2=(P-30)/5 \quad (3)$$

where x_2 is the specific code of X_2 (extracting pressure, P) and

$$x_3=(E-50)/25 \quad (4)$$

where x_3 is the specific code of X_3 (ethanol content, E)

and

$$x_4=(Q-50)/10 \quad (5)$$

where x_4 is the specific code of $X4$ (cosolvent quantity, Q) and

$$x_5=(F-11)/1 \quad (6)$$

where x_5 is the specific code of X_5 (flow rate, F).

Table 1 Factors and levels of experiments

Levels	Extracting temperature X_1 /℃	Extracting pressure X_2 /MPa	Ethanol content X_3 /%	Cosolvent quantity X_4 /mL	Flow rate X_5 /kg·h^{-1}
2	80	40	100	70	13
1	70	35	75	60	12
0	60	30	50	50	11
-1	50	25	25	40	10
-2	40	20	0	30	9

Table 2 Structured matrices and experiment results

No.	x_1 /℃	x_2 /MPa	x_3 /%	x_4 /mL	x_5 /kg·h^{-1}	Daidzein yield /%
1	1	1	1	1	1	0.051
2	1	1	1	-1	-1	0.031
3	1	1	-1	1	-1	0.055
4	1	1	-1	-1	1	0.047
5	1	-1	1	1	-1	0.041
6	1	-1	1	-1	1	0.038
7	1	-1	-1	1	1	0.045
8	1	-1	-1	-1	-1	0.036
9	-1	1	1	1	-1	0.059
10	-1	1	1	-1	1	0.058
11	-1	1	-1	1	1	0.043
12	-1	1	-1	-1	-1	0.049
13	-1	-1	1	1	1	0.045
14	-1	-1	1	-1	-1	0.048
15	-1	-1	-1	1	-1	0.041
16	-1	-1	-1	-1	1	0.041
17	2	0	0	0	0	0.036
18	-2	0	0	0	0	0.043
19	0	2	0	0	0	0.053
20	0	-2	0	0	0	0.037
21	0	0	2	0	0	0.051
22	0	0	-2	0	0	0.054
23	0	0	0	2	0	0.048
24	0	0	0	-2	0	0.043
25	0	0	0	0	2	0.040
26	0	0	0	0	-2	0.036
27	0	0	0	0	0	0.040
28	0	0	0	0	0	0.039
29	0	0	0	0	0	0.039
30	0	0	0	0	0	0.039
31	0	0	0	0	0	0.038
32	0	0	0	0	0	0.037
33	0	0	0	0	0	0.037
34	0	0	0	0	0	0.038
35	0	0	0	0	0	0.038
36	0	0	0	0	0	0.036

2.5 Verification of optimal conditions

Three groups randomly chosen from optimal extracting conditions mentioned in 3.2 were conducted in duplicate, in order to validate the optimal extracting condition.

2.6 Control experiments

Pulverized soybean seed (5 g) was weighted and placed in a distillation flask. Extraction was carried out using 10 mL methanol 80% in water (V/V) as the solvent for 2 h with agitation by a magnetic stirrer. The crude extract was centrifuged at 400×g for 20 min. This was control Experiment 1(C_1). Extracting condition and operating procedure in control Experiment 2 (C_2) were the same to C_1 except that the experiment matrix was extruded soybean seed (5 g). The experiment matrix was pulverized soybean seed (100 g) in control Experiment 3 (C_3). SFE extracting conditions were randomly chosen from optimal extract conditions mentioned in 3.2.

2.7 Statistical analyses

The average yield of the duplicate values obtained was taken as the dependent variable, Y. The model proposed for the yield of daidzein is given below:

$$Y_d=\alpha+\alpha_1x_1+\alpha_2x_2+\alpha_3x_3+\alpha_4x_4+\alpha_5x_5+\alpha_{11}x_1^2+\alpha_{12}x_1x_2+\alpha_{13}x_1x_3+\alpha_{14}x_1x_4+\alpha_{15}x_1x_5+\alpha_{22}x_2^2+\alpha_{23}x_2x_3+\alpha_{24}x_2x_4+\alpha_{25}x_2x_5+\alpha_{33}x_3^2+\alpha_{34}x_3x_4+\alpha_{35}x_3x_5+\alpha_{44}x_4^2+\alpha_{45}x_4x_5+\alpha_{55}x_5^2 \quad (7)$$

where Y_d is predicted yield of daidzein; α is offset term; α_1, α_2, α_3, α_4 and α_5 is linear effect terms; α_{11}, α_{22}, α_{33}, α_{44} and α_{55} are squared effects; α_{12}, α_{13}, α_{14}, α_{15}, α_{23}, α_{24}, α_{25}, α_{34}, α_{35} and α_{45} are interaction effects.

The proportion of variance explained by the polynomial models is given by the correlation coefficients (R^2) of determination. The significance of each coefficient was determined using the Students t-test and specified according to p value. The function (Y_d) =g daidzein from extract/100 g soybean seed extruded, according to the regression equation (7).

The fitted polynomial equation was expressed as model equations of yield in order to visualise the relationship between the yield and experimental levels of each factor and to deduce the optimal conditions. The statistical software used for this study was SAS, version 8.1, by SAS Inc (North Carolina, America).

3 Results and discussion

3.1 Model equations of supercritical extraction of daidzein after extrusion

The following regression equation (8) is an empirical relationship between daidzein yield and the test variables, respectively:

$$Y_d = 0.038111-0.002250x_1+0.003750x_2+0.000333x_3+0.001750x_4+0.000667x_5+0.000333x_1^2-0.000625x_1x_2-0.003625x_1x_3+0.003000x_1x_4+0.001750x_1x_5+0.001708x_2^2-0.000250x_2x_3+0.000875x_2x_4+0.000125x_2x_5+0.003583x_3^2+0.000625x_3x_4+0.001125x_3x_5+0.001833x_4^2-0.002000x_4x_5-0.000041667x_5^2 \quad (8)$$

The value of Pr > F (<0.0001) is under 0.01 in Table 3 and Table 4, which indicates a close agreement between experimental and predicted values of the daidzein yield. The total determination coefficient, R^2 = 0.9795 implies that the sample variations of 91.57% for daidzein are attributable to the experiment factors, namely extracting temperature, extracting pressure, ethanol content, cosolvent quantity and flow rate, respectively.

Table 3 Analysis of variance of daidzein equation

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	0.00167	0.00008374	35.77	<.0001
Error	15	0.00003511	0.00000234		
Corrected Total	35	0.00171			

R^2=0.9795.

Table 4 Daidzein equation analysis of partial regression coefficient significance

Variable	DF	Parameter estimate	Standard error	t value	Pr > |t|
Intercept	1	0.03811	0.00047705	79.89	<0.0001
x_1	1	-0.00225	0.00031230	-7.20	<0.0001
x_2	1	0.00375	0.00031230	12.01	<0.0001
x_3	1	0.00033333	0.00031230	1.07	0.3027
x_4	1	0.00175	0.00031230	5.60	<0.0001
x_5	1	0.00066667	0.00031230	2.13	0.0497
x_1^2	1	0.00033333	0.00027046	1.23	0.2367
x_1x_2	1	-0.00062500	0.00038249	-1.63	0.1231
x_1x_3	1	-0.00363	0.00038249	-9.48	<0.0001
x_1x_4	1	0.00300	0.00038249	7.84	<0.0001
x_1x_5	1	0.00175	0.00038249	4.58	0.0004
x_2^2	1	0.00171	0.00027046	6.32	<0.0001
x_2x_3	1	-0.00025000	0.00038249	-0.65	0.5233
x_2x_4	1	0.00087500	0.00038249	2.29	0.0371
x_2x_5	1	0.00012500	0.00038249	0.33	0.7483
x_3^2	1	0.00358	0.00027046	13.25	<0.0001
x_3x_4	1	0.00062500	0.00038249	1.63	0.1231
x_3x_5	1	0.00113	0.00038249	2.94	0.0101
x_4^2	1	0.00183	0.00027046	6.78	<0.0001
x_4x_5	1	-0.00200	0.00038249	-5.23	0.0001
x_5^2	1	-0.00004167	0.00027046	-0.15	0.8796

The significance of each coefficient in equation (7) was determined using the Student *t* test and *p* value in Table 4. The corresponding variables will be more significant if the absolute *t* value becomes larger and the *p*-value becomes smaller. It can be seen from Table 4 that the variable with the largest effect on yield of daidzein was the quadratic of ethanol content, (x_3^2); followed by the interaction effect of extracting pressure, (x_2); and the interaction effect of extracting temperature and ethanol content, (x_1x_3). Table 5 showed that all five experiment factors had significantly effects on extraction efficiency, synthetically analysed interaction effects, linear effects and quadratic effects of each factor.

Table 5 Analysis of variance of experiments factors

Factor	DF	Sum of squares	Mean square	F value	Pr > F
x_1	6	0.000535	0.000089093	38.06	<.0001
x_2	6	0.000451	0.000075106	32.09	<.0001
x_3	6	0.000651	0.000109	46.37	<.0001
x_4	6	0.000408	0.000067926	29.02	<.0001
x_5	6	0.000144	0.000024037	10.27	0.0001

3.2 Effect analysis of single factors

Equations which show single-factor relations with daidzein yield were obtained through reduction of dimensions.

$$Y_1=0.038111-0.002250x_1+0.000333x_1^2 \quad (9)$$

$$Y_2=0.038111+0.003750x_2+0.001708x_2^2 \quad (10)$$

$$Y_3=0.038111+0.000333x_3+0.003583x_3^2 \quad (11)$$

$$Y_4=0.038111+0.001750x_4+0.001833x_4^2 \quad (12)$$

$$Y_5=0.038111+0.000667x_5-0.000041667x_5^2 \quad (13)$$

where Y_1 is predicted yield of daidzein when single factor is x_1 (extracting temperature). Y_1 is calculated according to equation (9) when x_1 is -2, -l, 0, 1 and 2, respectively. The same calculations were carried out for Y_2, Y_3, Y_4 and Y_5. Table 5 and Figure 1 show: 1) the effect of x_1 (extracting temperature) on daidzein yield is greater than other factors. Daidzein yield decreases with increasing x_1. Because there is negative relation between extracting temperature and solubility of supercritical CO_2, that is to say, solubility of supercritical CO_2 decreases while extracting temperature increases. Thus, daidzein yield decreases with increasing x_1, which agrees with the literature[11]. 2) the effect of x_2 (extracting pressure) on daidzein yield is bigger than other factors.

Solubility of supercritical CO_2 increases with increasing extracting pressure. So daidzein yield increases with increasing x_1, which accords with literature reported[11].

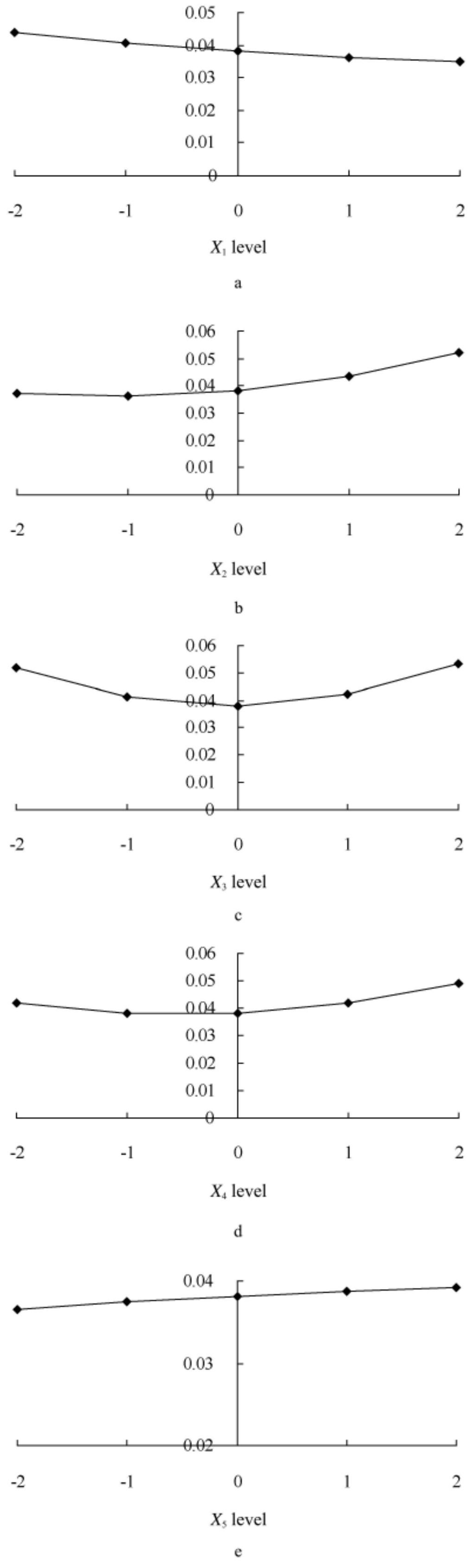

Figure 1 Influences of different supercritical CO_2 parameters on daidzein yield

3) x_3 (ethanol content) represents co-solvent used. The effect of ethanol content on daidzein yield is the biggest of all experiment factors. The choice of ethanol was based on literature data[16-18] that show ethanol to be a very effective co-solvent for the supercritical extraction of isoflavone from different matrices, because ethanol is a highly polar and biocompatible substance. The presence of ethanol (in traces) in the final extracts does not compromise the use of the products in nutraceutical or pharmaceutical applications[19]. Furthermore, methanol, a strongly polar compound, has been shown as a powerful solvent for many isoflavone at ambient condition. According to the molecular structures, hydrogen bonds are expected to be formed between the co-solvent (either ethanol or methanol) and daidzein molecules. Results showed in Figure 1c that daidzein yield is higher when x_3 is at level -2 or 2 than at level 0, because polarity of co-solvent is higher when x_3 was at level -2. On the contrary, when x_3 was at level 2, solvency of co-solvent is higher. 4) the effect of x_4 (co-solvent quantity) on daidzein yield is less of all experiment factors. Results showed in Figure 1d that the lower level of x_4 below level 0 is, the higher daidzein yield is. Oppositely, the higher level of x_4 above 0 is, the higher daidzein yield is. 5) the effect of x_5 (flow rate) on daidzein yield is the least of all experiment factors. Results showed in Figure 1e that the higher level of x_5 is, the higher daidzein yield is. x_5 was so low that polarity or solubility did not significantly change. But the increase in daidzein yield was very small and insignificant.

3.3 Optimal extraction conditions confirmed by simulation

According to the regression equation, 27 extract conditions under which daidzein yield was higher than 0.092% were obtained through simulation. The 27 extract conditions were shown in the Table 6 and were given by thorough consideration of the operating conditions and experimental factors.

3.4 Verification of optimal conditions

The suitability of the model for predicting the optimal yields was tested using the recommended optimal conditions. This set of conditions were determined to be optimal by a quadratic orthogonal rotation design with five factors optimization approach, which were also used to experimentally validate and predict the yields using model equations. The experimental values were found to be in agreement with the predicted ones (Table 7).

Table 6 Extract conditions of daidzein yield higher than 0.092%

No.	x_1 /℃	x_2 /MPa	x_3 /%	x_4 /mL	x_5 /kg·h^{-1}	Predicted yield /%
1	-2	-2	2	-2	2	0.093
2	-2	0	2	-2	2	0.092
3	-2	1	2	-2	1	0.094
4	-2	1	2	-2	2	0.093
5	-2	1	2	2	-2	0.093
6	-2	2	2	-2	-2	0.093
7	-2	2	2	-2	-1	0.096
8	-2	2	2	-2	0	0.095
9	-2	2	2	-2	1	0.093
10	-2	2	2	-2	2	0.093
11	-2	2	2	-1	0	0.093
12	-2	2	2	-1	1	0.095
13	-2	2	2	-1	2	0.092
14	-2	2	2	1	-2	0.093
15	-2	2	2	1	-1	0.094
16	-2	2	2	2	-2	0.097
17	-2	2	2	-2	-1	0.093
18	-2	2	2	2	0	0.094
19	-1	2	2	2	-2	0.094
20	1	2	-2	2	-2	0.095
21	1	2	-2	2	-1	0.093
22	2	1	-2	2	-2	0.092
23	2	2	-2	2	-2	0.097
24	2	2	-2	2	-1	0.096
25	2	2	-2	2	0	0.095
26	2	2	-2	2	1	0.094
27	2	2	-2	2	2	0.093

Table 7 Results of validation experiments of daidzein yield

No.	x_1 /℃	x_2 /MPa	x_3 /%	x_4 /mL	x_5 /kg·h^{-1}	Predicted yield /%	Experimental yield /%
1	-2	2	2	2	0	0.094	0.095
2	-1	2	2	2	-2	0.094	0.096
3	2	1	-2	2	-2	0.092	0.094

3.5 Control experiments

The purpose of control experiments was to show effects of SFE and extrusion on daidzein extraction. Results of F test for daidzein yield showed that there were significant differences between C_1, C_2, C_3 and verification of experiment (VE), except that there was no significant difference between C_1 and C_3 (Table 8). The explanation for the above results was as follows: Firstly, statistical differences presented by both the experimental data of C_1 and C_2 and the experimental data of C_3 and VE showed extrusion technology could significantly enhance the extraction of daidzein, and compared with C_1, the daizein yield in C_2 increased by 366%. Secondly, SFE was not able to significantly increase the daidzein yield of unextruded soybean seed. Finally, Table 8 indicated that the effect of extrusion on daidzein yield was higher than that of SFE. These conclusions have not previously reported in the literature.

Table 8 Analysis of varacontrol experiments for daidzein

	C_1	C_2	C_3	VE
Daidzein mean yield /%	0.032	0.149	0.030	0.094
Duncan grouping	C	A	C	B

4 Conclusions

The mathematical model of daidzein yield was developed with a wide range of data obtained by combining all data sets. The correlation coefficient (R^2) was 0.9795, offering better predictive performance. Also, mathematical model of daidzein yield can give direct prediction of daidzein yield. Results of control experiments indicate that extrusion technology can significantly enhance the extraction of daidzein and increase in daizein yield by 366%. The daidzein yield by extrusion is better than by other technologies. However, the daidzein yield by supercritical CO_2 from extruded soybean seed is significantly lower by 36.9% than that by organic solvent. To unextruded soybean seed, there is no significant difference between by SFE and by organic solvent. The SFE extraction from extruded soybean seed resulted in over 0.092% yield of daidzein according to optimal extraction conditions. Compared with 13.87% of daidzein recovery from soybean hypocotyls by supercritical CO_2[16], daidzein recovery in this study increase by 43.8%, because soybean seeds were extruded and cosolvents for supercritical CO_2 were used. Increase in daidzein yield by extrusion results from soybean seed cell wall disruption during extrusion, thus daidzein can efficiently move from soybean cell to supercritical CO_2 or organic solvent[7]. On the other hand, cosolvents in this study comprise ethanol and methanol, so it has high solvency and polarity[14]. Thus, it is beneficial to change supercritical CO_2 polarity for extraction of daidzein. The extraction with supercritical CO_2 has a potential in the extraction of aglycones such as daidzein from extruded soybean seed products, as they have a high concentration of isoflavone aglycones[16]. Furthermore, supercritical CO_2 has advantages of no residual solvent and shorter extraction time[11].

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