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Optimization of solvent extraction conditions of Cambodian soybean oil using response surface methodology

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ABSTRACT

The conventional solvent extraction (CSE) method is commonly used to extract oil from oilseeds. Even though the extraction of oil from soybean seeds using the CSE method has been studied extensively, the application of response surface methodology (RSM) has not yet been widely explored. In this paper, the CSE method was employed using three different types of solvents – ethanol (Eth), n-hexane (Hex), and a mixture of Hex:Eth in a ratio of 2:1 – and optimized using RSM to extract the oil from Cambodian soybean seeds. For each solvent, a central composite design (CCD) was applied to optimize the extraction conditions, such as the extraction times (15, 30, and 45 min) and solvent-to-solid ratios (5:1, 10:1, and 15:1 mL/g). This CCD generated 11 experimental runs for each type of solvent. The optimum oil yields when using ethanol, Hex:Eth, and n-hexane were 20.53%, 18.78%, and 17.39%, respectively. The optimum condition was obtained at 15 min extraction time and 15:1 (mL/g) solvent-to-solid ratio for all solvent types. The coefficients of determination of ethanol, Hex:Eth, and n-hexane were 0.9710, 0.9954, and 0.9263, respectively, which mathematically indicated a good model for the prediction. Therefore, the model was considered accurate and reliable for predicting oil yields in this study.

Contribution/Originality: Cambodian soybean oil has not yet been introduced to the market even though Cambodia is an agricultural country. This is the first report on the use of RSM to optimize the interactive effect between solvent-to-solid ratio and extraction time on the Cambodian soybean oil extraction process using different types of solvents.

1. INTRODUCTION

Cambodia is an agricultural country where soybeans are grown for local consumption. In Cambodia, 92% of soybean growers are located in the two provinces of Ratanakiri (52%) and Preah Vihear (40%), with the remainder in the provinces of Kratie (4%), Stung Treng (3%), and Mondulkiri (1%) (Nget et al., 2021). However, Cambodian soybean oil has not yet been introduced to the market. Soybean oil is a vegetable oil extracted from soybean seeds. It is generally obtained through mechanical pressing or solvent extraction methods (Lima et al., 2008). Among these methods, solvent extraction is the method commonly used to extract oil from seed materials with lower oil content, such as soybean seeds (Keneni, Bahiru, & Marchetti, 2021). The conventional solvent extraction (CSE) method is the process by which a molecule transfers from one solvent to another due to the difference in solubility between two immiscible (or slightly soluble) solvents (Chen & Wang, 2017). The main advantages of CSE are that it provides a higher oil yield, larger processing capacity, and lower refinery losses. In the CSE method, n-hexane is the solvent commonly used in the oil processing industry because it is inexpensive and has high solubility. Previous studies (Mohammed & AI-Saddi, 2003; Nikolic, Stankovic, Cakic, & Mitic, 2009; Shittu, Mari, & Dangora, 2019) have explored the extraction of soybean oil using n-hexane. Their results proved that n-hexane is the solvent that provides the highest soybean oil extraction yield. However, the use of n-hexane as an extraction solvent has many drawbacks due to its non-renewable fossil origin and high flammability, leading to environmental and public health concerns (Nde & Foncha, 2020; Oliveira, Garavazo, & Rodrigues, 2012; Russin, Boye, Arcand, & Rajamohamed, 2011; Tabtabaei & Diosady, 2013). Normally, ethanol can be used instead of n-hexane to extract oil from soybean seeds. Certain studies (Oliveira et al., 2012; Rodrigues, Aracava, & Abreu, 2010; Sawada, Venâncio, Toda, & Rodrigues, 2014; Toda, Sawada, & Rodrigues, 2016) have shown that ethanol is a solvent that can serve as a substitute for n-hexane in oil production as it has a proven ability to extract oil from the seeds and offers environmental benefits. However, although many researchers have studied the extraction of soybean oil using the CSE method, response surface methodology (RSM) has not yet been widely explored. RSM is one of the most used tools for optimizing the influence process variables in experimental designs (Khuri, 2017; Kumari & Gupta, 2019). RSM mainly identifies the response by using given process variables following experimental designs, such as central composite design (CCD), that fit an empirical full second-order polynomial model. In previous studies (Aydar, 2018; Bokhari, Yusup, & Ahmad, 2012; Nde & Foncha, 2020; Popoola, Akinoso, & Raji, 2016; Yolmeh & Jafari, 2017), CCD has been used for the optimization of process parameters during oil extraction and provided successfully optimized conditions. Therefore, the current study aims to determine the effect of different solvents on soybean oil yield and to optimize the extraction conditions, including solvent-solid ratio and extraction time, using RSM.

2. METHODOLOGY

2.1. Chemicals and Reagents

N-hexane of 99% purity (RCI Labscane, Thailand) and ethanol of above 99.9% purity (DAEJUNG, South Korea) were used to extract the oil from Cambodian soybean seeds.

2.2. Sample Preparation

Soybean seeds were collected from Ratanakiri province, Cambodia. The soybean seeds were cracked and then manually dehulled to remove the hulls and impurities from the seeds. After that, the dehulled soybeans were ground and sieved to obtain soybean powder with a particle size lower than 0.425 mm. The moisture content of the dried soybean seeds was $8.16 \pm 0.3\%$ as measured by a moisture analyzer (MOC63u, Shimadzu).

2.3. Experimental Design

RSM with CCD was used for the analysis and optimization of the interaction effects between variables for Cambodian soybean oil extraction using the CSE method. A CCD was applied to two independent variables with three coded levels (-1, 0, +1) and two un-coded levels ($-\alpha$, $+\alpha$), in which α was 1.4142. The independent variables were extraction time (A), ranging from 15 to 45 (min), and solvent-to-solid ratio (B), ranging from 5:1 to 15:1 (mL/g). The three types of solvents used in this study were ethanol, n-hexane, and a mixture of n-hexane and ethanol (Hex:Eth) in a ratio of 2:1. The independent variables with their coded and uncoded values are shown in Table 1.

Table 1. Coded and uncoded factor levels generated from the CCD for each solvent.

Variables	Levels				
	-1.4142	**-1**	**0**	**1**	**+1.4142**
A: Extraction time (Min)	8.7868	15	30	45	51.2131
B: Solvent-to-solid ratio (mL/g)	2.9289	5:1	10:1	15:1	17.071

This CCD comprised three center points with a one-star point, and the response for CCD optimization was soybean oil yield. The CCD was based on the second-order polynomial model as in the following equation:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2$$

Where Y represents the response variables of soybean oil yield (%), β_0 is the model intercept, A and B denote a code of extraction time and solvent-to-solid ratio, β_1 and β_2 are linear coefficients, β_{12} is interaction coefficients for each variable, and β_{11} and β_{22} are quadratic coefficients.

2.4. Oil Extraction Procedure

In this study, soybean oil was extracted using the CSE method with three different solvent types: ethanol, n-hexane, and a mixture of n-hexane and ethanol (Hex:Eth) in a ratio of 2:1. The soybean powder was added to the solvent at the defined solvent-to-solid ratios (5:1 to 15:1 mL/g). During the extraction, the mixture of sample and solvent was heated at 60 ± 2°C and stirred at 500 rpm for the defined extraction times (15 to 45 min). After the extraction, the mixtures were centrifuged at 4,000 rpm for 15 min. After centrifugation, the liquid extracts were filtered with 25 µm filter paper. Next, the miscella was evaporated at 40°C, 72 rpm, and 30 min. The collected oil was dried at 105°C for 30 min in a hot-air drying oven and then transferred into a desiccator for 15 min.

2.5. Determination of Soybean Oil Yield

The extracted oil was calculated and expressed as a percentage, which was defined as the weight of the extracted oil over the wet weight of the sample taken:

$$\%\text{Yield} = \frac{m_f - m_i}{m} \times 100$$

Where %Yield is the percentage by weight of extraction yield in wet basis (% wet weight basis), m_f is the weight of round bottom flask containing distilled soybean oil (g), m_i is the tare weight of round bottom flask (g), and m is the weight of the sample used for extraction (g).

2.6. Statistical Analysis

In this study, Design-Expert software version 13.05.0 (State-Ease Inc., USA) was used for experimental design and statistical analysis. Analysis of variance (ANOVA) was used to estimate the suitability and validity of the model with an above 95% confidence level. The optimal condition was then chosen based on the desirability (numerical optimization) of the response variable.

3. RESULTS AND DISCUSSION

3.1. Soybean Oil Yield Using Different Types of Solvent

The results of the experiments in terms of un-coded values with independent variables and responses are depicted in Table 2. The variation in the oil values indicated that the extraction factors affected the extracted oil yields.

3.2. Effect of Solvent Types on Extracted Oil Yield

The extraction solvent is one of the most significant factors affecting the extraction efficiency of oil from oilseeds. Oil can be extracted from plant seeds using different solvents (Bardone et al., 2012; Shivani, Khushbu, Faldu, Thakkar, & Shubramanian, 2011). The extraction of soybean oil was performed using the CSE method and three different extraction solvents (ethanol, n-hexane, and Hex:Eth). The results showed that ethanol was the best solvent, providing the highest crude oil yield, followed by the Hex:Eth mixture and, finally, n-hexane. This result could be partially explained by the difference in solvent polarities (Li et al., 2014). Ethanol is polar, while n-hexane is a non-polar solvent. Therefore, ethanol can extract both non-polar and polar lipids, such as triglycerides, phospholipids, tocopherols, waxes, and sugars, while n-hexane can extract only non-polar lipids. This explains the higher yield obtained when using ethanol as an extraction solvent.

Table 2. Results of soybean oil yields extracted using various types of solvents.

Std.	Runs	Independent variables		Oil yields (%)		
		A	B	Ethanol	N-hexane	Hex:Eth (2:1)
7	1	30	2.929:1	11.65	14.81	15.01
4	2	45	15:1	21.74	17.63	18.69
6	3	51.213	10:1	19.8	17.19	17.97
9	4	30	10:1	20.09	16.8	17.29
1	5	15	5:1	13.52	13.8	15.38
8	6	30	17.071:1	20.14	18.01	19.77
3	7	15	15:1	20.47	17.41	18.93
11	8	30	10:1	19.12	16.64	18.11
5	9	8.787	10:1	18.7	15.36	16.64
10	10	30	10:1	18.85	16.88	17.01
2	11	45	5:1	16.41	16.2	15.40

Note: A: Extraction time (Min), B: Solvent-to-solid ratio (mL/g).

3.3. Regression Model for Soybean Oil Yield

The linear terms, interaction term, and quadratic terms were employed in multiple regression analysis to obtain predicted responses. The second-order polynomial equations in terms of coded and actual factors for the various types of solvent were presented as follows:

For ethanol:

$$Y_{coded\ (1)} = +\ 19.3533 + 0.714456A + 3.03584B - 0.405AB + 0.0639617A^2 - 1.61355B^2$$

$$Y_{actual\ (1)} = +\ 4.03440 + 0.084574A + 2.06001B - 0.0054AB + 0.000284A^2 - 0.064542B^2$$

For n-hexane:

$$Y_{coded\ (2)} = +\ 16.7733 + 0.651002A + 1.19569B - 0.545AB - 0.269793A^2 - 0.202292B^2$$

$$Y_{actual\ (2)} = +\ 9.01162 + 0.188012A + 0.618971B - 0.007267AB + 0.001199A^2 - 0.008092B^2$$

For the mixture (Hex:Eth):

$$Y_{coded\ (3)} = +\ 17.47 + 0.207613A + 1.69646B - 0.065AB - 0.144376A^2 - 0.101876B^2$$

$$Y_{actual\ (3)} = +\ 12.41685 + 0.061008A + 0.446792B - 0.000867AB + 0.000642A^2 - 0.004075B^2$$

The coded equation was used to determine the relative effect of the factors on the response by comparing the coefficients of factors. Therefore, the influence of the factors on the oil yields extracted using ethanol, n-hexane, and the Hex:Eth mixture were $B > B^2 > A > AB > B^2$, $B > A > AB > A^2 > B^2$, and $B > A > A^2 > B^2 > AB$, respectively. Additionally, the equation in terms of actual factors can be used to predict the response for given levels of each factor. The given levels should be specified in the original units for each factor. Moreover, the sign of the regression coefficients can be used to show how the terms influenced oil yield. A positive coefficient sign indicates that the term's effect favors the extracted oil yield, while a negative sign indicates that the term's effect will be antagonistic to the extracted oil yield. The results of the statistical analysis of the regression models are indicated in Table 3, with F-test and *p*-values calculated using ANOVA. Various statistical parameters, such as the probability value (*p*-value), the regression coefficient (R^2 value), the Fisher model value (F-value), and the lack of fit F value, were used to modify the responses to fit the mathematical model.

The result of ANOVA for soybean oil extraction using ethanol, n-hexane, and Hex:Eth is thus indicated in Table 3. The *F*-values of the models $Y_{coded(1)}$, $Y_{coded(2)}$, and $Y_{coded(3)}$ were 44.47, 215.4, and 17.7, respectively. Therefore, there was only a 0.04%, 0.01%, and 0.34% chance that a strong F-value of the models $Y_{coded(1)}$, $Y_{coded(2)}$, and $Y_{coded(3)}$ could occur due to noise. Moreover, the p-values of the regression model of the oil extracted using ethanol (p = 0.0004), n-hexane (p < 0.0001), and Hex:Eth (p = 0.0034) were all lower than 0.05, indicating that the model was significant. In addition, the p-values of lack of fit of oil extraction using ethanol (p = 0.5332), n-hexane (p = 0.5229), and Hex:Eth (p = 0.6376) were all higher than the significant level.

Table 3. Multiple regression analysis results for the significant variables and interactions on the response of soybean oil yield using various types of solvents.

SM	SS	DF	MS	F-value	*P*-value	Remark
Ethanol ($Y_{ethanol}$)						
Model	94.96	5	18.99	44.47	0.0004	Significant
A	4.08	1	4.08	9.56	0.0271	
B	73.73	1	73.73	172.63	< 0.0001	
AB	0.6561	1	0.6561	1.54	0.2702	
A^2	0.0231	1	0.0231	0.0541	0.8253	
B^2	14.7	1	14.7	34.42	0.002	
Residual	2.14	5	0.4271			
Lack of fit	1.28	3	0.4283	1.01	0.5332	Not significant
Pure error	0.8505	2	0.4252			
Cor total	97.1	10				
Std. dev.	0.6535		R^2 = 0.978			
Mean	18.23		Adjusted R^2 = 0.956			
C.V. %	3.59		Predicted R^2 = 0.886			
Press	11.05		Adeq precision = 19.301			
n-hexane ($Y_{n\text{-}hexane}$)						
Model	16.52	5	3.3	215.4	< 0.0001	Significant
A	3.39	1	3.39	221.03	< 0.0001	
B	11.44	1	11.44	745.63	< 0.0001	
AB	1.19	1	1.19	77.46	0.0003	
A^2	0.411	1	0.411	26.8	0.0035	
B^2	0.2311	1	0.2311	15.07	0.0116	
Residual	0.0767	5	0.0153			
Lack of fit	0.0468	3	0.0156	1.05	0.5229	Not significant
Pure error	0.0299	2	0.0149			
Cor total	16.6	10				
Std. dev.	0.1239		R^2 = 0.9954			
Mean	16.43		Adjusted R^2 = 0.9908			
C.V. %	0.7538		Predicted R^2 = 0.9759			
Press	0.4002		Adeq precision = 45.3716			
Hex:Eth ($Y_{Hex:Eth}$)						
Model	23.52	5	4.7	17.7	0.0034	Significant
A	0.3448	1	0.3448	1.3	0.3064	
B	23.02	1	23.02	86.6	0.0002	
AB	0.0169	1	0.0169	0.0636	0.811	
A^2	0.1177	1	0.1177	0.4427	0.5353	
B^2	0.0586	1	0.0586	0.2204	0.6585	
Residual	1.33	5	0.2659			
Lack of fit	0.6757	3	0.2252	0.6892	0.6376	Not significant
Pure error	0.6536	2	0.3268			
Cor total	24.85	10				
Std. dev.	0.5156		R^2 = 0.9465			
Mean	17.29		Adjusted R^2 = 0.893			
C.V. %	2.98		Predicted R^2 0.7475			
Press	6.28		Adeq precision = 12.6003			

Note: A: Extraction time (Min), B: Solvent-to-solid ratio (mL/g), DF: Degree of freedom, SM: Source of model, MS: Mean of squares, SS: Sum of squares, Adeq precision: Adequate precision.

Thus, the lack of fit was insignificant, and the insignificant lack of fit was good. The fitness of the polynomial model was expressed by the coefficient of determination R^2, coefficient of adjusted R^2 and predicted R^2. It has been suggested that these values should be at least 0.80 for a good fit of the model, and the difference between adjusted R^2 and predicted R^2 should be less than 0.2 (Yujie, 2011). Therefore, the value of R^2, adjusted R^2, and predicted R^2 of the model indicated that this regression model was a good fit for the mathematical model. The signal-to-noise ratio was measured with adequate precision. It is preferable to have a ratio of more than 4. The adequate precisions of the regression model using ethanol, n-hexane, and Hex:Eth were 19.30, 45.37, and 12.60, respectively. Therefore, this model represented a good sign and had a strong enough signal to be used for optimization.

3.4. Effect of Extraction Time on Soybean Oil Yield

The extraction time is an important parameter of the oil extraction process (Panchal, Deshmukh, & Sharma, 2014). The effect of extraction time on soybean oil yield varied depending on the type of solvent used. Based on the results of the statistical analysis in Table 3, the extraction time had a highly significant effect on oil yield when n-hexane was used to extract the oil ($p < 0.0001$), a significant effect for ethanol ($p = 0.0271$), and an insignificant effect for the Hex:Eth mixture ($p = 0.3396$). Figure 1 shows the effect of extraction time and solvent-to-solid ratio on oil yield extracted using the various types of solvents. It was observed that when n-hexane and ethanol were used in the extraction, the oil yield increased slowly due to the increase in the extraction time. However, when the mixture of Hex:Eth was used, the oil yield did not change in response to an increase in extraction time. The effect of extraction time on oil yield can be explained by the reduction of residual oil in the sample. At the beginning of the extraction process, the solvent starts to dissolve the oil in the materials. The higher (total) oil content in the seed materials in contact with the solvent results in the higher oil molecules being diffused into the solvent. Thus, the extraction time should be long enough to let the oil molecules diffuse into the solvent (Abed, Kurji, & Abdul-Majeed, 2015). However, when the equilibrium time was reached, the oil yield remained stable in response to an increase in extraction time (Shao et al., 2012). This is due to the low solvent density and oil content remaining in the materials after the extraction time has been extended (Saxena, Sharma, & Sambi, 2011).

3.5. Effect of Solvent-to-Solid Ratio on Soybean Oil Yield

The solvent-to-solid ratio is also an important parameter of the oil extraction process (Lawson, Oyewumi, Ologunagba, & Ojomo, 2010; Panchal et al., 2014). According to the statistical analysis, as shown in Table 3, the solvent-to-solid ratio had a strong significant effect on the oil yield extracted from the soybean seeds. The effects of the solvent-to-solid ratio on the oil yield extracted using ethanol, n-hexane, and the Hex:Eth mixture are shown in Figure 1. It was observed that an increase in solvent-to-solid ratio led to a significant increase in the extraction yield of soybean oil. The increase in oil yield due to the increase in the solvent-to-solid ratio can be explained by the fact that at a lower solvent-to-solid ratio, the amount of solvent in contact with the sample decreases. Then the oil is less soluble in the solvent (Jisieike & Betiku, 2020). However, at a higher solvent-to-solid ratio, the amount of solvent contacting the sample increases, which can improve the rate of mass transfer, resulting in higher extraction productivity (Bokhari et al., 2012). Additionally, the increased solvent-to-solid ratio results in a faster diffusion rate of oil from the extracted materials to the solvent (Predescu et al., 2016).

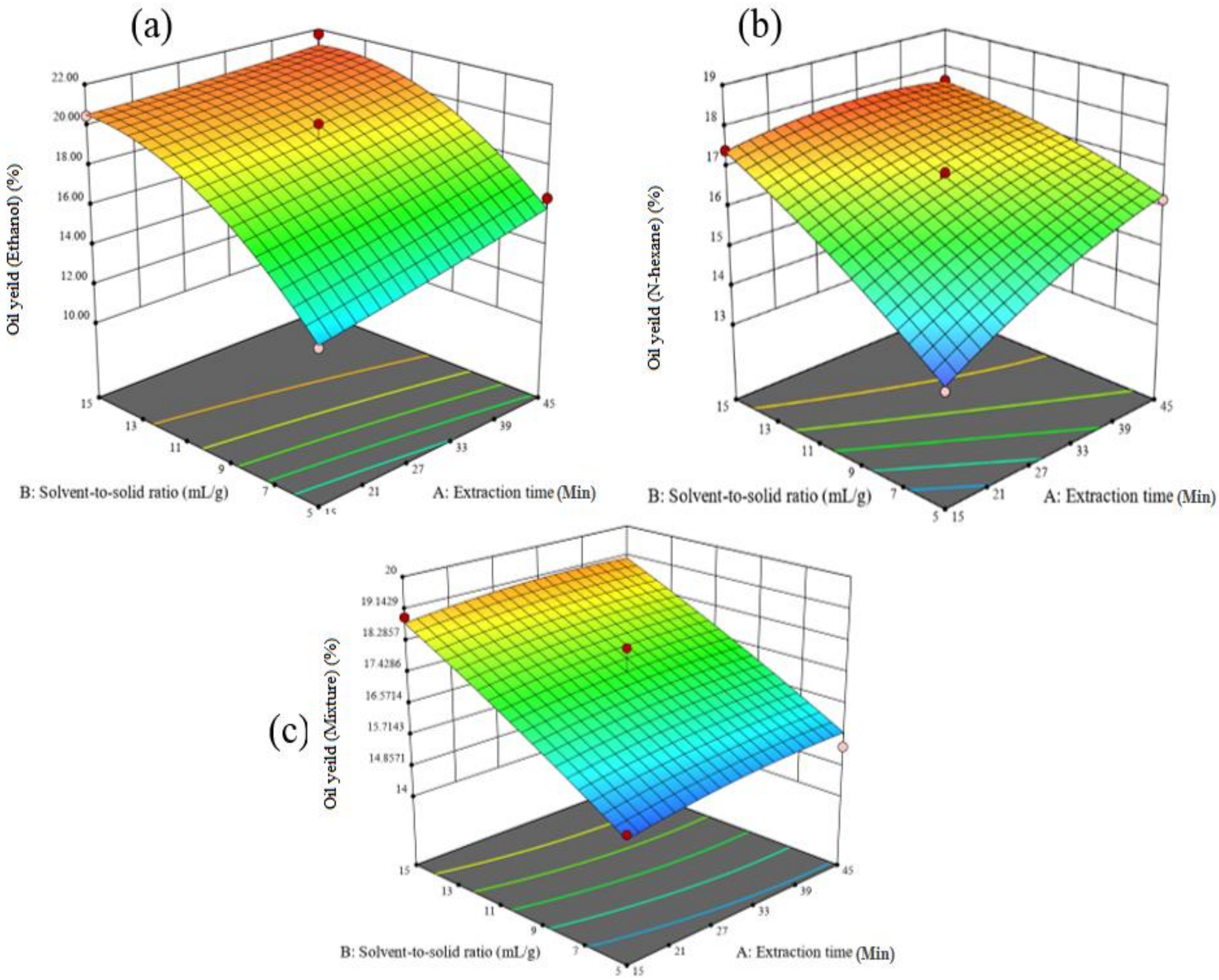

Figure 1. 3D response surface plots showing the effect of solid-to-solvent ratio and extraction time on the oil yield using different types of solvents: (a). Ethanol, (b). N-hexane, and (c). Mixture of Hex:Eth

3.6. Model Reduction

Model reduction is the elimination of the not significant terms from the model, such as the term for a predictor variable or the interaction between predictor variables. Model reduction helps to increase the precision of the predictions by enhancing the fit between data and model. The regression model of the soybean oil extraction using n-hexane was not reduced because all terms of the model were significant. The reduced regression models of the soybean oil extraction in terms of coded and actual factors are presented in equations as follows:

For ethanol:

$$Y_{actual\ (4)} = + 5.38351 + 0.047630A + 1.91305B - 0.065294B^2$$

$$Y_{coded\ (4)} = + 19.41 + 0.7145A + 3.04B - 1.63B^2$$

For the mixture of (Hex:Eth):

$$Y_{actual\ (5)} = + 13.89799 + 0.339292B$$

$$Y_{coded\ (5)} = + 17.29 + 1.70B$$

The indicators used to assess the fit and quality of the reduced models are presented in Table 4.

Table 4. Indicators to assess the fit and quality of reduced models.

Indicators	Reduced $Y_{ethanol}$	Reduced $Y_{Hex:Eth}$
R^2	0.9710	0.9263
Adjusted R^2	0.9586	0.9182
P-value of the model	<0.0001	<0.0001

The results showed that the reduced models had good accuracy as the R^2 values were close to 1 and the values of the adjusted R^2 were high enough to suggest a satisfactory correlation between the values predicted by the model and the values observed in the experiments. Additionally, the *p*-values of the reduced models generated by ANOVA on the reduced regression models were less than 0.0001, indicating that the models obtained were acceptable.

3.7. Optimization of Extraction Conditions Using RSM with CCD

Evaluation of the optimum oil yield was based on numerical optimization using Design-Expert software. The optimization was achieved by setting the optimization goals as follows: minimize the extraction time, maximize the solvent-to-solid ratio, and maximize the oil yield, and the importance of all goals was 3 pluses (+++). The optimal formulation was chosen based on the desirability of the response variable (Pal & Gauri, 2018). The resulting optimum oil yields of soybean oil extraction using ethanol, n-hexane, and the Hex:Eth mixture were 20.10%, 17.39%, and 18.99%, with a desirability of 0.943, 0.930, and 0.942, respectively. The optimum oil yield of all solvents used was obtained at 15 min of extraction time and a 15:1 (mL/g) solvent-to-solid ratio.

3.8. Model Validation

To verify and confirm the agreement between the optimum results obtained from the model and the actual values, three new confirmation experiments were carried out under the suggested optimal conditions, using the same extraction procedure, and the results are presented in Table 5 (Choudhary & Pramanik, 2021).

Table 5. Comparison of predicted and experimental oil yield values from soybean seeds extracted by CSE using different types of solvents.

Solvent	A: Extraction time (Min)	B: Solvent-to-solid ratio (mL/g)	Predicted value	Actual value	RSE (%)
Ethanol	15	15:1	20.10	20.52 ± 0.65	2.039
N-hexane	15	15:1	17.39	17.23 ± 0.12	0.925
Hex:Eth	15	15:1	18.99	18.02 ± 0.52	5.093

Noted: The data are expressed as mean ± standard deviation for experimental value, RSE: Residue standard error.

The average oil yields that were obtained from the extraction using ethanol, n-hexane, and the mixture were 20.52 ± 0.65%, 17.23 ± 0.12%, and 18.02 ± 0.52%, respectively, while the predicted oil yields were 20.10%, 17.39%, and 18.99%, respectively. The differences in maximum oil yield between the predicted values and the experimental values using ethanol, n-hexane, and the Hex:Eth mixture were 2.039%, 0.925%, and 5.093%, respectively. These errors were within an acceptable range of 10% and confirmed that the predicted model was sufficiently accurate within the 99% prediction interval. Therefore, the model based on the CCD was considered to be accurate and reliable for predicting oil yield extracted from soybean seeds.

4. CONCLUSION

The application of RSM with CCD in this study provided a good fit for the model based on the predicted and experimental data. Among the three solvent types, the highest crude soybean oil yield was obtained using ethanol, followed by the Hex:Eth mixture and then n-hexane. According to the statistical analysis, the solvent-to-solid ratio had a highly significant effect on the extraction process for all solvent types. Similarly, the extraction time had a highly significant effect on the extraction when n-hexane was used. However, when ethanol and Hex:Eth were used in the extraction process, the extraction time had a slightly significant and insignificant effect on the extraction process, respectively. Based on the numerical optimization, the optimum oil yields when using ethanol, the mixture, and n-hexane were 20.53%, 18.78%, and 17.39%, respectively. The optimum condition was obtained at 15 min extraction time and 15:1 (mL/g) solvent-to-solid ratio for all solvent types. In future research, the quality of oils extracted from Cambodian soybean seeds using n-hexane, ethanol, and the mixture of n-hexane and ethanol should be determined, and the refining process should be optimized.

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