Research Article

Utilization of Supercritical Carbon Dioxide and Co-solvent n-hexane to Optimize Oil Extraction from *Gliricidia sepium* Seeds for Biodiesel Production

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DOI: 10.14416/j.asep.2021.09.003
Received: 6 April 2021; Revised: 15 May 2021; Accepted: 9 June 2021; Published online: 6 September 2021

Abstract

This study was conducted to optimize the supercritical carbon dioxide ($scCO_2$) extraction of oil from *Gliricidia sepium* seeds using response surface methodology. Initial experiments were carried out using $scCO_2$ and $scCO_2$ with co-solvent n-hexane to determine the effect of co-solvent addition in oil yield. In order to obtain the maximum yield, experiments were conducted using Response Surface Methodology - Face Centered Central Composite Design (RSM – FCCD) under the following conditions: pressure of 20, 30, and 40 MPa, temperature of 50, 60, and 70°C, and CO_2 flow rate of 2, 2.5, and 3 mL/min. A second-order polynomial with extended cubic interaction model was significantly fitted ($p < 0.05$), and a high coefficient determination value ($R^2 = 0.98$) was recorded. At a constant extraction time of 60 minutes, the best extraction yield (12.12%) was obtained at 60°C, 40 MPa, and 2.5 mL/min. The pressure, temperature, and CO_2 flow rate were all found to have a significant effect on the oil yield. The oil was used in biodiesel production and its methyl ester composition was analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID).

Keywords: Biodiesel, *Gliricidia sepium*, Kakawate, Response surface methodology, Supercritical carbon dioxide

1 Introduction

Gliricidia sepium is a fast-growing multipurpose legume tree cultivated in Central and South America, East and West coast of Mexico, Philippines, and South India. It was named "kakawate" or "Madre de cacao" (mother of cocoa) to describe its use as shade for cocoa and coffee plantations. It is also one of the best species for reforestation of denuded or grassland areas [1]. The many parts of this tree are used as fuelwood, poles, live fencing, and green manure as animal fodder [2]. This leguminous tree has been subject in agroforestry research, particularly the maize-based intercropping system, in Southern Malawi, Southern Africa, and Northeastern Brazil [3]–[6]. Numerous studies have demonstrated interest in its chemical composition and nutritive values [2], [7], [8]. Among the components identified for the most recent years were hederagin-based acetylated saponins from the fruits [1], isoflavan, isoflavonoids, isovestisol, formononetin and afrormosin, a pterocarpan, medicarpin, 4-hydroxy-3-methoxy-cinnamaldehyde [9], stigmastanol glucoside and

3’,4‘-dihydroxy-trans-cinnamic acid octacosylester from the heartwood and 2H-chromen-2-one from the leaves [10]. Some studies have proven that *Gliricidia sepium* extract has larvicidal, ovicidal, and pupicidal properties providing the same level of protection as chemical insecticides [11], [12].

Mechanical pressing or extraction with organic solvents have traditionally been used to recover oils. One of the most common organic solvents used in the industry for the collection of oil extracts is n-hexane. This solvent is known for its high yield recovery and low production cost [13]. In biodiesel production, for example, oil from *Gliricidia sepium* seed was extracted using Soxhlet extractor with n-hexane. This was successfully converted into methyl esters using methanol and sodium methoxide catalyst [14]. The popularity of n-hexane as a solvent for oil extraction is presented in literature, comparing its efficiency with other solvents. Hexane produced a higher yield than petroleum ether in extracting oil from soybean, sunflower, canola, and crambe [15]. Likewise, oil from dried spent coffee grounds was extracted using four different solvents: n-hexane, anhydrous ethanol, hydrous ethanol, and methanol. The solvent n-hexane extracted mostly triglycerides (>80%), while the three other solvents extracted more monoglycerides (80%) [16]. Similarly, n-hexane was considered the best organic solvent in wax extraction from wheatgrass [17]. Although n-hexane was found to be an effective extracting solvent, it also had drawbacks. Traditionally, hexane-extraction requires a large amount of solvent and a long reaction time. One study described n-hexane as a solvent that yields higher amounts of non–extractive compounds when compared to other extracting methods such as supercritical CO_2 [18].

Supercritical fluid method of extraction became popular because it is faster, more efficient, has better potential for automation, and reduces the need for large volumes of potentially hazardous liquid solvents [19]. Among the supercritical fluids, one that has gained a lot of attention is the use of CO_2 under supercritical conditions. Its low critical constants (T_c = 304.15 K, P_c = 7.38 MPa) allow performing the reaction at low temperatures and moderate pressures. Moreover, the supercritical method offers other advantages: 1) the medium facilitates the transport of the precursors to the internal surface of nanopores without structural damage due to its low viscosity, high diffusivity, and very low surface tension and 2) CO_2 is faster than toluene or most solvents mainly because of the good transport properties. However, compressing CO_2 is energy-intensive, and the energy costs can make the process uncompetitive [20].

The choice between utilizing a conventional extraction technique using an organic solvent or a promising technology using $scCO_2$ has been another area of research. Several studies comparing the performance of the conventional method of extraction using n-hexane to the $scCO_2$ extraction method were published. The total yield of lipids obtained from sorghum distiller grains by $scCO_2$ extraction was almost doubled compared to the yield obtained using recirculated solvent extraction with n-hexane [21]. In the same way, supercritical extracts from *Pterodon pubescens benth* fruit were more effective against *L. amazonensis* than conventional extracts using n-hexane [22]. Also, higher phytosterol and tocopherol concentrations were extracted from the Macauba kernel using $scCO_2$. [23]. The $scCO_2$ extraction of β-sitosterol from *Morus alba* leaves resulted in a 1.11% yield as compared to 0.83% using ultrasound-assisted n-hexane extraction [24]. Similarly, the effectiveness of $scCO_2$ over other organic solvents such as dichloromethane and n-hexane was also exhibited in the extraction of *L*-Dopa from Mucuna seeds [25]. Although $scCO_2$ is well-known for its high extraction capability, multiple investigations have found that the conventional method of employing n-hexane is preferable. The chemical compound composition of *Dracocephalum kotschyi* seed oil showed no significant difference, whether extracted using $scCO_2$ or n-hexane solvent [26]. Also, higher oil yield was obtained using Soxhlet n-hexane method in Tunisian *Opuntial ficus indica* seeds when compared to $scCO_2$. The $scCO_2$ fatty acid profile shows that it contains other compounds not present using n-hexane, such as C20:1, C20:2, and C22:0 [27]. Moreover, the highest $scCO_2$ oil yield from *Cnidoscolus quercifolius* had corresponded to 87% of the Soxhlet yield [28]. Athukorala and Mazza also reported on the yield of extracts from wax and other lipophilic compounds from triticale straw. They observed no significant statistical difference whether using $scCO_2$ or Soxhlet extraction using n-hexane [29]. In this context, both methods of extraction-$scCO_2$ method and chemical solvent n-hexane can produce a high oil yield.

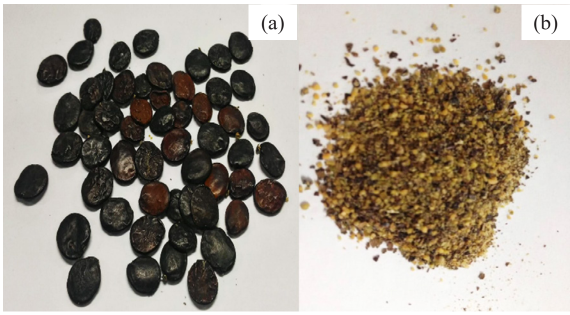

Figure 1: Photos of (a) *Gliricidia sepium* seed and (b) ground *Gliricidia sepium* seed.

To date, no research has been reported on the optimization of process parameters for $scCO_2$ with n-hexane extraction of *Gliricidia sepium* seed oil. The n-hexane was kept at a low concentration, just enough to saturate the sample's surface and start the oil desorption process. In this study, Response Surface Methodology – Face Centered Central Composite Design (RSM – FCCD) was used to develop a model for oil yield as a function of temperature, pressure, and CO_2 flow rate.

2 Materials and Methods

2.1 *Material and preparation of sample*

The seeds of *Gliricidia sepium* were collected in Laguna, a southern province in the Philippines. It was oven-dried at 105°C to a constant mass, ground into powder using a fast rotating mill (Thomas Wiley Mill Model 4) at 800 rpm, and passed through a set of standard mesh sieves. The ground seeds were stored in a clean and dry container at room temperature. Figure 1(a) shows the *Gliricidia sepium* seed while Figure 1(b) presents the seeds after it was milled.

2.2 *Reagents*

Carbon dioxide (99.97%, Linde Philippines, Inc.) and n-hexane (95%, RCI Labscan Inc.) were used as solvents in supercritical fluid extraction. Other chemicals such as methanol (RCI Labscan Inc.), NaCl (Merck Inc.), Na_2SO_4 (Fisher Scientific), NaOH (RTC Supply House), and BF3-methanol-complex solution (Sigma-Aldrich®) were used in the conversion of triglycerides into methyl esters. All chemicals were of analytical grade and used without any further purification.

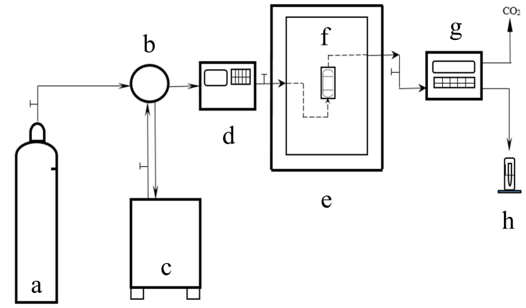

Figure 2: Schematic diagram of the supercritical CO_2 system.

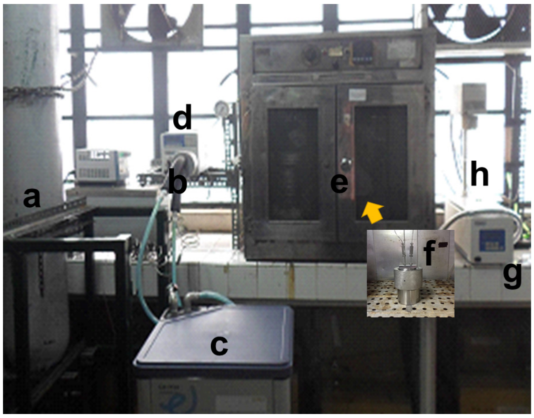

Figure 3: Actual photo of the supercritical CO_2 system.

2.3 *Supercritical CO_2 operation*

Figures 2 and 3 demonstrate the supercritical fluid extraction set-up used to extract oil from *Gliricidia sepium* seeds. The system is made of the following components: (a) CO_2 source, (b) cooling jacket, (c) chiller (Eyela Model no: CA115), (d) intelligent, high-performance liquid chromatography (HPLC) pump (PU-2080 Plus, Jasco International Co., Ltd, Japan), (e) laboratory oven (Memmert GmbH + Co. kG, Germany) (f) 10 mL reactor cell, (g) back pressure regulator (BPR) (BP-2080 Plus, Jasco International Co. Ltd, Japan) and (h) oil collector.

The supercritical CO_2 extraction was performed at different working conditions of pressure, temperature, and CO_2 flow rate. Prior to oil extraction, parameter values were manually encoded into the HPLC and BPR. A constant weight of approximately 3 g of ground seeds was placed inside the cell reactor. For experimental runs with co-solvent n-hexane, 3 mL of the organic solvent was added together with the seeds. This small amount of n-hexane is just enough to wet the seeds inside the reactor. The reactor cell was immediately sealed and placed inside the oven. Carbon dioxide was pumped into the reactor cell and extracted oil in its critical temperature and critical pressure. A back pressure regulator was maintained at constant pressure in the collector. After each runs, the whole system was purged with carbon dioxide. A 10 mL glass vial was utilized as a collection vessel and was kept at room temperature. Each extraction experiment was conducted twice, and the average value of two replications was presented in this study.

2.4 *Oil yield calculation*

The amount of extracted oil from each experimental run was determined gravimetrically, and extraction yield was expressed as the percent ratio of the mass of extracted oil to the mass of seeds loaded to the vessel following Equation (1).

$$Gliricidia\ sepium\ seed\ oil\ yield\ (wt\ \%) = \frac{mass\ of\ extracted\ oil\ (g)}{mass\ of\ seed} \times 100 \tag{1}$$

2.4.1 Factorial design

Identifying the workable range of parameters is one aspect to consider before optimization. The use of Design of Experiment, known as a more structured approach in conducting experiment could lower operating costs, lower operating time, and lower cost of poor quality. Initially, a 2^k factorial design (k = 3) was employed to determine the effects of temperature, pressure, and co-solvent in the extraction of oil using $scCO_2$. Moreover, the results of this experiment became the basis of succeeding tests in oil extraction. The actual values of these parameters are summarized in Table 1, while the CO_2 flow rate and extraction time were held constant at 3 mL/min and 60 min.

Table 1: Summary of variables used in factorial design

Variables	**Unit**	**Coded and Actual Values**	
		–1	**+1**
Temperature	°C	60	80
Pressure	MPa	20	30
Amount of co-solvent	mL	0	3

2.4.2 Response surface methodology – face-centered central composite design

Determining the optimal conditions is a part of any experimental research constantly considered when presenting a new process and comparing it to an existing one. In this study, a total of 14 experimental runs and five replicates at the center points were done in the optimization of oil extraction with co-solvent n-hexane. The variables considered were temperature, pressure, and CO_2 flow rate which were coded as Z_1, Z_2, Z_3, respectively. The range and levels of the variables are shown in Table 2.

Table 2: Summary of variables used in RSM – FCCD

Variables	**Unit**	**Symbol**	**Coded and Actual Values**		
			–1	**0**	**1**
Temperature	°C	Z_1	50	60	70
Pressure	MPa	Z_2	20	30	40
CO_2 flow rate	mL/min	Z_3	2	2.5	3

2.5 *Statistical data analysis*

The effect and interaction of individual variables were determined from the regression coefficients and statistical model terms given by *Design-Expert*® 7.0.0 Trial (Stat-Ease Inc., Minneapolis, MN, USA), and these were observed in 2^k Factorial Design and RSM – FCCD. A response surface analysis was used to determine which factors and interactions are significant and to model response as a mathematical function of selected independent variables.

2.6 *Transesterification of oil*

The preparation of methyl ester from *Gliricidia sepium* seed oil was adapted with modification from the standard procedure of the Association of Official Analytical Chemists (AOAC) 969.33. A 250 mg of oil sample and 4 mL of methanolic sodium hydroxide solution were placed in a 15 mL screw-cap glass vial. The mixture was ultrasonicated for 10 min at 65–70°C, added with 4.5 mL of boron trifluoride solution, and mixed for another 1 min A 5 mL of n-hexane was then added, and continuous mixing was observed for another 1 min at 65–70°C. Saturated sodium chloride solution was added to separate the n-hexane solution. Lastly, a 1 mL n-hexane solution was removed, and Na_2SO_4 was added to remove traces of water.

2.7 *Fatty acid methyl ester analysis*

Quantitative and qualitative analyses of methyl esters were performed by GC - FID. The *Gliricidia sepium* methyl ester composition was analyzed using a Perkin Elmer Clarus 500 GC equipped with a flame ionization detector and a capillary column of Elite 5 capillary column (30 m length, 0.32 mm ID, and 0.25 µm thickness). The injector and detector temperatures were set at 250°C and 280°C, respectively. The GC oven was initially programmed at 60°C for 3 min, then increased to 140°C with a rate of 15°C/min, and then finally ramped to 220°C with a rate of 4°C/min for 15 min.

3 Results and Discussion

3.1 *scCO₂ oil extraction using factorial design*

Experimental values for *Gliricidia sepium* seed oil extraction using 23 factorial design varied from 7.00% to 12.68%. Higher oil yields were obtained for experimental runs added with n-hexane. Table 3 gives the matrix for 23 factorial design and the results of oil extraction. The best condition for obtaining the highest oil yield of 12.68% was at extraction pressure of 30 MPa, temperature of 80°C, and 3 mL of n-hexane.

3.1.1 Effect of n-hexane addition

Referring to Table 3 and comparing the oil yield results of experimental run nos. 1 and 5, 2 and 6, 3 and 7, and 4 and 8, it is evident that the addition of n-hexane increases the oil yield at any given sets of temperature and pressure. A difference of 2.76% oil yield (experimental run nos. 3 and 7) was observed at extraction temperature of 80°C and pressure of 20 MPa, the highest yield recorded when these two variables were held constant, and only the amount of n-hexane was varied. The addition of co-solvent during the extraction process accelerates desorption of oil from the ground seed allowing $scCO_2$ to dissolve more oil and carry it out of the reactor cell. A co-solvent is added to an extraction process mainly for two reasons: 1) to improve the solubility of the essential oil by increasing the polarity of the supercritical fluid extraction and 2) to facilitate desorption of the essential oil from the plant matrix [30], [31].

Table 3: The matrix for 23 factorial design and experimental data for oil extraction from *Gliricidia sepium* seed

Run	Independent Variables			Dependent Variable
	Pressure (MPa)	Temperature (°C)	Amount of Co-solvent (mL)	Oil Yield (%)
1	20	60	0	7.31
2	30	60	0	10.56
3	20	80	0	7.00
4	30	80	0	10.15
5	20	60	3	8.68
6	30	60	3	11.26
7	20	80	3	9.76
8	30	80	3	12.68

3.1.2 Effect of pressure

The oil yield increased with increasing pressure, and this was observed for both extraction temperature conditions of 60 and 80°C, with or without n-hexane. It was observed that the effect of pressure is more apparent on the experimental runs conducted without n-hexane. Nonetheless, the pressure was found to be the most significant variable that greatly influences the response. This study conforms with those described by Ara and Raofie [31], Jokic *et al*. [32], and Sodeifiana *et al*. [33] that pressure increases the solvent density of CO_2, resulting in high solubility of analyte and subsequently producing higher oil yield.

Furthermore, at a lower pressure of 10 MPa, the extracted oil shade was initially yellow but changed to green with rising pressure. In other words, the amount of extracted non-volatile compounds such as pigment increases with increasing $scCO_2$ pressure and $scCO_2$ density.

3.1.3 Effect of temperature

Solvent density of CO_2 under supercritical conditions can be changed as a function of temperature and pressure. Thus, in any $scCO_2$ extraction operation, it was always these two variables that were considered.

In the presence of n-hexane and constant pressure, the oil yield increased upon increasing the temperature condition from 60°C to 80°C. This was observed for both extraction pressure of 20 MPa and 30 MPa. In these experiments, n-hexane became more effective as an extraction solvent when used at temperatures close to its boiling point of 69°C [26]. On the other hand, when $scCO_2$ is used as the sole solvent, the oil yield decreases as the operating temperature rises. The solvent density of $scCO_2$ decreased as the temperature was raised, resulting in decreased solvent power and oil yield [34]. These findings are consistent with those described in the extraction of oils from pomegranate peel [31] and grape seeds [32].

3.2 *Factorial design and statistical analysis*

The analysis of variance of the three variables and their interactions are shown in Table 4, while a summary of statistical analysis is presented in Table 5. It shows that the oil yield was influenced strongly by pressure ($p<0.05$), amount of co-solvent ($p<0.05$), and interaction of temperature and co-solvent ($p<0.05$). Although the temperature was found to be the least important among the three variables, adding n-hexane and raising the temperature from 60°C to 80°C enhanced the oil extraction. In Table 5, the values of the coefficient of determination (R^2) and adjusted coefficient of determination (Adjusted R^2) were computed to be 99.5 and 98.8%, respectively. A high value of R^2 indicates that the expected and experimental oil yield values are in good agreement.

Table 4: 23 Analysis of variance for oil yield using $scCO_2$

Source	Sum of Squares	Mean Square	F Value	*p*-value Prob > F
Pressure	17.70	17.70	400.33	0.0003
Temperature	0.40	0.40	8.96	0.0580
Amount of co-solvent	6.77	6.77	153.14	0.0011
Temperature and amount of co-solvent	1.30	1.30	29.31	0.0124

Table 5: Statistical analysis for oil yield using $scCO_2$

Standard Deviation	Mean	R^2	Adjusted R^2
0.21	9.68	0.995	0.988

Moreover, the adjusted R^2 value showed that only 1.2% of total variations failed to be supported by a model [35], [36]. The effects of temperature, pressure, and n-hexane addition on oil yield are shown in a Pareto chart (Figure 4). The chart supports the result on which among the variables are statistically significant. Likewise, Figure 5 shows a graphical representation of the effects of temperature and co-solvent addition.

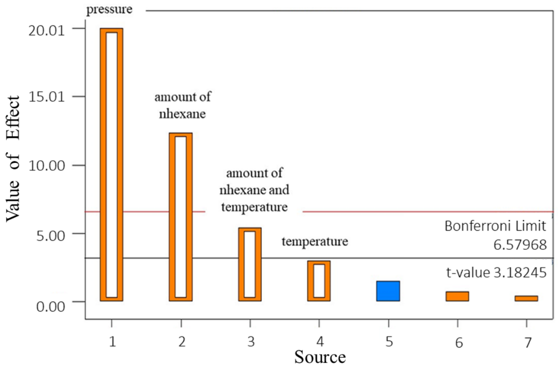

Figure 4: Pareto chart obtained from the 23 factorial design.

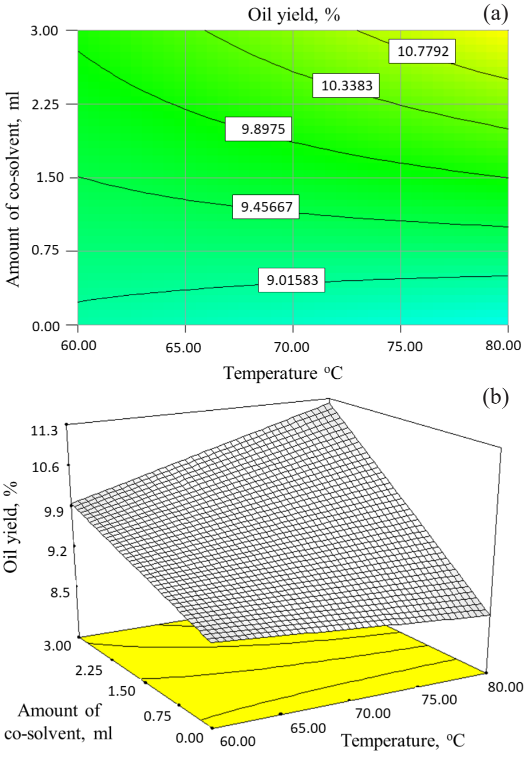

Figure 5: 2D Contour (a) and 3D surface images (b) of temperature and addition of n hexane in oil yield using supercritical CO_2 extraction.

3.3 *ScCO$_2$ and n-hexane using RSM FCCD*

The RSM-FCCD design and result of oil extraction are summarized in Table 6. The highest oil yield of 12.12% from this set of experimental runs was obtained at extraction pressure of 40 MPa, temperature of 60°C, 2.5 mL/min CO$_2$, extraction time of 60 min, and 3 mL of n-hexane.

Table 6: The matrix for RSM – FCCD design and experimental data for oil extraction from *Gliricidia sepium* seed

Runs	Independent Variables			Dependent Variable
	Temperature (°C)	Pressure (MPa)	CO$_2$ Flow Rate (mL/min)	Oil Yield (%)
1	50	20	2.0	8.08
2	70	20	2.0	6.27
3	50	40	2.0	10.75
4	70	40	2.0	9.96
5	50	20	3.0	9.96
6	70	20	3.0	7.52
7	50	40	3.0	10.64
8	70	40	3.0	9.28
9	50	30	2.5	7.26
10	70	30	2.5	11.28
11	60	20	2.5	9.50
12	60	40	2.5	12.12
13	60	30	2.0	11.06
14	60	30	3.0	11.27
15	60	30	2.5	11.8
16	60	30	2.5	11.95
17	60	30	2.5	11.34
18	60	30	2.5	11.45
19	60	30	2.5	11.79
20	60	30	2.5	11.60

3.3.1 Statistical analysis of regression models

Three models of Design-Expert® v 7.0.0 were fitted to interpret the oil yield as a function of temperature, pressure, and CO$_2$ flow rate. The mathematical models were linear [Equation (2)], quadratic [Equation (3)], and quadratic with an extended cubic [Equation (4)].

$$Y = \beta_o + \sum \beta_i Z_i \tag{2}$$

$$Y = \beta_o + \sum \beta_{ii} Z_i^2 + \sum \sum \beta_{ii} Z_i Z_j \tag{3}$$

$$Y = \beta_o + \sum \beta_{iii}^2 + \sum \sum \beta_{ij} Z_i Z_j + \sum \sum \beta_{iii} Z_i^3 + \sum \sum \beta_{ij} Z_i^2 Z_j \tag{4}$$

Where *Y* is the extraction yield (dependent variable), β is the coefficient that denotes the intercept (β_o), the main (β_i, β_{ii}, β_{iii}, β_{ij}, β_{iij}), and Zi, Zj, are coded values of the variables. Table 7 shows the analysis of variance of the three models that were considered in the study. The *p*-value denotes the probability of obtaining a Fisher ratio (F), which evaluates whether the independent variables have an effect on the response. In Table 7, both the linear and quadratic models show a significant lack of fit and low values of R^2. On the other hand, a quadratic model with extended cubic interaction was highly significant ($p<0.01$) and has a good fit at a confidence level of 95%. Thus, the quadratic model with extended cubic interaction of RSM-FCCD was selected to represent the oil yield calculation using scCO$_2$ and n-hexane solvents. The regression model was in good agreement with experimental data with an R^2 of 0.9800.

Table 7: Analysis of variance of three polynomial models

Type of model	F	pa	R^2	Lack of fit
Linear model	1.76	0.1946	0.2485	significant
Quadratic model	3.58	0.0296	0.7633	significant
Quadratic with extended cubic model	67.47	<0.0001	0.9800	insignificant

[a] p < 0.01 highly significant; 0.01 ≤ *p* ≤ 0.05 significant; *p* ≥ 0.05 insignificant

3.3.2 RSM-FCCD and statistical analysis

Table 8 shows the p-value of the independent variables - temperature, pressure, and CO$_2$ flow rate. The three variables listed had influenced ($p<0.05$) oil extraction, with temperature and pressure having the most significant effects on the response. Likewise, the temperature and pressure and the pressure and CO$_2$ flow rate have significant ($p<0.05$) effects on the yield among the interactions between factors. The model equation generated was found significant ($p<0.05$), and the lack of fit value of 0.1479 ($p>0.1$) indicates that only a maximum of 14.79% chance that lack of fit F value could occur due to unexplained variance and that the proposed statistical model fits well.

As it can be determined from Table 9, the low standard deviation of 0.32 shows that experimental data are clustered closely in the mean value of 10.24 and signifies reliability. The 3.15% relative standard deviation, also known as the coefficient of variation and ratio of standard deviation to mean, supported this claim. The values of predicted residual error sum of squares (PRESS), R^2, adjusted R^2, and predicted R^2, which are all close to value of 1.0, provide a summary measure of fitness of the model. At 95% confidence level, the selected regression model is reduced to Equation (5).

$$Y(oil\ yield) = 11.48 + 2.01Z_1 + 1.14Z_2 + 0.25Z_3 \\ +0.26Z_1Z_2 - 0.49Z_2Z_3 - 2.01Z_1^2 - 0.47Z_2^2 - 2.81Z_1Z_2^2 \quad (5)$$

Examining the coefficients in Equation (5), oil yield was positively proportional to temperature, pressure, CO_2 flow rate, and quadratic effects of pressure and temperature interactions; whereas, a negative effect on the oil yield was brought by quadratic effects in temperature and pressure. A positive coefficient value for the estimated effect indicates an increase in the extraction yield if the variable is at its high level. A negative coefficient value indicates that a better extraction yield is obtained at low levels of the variables [31].

Table 8: RSM – FCCD Analysis of variance for oil yield using $scCO_2$ + co-solvent n-hexane

Source Model (Symbol)	Sum of Squares	dF	Mean Square	F-value	p[a]
Model	56.24	8	7.03	67.47	< 0.0001
Temperature (Z_1)	8.08	1	8.08	77.56	< 0.0001
Pressure (Z_2)	13.04	1	13.04	125.18	< 00001
CO_2 flowrate (Z_3)	0.65	1	0.65	6.24	0.0296
Temperature Pressure (Z_1Z_2)	0.55	1	0.55	5.29	0.0420
Pressure CO_2 flowrate (Z_2Z3)	1.92	1	1.92	18.44	0.0013
Temperature^2 (Z_1^2)	12.9	1	12.90	123.78	< 0.0001
Pressure^2 (Z_2^2)	0.70	1	0.70	6.71	0.0251
Temperature Pressure^2 ($Z_1Z_2^2$)	12.63	1	12.63	121.26	< 0.0001
Residual	1.15	11	0.10		
Lack of fit	0.88	6	0.15	2.7	0.1479
Pure error	0.27	5	0.054		
Corr. Total		19			

[a] $p < 0.01$ highly significant; $0.01 \leq p \leq 0.05$ significant; $p \geq 0.05$ insignificant

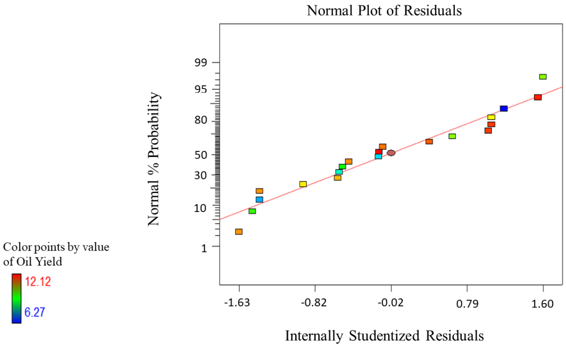

Figure 6: Normal plot of residuals.

Table 9: RSM- FCCD analysis of variance for oil yield using $scCO_2$ + co-solvent n-hexane

Statistical Parameter	Value
Standard Deviation	0.32
Mean	10.24
C.V.%	3.15
PRESS	5.76
R^2	0.9800
Adjusted R^2	0.9655
Predicted R^2	0.8997
Adequate Precision	26.168

The normal probability plot of the residuals is presented in Figure 6, where residuals tend to behave linearly, supporting the condition that error terms are normally distributed. On the other hand, the estimated response surface for pressure and temperature and pressure and flow rate versus oil yield (%) and their 2D and 3D contours are shown in Figure 7(a) and (b). It illustrates the interaction between variables and facilitates the location of optimal extraction conditions. It can be established from Figure 6(a) that extraction yield significantly increased with increasing temperature from 60–65°C and increasing pressure of 30–40 MPa. The highest oil yield is expected to occur in this same pressure and temperature range. Referring to Table 4 and comparing the oil yield results of experimental run nos. 2 and 1 and 17 and 16, the oil yield increased with increasing pressure at a constant temperature of 50°C or 70°C and a constant flow rate of 2 mL/min. This is similar to the observation made in oil extraction using 23 factorial design. Moreover, Figure 6(b) shows that maximum oil yield could be obtained at a high

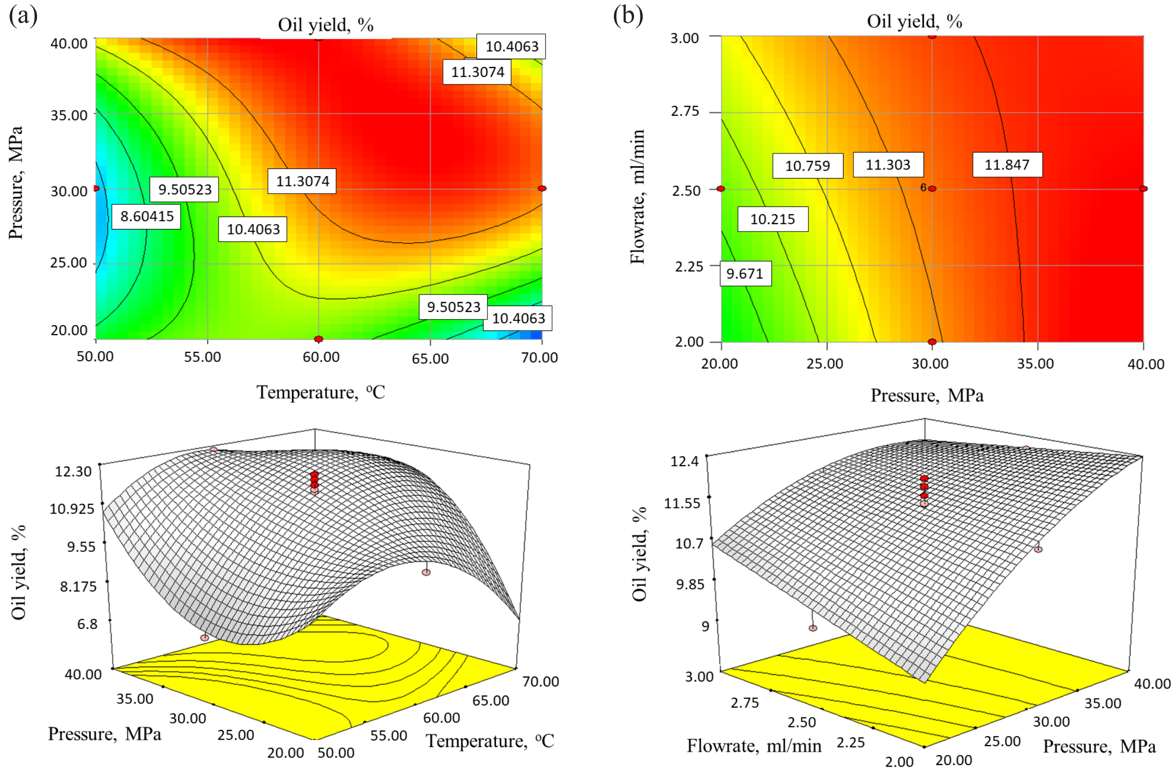

Figure 7: 2D Contour and 3D Surface images of (a) temperature and pressure and (b) pressure and CO_2 flow rate in oil yield using supercritical CO_2 extraction + co-solvent n hexane.

CO_2 flow rate and high-pressure conditions. Using *Design-Expert*® software, numerical optimization was performed through the desirability function method. This was conducted to determine the optimum extraction pressure, temperature, and CO_2 flow rate and obtain the maximum oil yield from *Gliricidia sepium* using $scCO_2$ and n-hexane. An extraction pressure of 35.8 MPa, temperature of 64.5°C, and CO_2 flow rate of 2 mL/min at constant 60 min of extraction time and of 3 mL n-hexane solvent addition were determined to be the optimal conditions for extraction. The maximum response was 12.15% oil yield (desirability = 1.0). The experiment was repeated in triplicate using these conditions, and the actual average oil yield resulted in 12.13% ($\sigma = 0.007$). This oil yield is higher compared to the 11.3% oil content of *Gliricidia sepium* (green seeds), and comparable to 13.9% oil content (brown seeds) produced using a Soxhlet extractor in a larger volume of n-hexane, and a longer extraction time [14].

3.4 *GC-FID analysis*

The *Gliricidia sepium* seed oil was successfully converted into methyl esters using the modified Standard Method of AOAC 969.33. The major fatty acid methyl esters identified after transesterification were palmitic acid methyl esters, heptadecanoic acid methyl esters, stearic acid methyl esters, oleic acid methyl esters, and linoleic acid methyl esters. This finding is similar to the reports of several authors [14], [37], [38].

4 Conclusions

The addition of n-hexane, a nonpolar solvent, significantly increased the oil yield in $scCO_2$ extraction. The amount of n-hexane used in this investigation was sufficient to saturate the sample's surface, eliminating the large volume and prolonged reaction time requirements of the traditional Soxhlet procedure. Among the variables considered in this process, pressure is the most significant factor that influences oil yield. According to the results provided using Face Centered Central Composite Design – Response Surface Methodology, the highest oil yield was achieved under the following conditions: pressure of 35.8 MPa, temperature of 64.5°C, and CO_2 flow rate of 2 mL/min at constant 60 min extraction time and 3 mL n-hexane solvent addition. The *Gliricidia sepium* seed oil, extracted with $scCO_2$ and n-hexane solvents, was used as feedstock and successfully converted into methyl esters.

Acknowledgments

This work was supported by the Department of Science and Technology – Philippine Council for Industry, Energy and Emerging Technology (DOST – PCIEERD) and e-Asia Joint Research Program (JRP) of Japan-Philippines-Thailand.

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