Biodiesel Production from Chia Seeds: A Sustainable and Renewable Energy Source

P. M. Namratha[1#], S. Vishnu[1], S. Myvizhi[1], B. Subhashini[1] and M. Y. Mohammed Shanawaz[1]

[1]*Department of Biotechnology, Sri Shakthi Institute of Engineering & Technology, Coimbatore, 641062*

Corresponding authors' details;

Ms. Namratha P. M

Assistant Professor

Department of Biotechnology

Sri Shakthi Institute of Engineering & Technology, Coimbatore

Email: namrathapmbt@siet.ac.in

Abstract:

The increasing global demand for sustainable energy solutions has fueled research into alternative and renewable sources of biodiesel. Chia seeds, derived from the Salvia hispanica plant, have gained attention as a potential feedstock for biodiesel production due to their high lipid content and favorable fatty acid profile. This study investigates the extraction of biodiesel from chia seeds through a comprehensive process involving oil extraction, transesterification, and purification. Chia seeds were subjected to oil extraction using environmentally friendly methods, such as cold pressing or solvent extraction, to obtain a lipid-rich feedstock. The extracted chia seed oil was then subjected to transesterification, converting triglycerides into biodiesel and glycerol. Various catalysts, including alkaline and enzymatic catalysts, were evaluated for their efficiency in promoting the transesterification reaction. The influence of key process parameters, such as reaction time, temperature, and catalyst concentration, on biodiesel yield and quality were systematically studied using experimental designs. The properties of the produced biodiesel, including viscosity, density, and cetane number, were analyzed to ensure compliance with international biodiesel standards. Furthermore, the environmental and economic aspects of chia seed biodiesel production were assessed to evaluate its viability as a sustainable energy source. Life cycle analysis and economic feasibility studies were conducted to determine the overall environmental impact and economic feasibility of chia seed biodiesel. The findings of this study contribute to the growing body of knowledge on alternative biodiesel feedstocks, providing valuable insights into the potential of chia seeds as a sustainable and renewable resource for biodiesel production. The environmentally friendly extraction methods and the optimization of the transesterification process highlight the feasibility of integrating chia seeds into the global biodiesel industry, contributing to a more sustainable and diversified energy portfolio.

Keywords: *Biodiesel, Chia seeds, Salvia hispanica plant, Solvent extraction*

1. Introduction

Conventional diesel occupies a great drawback of the total fuel usage around the world as it is now been used by many sources like ships, vehicles, diesel power planet, and some automatic sectors also diesel [1]. The importance of using alternate fuels instead of fossil fuels is to protect the environment from pollution and to preserve the non-renewable resource as it is been exploited more in recent days [2]. In recent days the world is been moving towards the usage of naturally produced biodiesel that is renewable and low-polluting. The biodiesel is a mono-alkyl ester of long-chain fatty acids and is typically produced through transesterification of oil and fats with methanol or ethanol in the presence of different acidic and alkaline catalysts [4]. However, biodiesel lacks sulfur and aromatic compounds that are present in conventional fuels [4]. Apart from controlling the production of greenhouse gases, it is also used in reducing the vibrations and noise in the compression ignition engine [1]. According to the National Wasteland Development Board, Ministry of Rural Areas and Employment Board India has about 75 mha of wasteland in the country that can be used for growing oil seed crops as avenue trees [2]. Over 350 oil crops have been identified for the production of biodiesel [4]. Biodiesel usage also helps in decreasing cancer-causing agents and air toxicity in a considerable range [4]. The production of biodiesel is done from different sources that are oil, vegetable oil, edible seed oil, and edible seed oil [4]. But in recent times it is found that biodiesel can also be produced from fresh water and marine algae species which seems to be very promising and could be used in the ignition engine by blending that with conventional fuel [1]. Biodiesel derived from edible sources not only increases the price of the food but also increases the price of the food materials. The Chia seeds which are *Salvia hispanica* are from the mint family and are a very good source of nutrients, fiber, protein, fatty acids, etc are used as raw material for the production of biodiesel. Chia seeds are found to be produced in large amounts in Australia, Argentina, and Columbia [3]. Chia seeds-based biodiesel are renewable source of energy which is a very good product that can be used instead of diesel which is a non-renewable source of energy. Chia-based biodiesel is found to be a great alternative compared to fossil fuel because it is highly biodegradable, non-toxic, non-polluting, and has a high calorific value [1]. The calorific value of chia seeds is high compared to many other seeds because they contain a lot of healthy fat as well as omega-3 fatty acids [3]. Chia seeds contain a lot of the best combinations of polyunsaturated fatty acids (PUFA) compared to other vegetable oils [3]. Chia seeds are classified based on the highest amount of PUFA and have a potential source of tocopherol [3]. Chia seeds are a very good source that helps in preventing obesity [3].

2. Methodology

2.1 Materials:

The seeds of the *Salvia hispanica* plant were gathered in the vicinity of Coimbatore, Tamil Nadu, India. The study utilized petroleum ether, hexane, methanol, sodium hydroxide, and sulfuric acid, all procured. Analytical reagent (AR) grade reagents and chemicals were employed for this research.

2.2 Particle size:

Seeds from the Salvia hispanica plant were obtained, dehulled, and subjected to drying at 105°C for moisture removal. A 40-mesh size sieve plate was employed to finely grind and separate the thoroughly dried seeds. The meshed seeds were categorized based on particle sizes: 1mm (coarse), 0.5–1 mm (medium), and below 0.5mm (powdered).

2.3 Extraction of Chia Seed oil:

Chia seed oil was obtained through Soxhlet extraction, utilizing petroleum ether as solvents. Subsequently, the extracted oil underwent a processing step to minimize the presence of free fatty acids. Optimization of the oil extraction process was achieved through Response Surface Methodology (RSM). The resulting oil was subjected to characterization and evaluation of its physicochemical properties. The fatty acid content of the extracted oil was determined using a Perkin Elmer Model GC-MS.

2.3.1. Oil extraction by Soxhlet Apparatus: Various methods are employed to extract crude oil from seeds, including mechanical extraction, solvent extraction, and biological extraction [5]. Among these, the Soxhlet extraction method, a solid-liquid extraction technique, is considered the most efficient [6]. This efficiency is attributed to the reusability of the solvent post-procedure and its ability to yield a higher quantity of oil. Co-solvents such as hexane, petroleum ether, methanol, chloroform, isopropanol alcohol, ethanol, and toluene are also utilized in the crude oil extraction process [7]. Leaching is conducted at the boiling point of each solvent until a clear liquid indicates complete bio-oil leaching [8]. Following a batch run, the packed bed of seeds is removed. A basic batch distillation process is then employed to further distill the oil-solvent mixture, which is subsequently separated using a rotary evaporator [9]. The percentage of crude oil yield is calculated using the formula,[10]

$$\% \text{ Oil Yield} = (W2/W1) \times 100 \quad (1)$$

where W1 represents the weight of the seeds and W2 represents the weight of the extracted oil.

2.4. Determination of characteristics of the oil:

To ensure the suitability of the seed oil for biodiesel synthesis, it is crucial to assess and characterize its physicochemical properties [11]. This includes evaluating its kinematic viscosity, peroxide value, saponification value, iodine value, free fatty acid (FFA) content, acid value (AV), and refractive index [12]. Among these, the free fatty acid and acid values are particularly vital, being assessed both before and after biodiesel production to ascertain the sustainability of the oil type. The remaining factors serve to prevent the formation of soap during the biodiesel synthesis process.[13]

2.4.1. Acid Value (AV) determination: To determine the Acid Value of the sample, phenolphthalein was used as an indicator, and NaOH served as the titrant. A 1g sample was weighed and dissolved in hot neutralized alcohol. The sample was then maintained at 60°C for 30 minutes to facilitate the reaction. Following this, a few drops of phenolphthalein indicator were introduced, and the sample was neutralized by titrating it with 0.1N NaOH. The Acid Value of the sample was calculated using the formula,[14]

$$\text{AV (\%)} = (\text{Amount of NaOH used} \times N \times 28.2) / (\text{Amount of oil used in g}) \quad (2)$$

where N represents the normality of NaOH used

2.4.2. Determination of free fatty acid (FFA) value: The FFA value of crude oil extracted from chia seeds was estimated using

FFA (%) = AV/ 2 (3)

2.4.3. Saponification Value: The process began by weighing 2g of the sample and placing it into a conical flask, followed by the addition of 25 ml of 0.1N ethanolic potassium hydroxide. The mixture was heated to boiling for 60 minutes with continuous stirring, and a reflux condenser was positioned atop the flask. After introducing a few drops of phenolphthalein indicator, the mixture was titrated with 0.5 M HCl until the pink color of the indicator rapidly disappeared, indicating the endpoint. This procedure was repeated for both the samples and the blanks. The formula for determining the saponification value was expressed as[14]

Saponification Value = 56.1 × N × (Vo-V1) / M (4)

where N represents the normality of HCl used, Vo is the volume of solution used for the blank solution, V1 is the volume of solution used for sample determination, and M represents the mass of the samples used.

2.4.4. Iodine value determination: The iodine value of the oil was determined using the Hanus method. In a conical flask, 0.1g of the oil sample was combined with 10 ml of anhydrous chloroform and 30 ml of the Hanus solution. The mixture was thoroughly mixed and left in a closet for approximately 30 minutes. Subsequently, a potassium iodide solution (20 ml of 15% weight volume) was added to rinse the iodine from the flask stopper. The entire solution was titrated with 0.14M $Na_2S_2O_3$ until a light-yellow color became visible. Following this, 2 ml of 1% starch indicator was introduced, resulting in the formation of a blue color. The titration continued until the blue color disappeared. This process was repeated for the blank solution under the same conditions, and the titration value was recorded. The iodine value was then calculated using the equation [13]

Iodine Value = (B2-R2) × Normality of $Na_2S_2O_3$ × 12.69 / Weight of the sample used (5)

where B2 and R2 represent the volumes of $Na_2S_2O_3$ used for titrating the blank and sample solutions.

2.4.5. Peroxide value: To determine the peroxide value of the sample, 1.0g of the sample was placed in a 100 ml conical flask, followed by the addition of 30 ml of chloroform (in a ratio of 3:2 v/v). After thorough mixing, 1ml of potassium iodide solution and 0.5ml of starch indicator solution were added. The mixture was titrated with 0.1N sodium thiosulphate until the solution turned dark blue. A similar process was carried out with a blank solution. The peroxide value was calculated using the formula,[13]

Peroxide value = (B2-R2) × Normality of $Na_2S_2O_3$ / Weight of the sample used (6)

where B2 and R2 represent the volumes of $Na_2S_2O_3$ used for titrating the blank and sample solutions.

2.5. Biodiesel Production:

In this study, biodiesel was synthesized in two stages: (i) pre-treatment acid-catalyzed esterification and (ii) base-catalyzed transesterification. The ultimate goal of the pre-treatment process is to reduce the FFA content of the oil sample to less than 2%, paving the way for the transesterification reaction. The main objective of the transesterification reaction is to increase the yield of biodiesel.

2.5.1. Pre-treatment process (acid-catalyzed-esterification): In each experiment of acid-catalyzed esterification, 100 ml of chia seed oil was utilized. Before initiating the reaction, the oil sample underwent filtration with Whatman filter paper to eliminate any unwanted components [15]. Before the reaction commencement, chia seed oil was placed in a round-bottom flask and preheated at 60°C for 30 minutes to remove undesirable products and water elements [16]. A mechanical stirrer facilitated the mixing process to expedite the reaction. H2SO4 served as both a catalyst and reagent in this procedure [17]. The preheated solution, with a molar ratio of 12:1 (alcohol to oil), was gradually added by H2SO4 at a ratio of 1% (v/v) in a drop-by-drop manner into the reactor. The reaction was maintained at a temperature of 60°C and a rotational speed of 600 rpm throughout the two-hour reaction period [18]. Upon completion, the reaction mixture was transferred to a separating funnel and allowed to stand for approximately 4 hours to eliminate excess alcohol, free fatty acid (FFA) content, and impurities. The esterified product, characterized by low FFA content, settled at the bottom of the funnel, while excess alcohol, H2SO4, and FFA were observed in the upper layer [19]. The esterified product was separated, and analysis revealed an FFA content of less than 2 mg KOH/g, falling within the suitable range for the transesterification reaction [20].

2.5.2. Transesterification (Base-catalyzed or alkali-catalyzed transesterification): The transesterification process, employed for biodiesel production, takes place in the same reactor and experimental facilities utilized for the esterification process [13]. In this procedure, methanol and a catalyst are used to conduct a transesterification reaction on highly purified chia seed oil [14]. To maintain a consistent temperature within the flask, a water bath is employed. The investigation of key parameters, including catalyst load, reaction time, and methanol-to-oil ratio, was undertaken to determine the optimum values for enhanced biodiesel yield. [16

Initially, 100ml of chia seed oil was placed in the reactor and gradually heated to 60oC. Subsequently, a preheated mixture containing methanol and sodium hydroxide (NaOH) was added to the chia seed oil. The mixture was thoroughly stirred at 600 rpm with a magnetic stirrer [20]. The time was recorded upon the mixing of the methoxide solution with oil. Maintaining the temperature at 60oC, just below the boiling point of methanol (65oC), facilitated the reaction. After the designated reaction time, the mixture was allowed to cool, and the resulting mixtures of biodiesel, glycerol, and undesirable compounds were carefully separated using a separating funnel [22]. The yield of biodiesel was calculated using the equation,

Biodiesel yield (%) = (Weight of biodiesel × 100) / Weight of oil used (7)

2.6. Properties and Characterization of Biodiesel:

Following the transesterification procedure, the biodiesel was analyzed to acquire its physicochemical properties, which are listed below.

2.6.1. Flash Point: Biodiesel was carefully poured into an Erlenmeyer flask, and the flask was then gradually heated on a hot plate. The temperature at which a flash is observed in a biodiesel sample is known as the flash point [23].

2.6.2. Pour Point: Biodiesel was filled to a specific level in a conical flask and attached to a wooden clamp along with a thermometer. The flask, containing the sample, was then cooled in an ice bath to temperatures below 0 °C. Subsequently, the flask with the sample was removed from the cold bath, clamped securely, and analyzed at various intervals. The pour point is identified as the temperature at which the sample initiates flow [24].

2.6.3. Cloud Point: Biodiesel was added to an Erlenmeyer flask and positioned on a stand alongside a thermometer. The flask was then immersed in an ice bath to achieve cooling below 0°C until the color turned white or cloudy. The temperature at which this cloudiness or whiteness becomes noticeable is referred to as the cloud point [23].

2.6.4. Determination of physical and fuel properties: The Koehler Bath Model K 23377 was employed to assess the kinematic viscosity of the sample at 40°C. Additionally, the K77001 Automated Cloud Point Analyzer, Ducom Pour Point Tester (DPP), and Flash Point Tester PMA50 were utilized to measure Pour Point, Cloud Point, and Flash Point, adhering to ASTM standards. The density at 15°C was determined with a Metler-Toledo densimeter. The physicochemical characteristics of the sample, including acid value, iodine value, free fatty acid (FFA) value, and saponification value, were analyzed using titration procedures. The functional group composition of the biodiesel was investigated through FT-IR analysis. Gas chromatography was employed to quantify the number of fatty acids in the oil sample by converting them into methyl esters.[23].

3. Results and Discussion:

3.1. Optimization of crude oil yield by response surface methodology:

The optimization of crude oil yield from *Salvia hispanica* (SH) seeds was conducted using Box Behnken Design (BBD) model, as illustrated in Table 1. The extraction process resulted in crude oil yields ranging from 20% to 30%, demonstrating the variability influenced by reaction conditions. Randomization of all runs was implemented to mitigate systematic errors. The design matrix in Table 1 presents experimental run order, predicted yields, and actual yields, affirming the variability in crude oil extraction from SH seeds based on reaction conditions.

Run order	Parameters		Responses			
	A: Seed to Solvent B:		Time (hr)	Actual Value (%)	Predicted Value (%)	Residual
	ratio	Temperature (ºC)				
1	0.14	90	0.75	4.29	2.93	1.36
2	0.14	72.5	3.375	5.73	5.73	0
3	0.2	72.5	0.75	4.52	5.27	-0.745
4	0.14	55	0.75	1.42	0.515	0.905
5	0.2	90	3.375	9.06	7.68	-0.615
6	0.14	55	6	9.88	11.24	-1.36
7	0.14	72.5	3.375	5.73	5.73	0
8	0.08	72.5	0.75	2.86	4.38	-1.52
9	0.2	55	3.375	7.06	7.22	-0.16
10	0.14	72.5	3.375	5.73	5.73	0
11	0.14	72.5	3.375	5.73	5.73	0
12	0.08	90	3.375	19.06	18.9	0.16
13	0.08	72.5	6	26	25.26	0.745
14	0.14	90	6	19.58	20.48	-0.905
15	0.2	72.5	6	14.19	12.67	1.52
16	0.14	72.5	3.375	5.73	5.73	0
17	0.08	55	3.375	8.31	7.7	0.615

Table 1: Experimental design (BBD) along with Residual, RSM predicted, and SH crude oil yield (%)

Through Response Surface Methodology (RSM) using a quadratic regression model predicted crude oil yields from SH seeds were generated, facilitating statistical analysis of experimental data. Equation (8) expresses the relationship between seed-to-solvent ratio (A), temperature (B), time (C), and the expected yield (Y) of SH crude oil.

$$Y = 5.73 - 2.93A + 2.92B + 7.07C - 2.69AB - 3.37AC + 1.171AC + 3.87A2 + 0.7713B2 + 2.29C2 \quad (8)$$

The experimental and projected SH oil production comparison, illustrated in Fig. 1b, demonstrates a well-fitting line, with points indicating minimal error, confirming the model's accuracy.

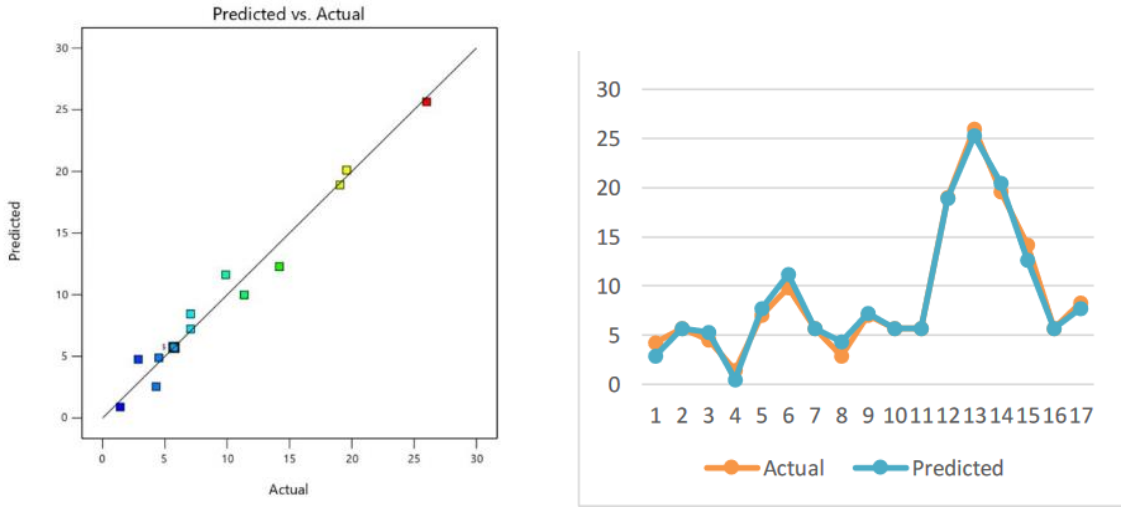

Figure 1a & b. SH crude oil yield between Actual and Predicted values

Table 1 presents the significance of parameters using P values, indicating the impact of individual parameters and their interactions on SH crude oil yield. The model's significance was confirmed at a 95% confidence level ($P < 0.05$). Additionally, Table 2 provides the analysis of variance (ANOVA) results, confirming the model's significance and identifying key parameters influencing SH crude oil production. Parameters such as seed-to-solvent ratio (A), temperature (B), time (C), and their quadratic effects demonstrated significant impacts on SH crude oil production. Interaction parameters, particularly the seed-to-solvent ratio (A), exhibited significant effects on crude oil yield. The model's lack of fit was found to be insignificant, further validating the relationship between independent and dependent parameters. With R^2 and adjusted R^2 values of 98.37% and 96.28% respectively, the regression model effectively explains variations in experimental data, indicating a robust relationship between parameters and response in SH crude oil production.

Table 2: The analysis of variance (ANOVA) results for SH crude oil

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value	Remarks
Model	716.85	9	79.65	46.95	<0.0001	Signicant
Linear	178.77	3	178.77	105.37	<0.0001	
A-seed to Solvent ratio	68.45	1	68.45	40.34	0.0004	
B-temperature	67.98	1	67.98	40.34	0.0004	
C-Time	399.88	1	399.88	40.07	<0.0001	
2-Way Interaction	28.63	1	28.63	16.88	0.0133	
AB	28.89	3	28.89	17.03	0.0044	
AC	45.36	1	45.36	26.74	0.0013	
BC	11.66	1	11.66	6.87	0.0343	

Square	29.23	1	29.23	17.23	0.090	
A^2	63.10	1	63.10	37.19	0.0005	
B^2	2.10	1	2.50	1.48	0.2637	
C^2	22.10	1	22.10	13.03	0.0086	
Residual	11.88	7	1.70			
Lack of Fit	11.88	3	3.96			
Pure Error	0.0000	4	0.0000			
$R^{2=0.9837}$			Adj $R^{2=0.9628}$			
Cor Total	728.73	16				

3.1.1 SH crude oil production's response surface plots:

To evaluate the influence of each independent variable on the optimization of *Salvia hispanica* (SH) crude oil production, it's essential to examine the interaction of the two factors involved in the process. This assessment is carried out through a regression model, which enables the generation of contour and surface plots depicting SH crude oil yield based on the equation provided. Surface plots are constructed by plotting any two independent variables against a three-dimensional (3D) surface curve while maintaining the other variables constant at their intermediate values. These plots visually represent how changes in two parameters affect SH crude oil yield. On the other hand, contour plots are generated by fixing one parameter constant and illustrating the interaction between the two remaining parameters. By observing contour plots, the variation in SH crude oil production due to alterations in experimental parameters can be identified.

3.1.1.1 Interaction effect of seed-to-solvent ratio with temperature:

Figure 2 illustrates the interaction effects of seed-to-solvent ratio and temperature on the *Salvia hispanica* (SH) crude oil yield while maintaining a constant reaction time of 6 hours. The plot demonstrates that SH crude oil yield decreases with an increase in seed-to-solvent ratio and a decrease in temperature. Conversely, the yield increases when the seed-to-solvent ratio decreases with an increase in temperature. However, beyond the optimal range, SH crude oil yield begins to decline. This phenomenon occurs because both parameters significantly impact SH oil yield, as indicated by the ANOVA table's p-values (<0.05). Run order 13 exhibits a higher yield (26%) resulting from the interaction between seed-to-solvent ratio (A) and temperature (B). A higher seed-to-solvent ratio affects the leaching process, leading to a lower yield, while an increase in temperature affects the process due to the lower boiling point of the solvent relative to the temperature. The 2D contour plot depicting the interaction between AB, as shown in Figure 2, carries significant implications for SH crude oil production.

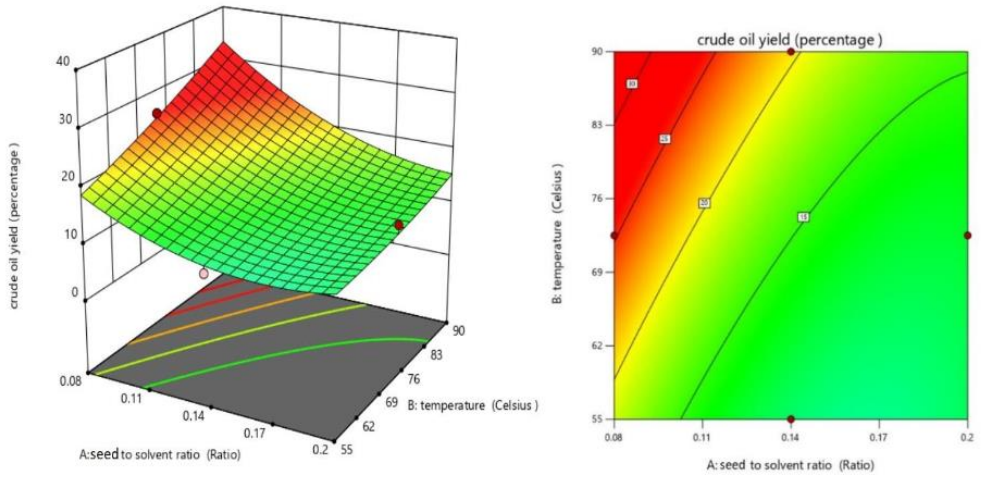

Figure 2. Interaction effect of seed to solvent ratio and temperature on SH crude oil extraction.

3.1.1.2 Interaction effect of seed-to-solvent ratio with time:

Figure 3 illustrates the interaction effect of seed-to-solvent ratio with time on the production of *Salvia hispanica* (SH) crude oil while maintaining a constant temperature of 72.5°C. The plot shows that SH crude oil yield decreases with increasing seed-to-solvent ratio and decreasing time, while it increases with decreasing seed-to-solvent ratio and increasing time. However, exceeding the optimal range of parameters leads to a decline in SH crude oil yield. This scenario arises due to both parameters having a significant p-value (<0.05) in the ANOVA table for SH oil yield. Within the optimal range of seed-to-solvent ratio and time, run orders 13, 14, and 15 demonstrate better yields compared to others. Lower yields are attributed to the leaching process being affected by a higher seed-to-solvent ratio. Despite the temperature being optimal, time emerges as more crucial since it has the most significant impact on crude oil production, as evidenced by its lower p-value of 0.0001, which is deemed significant. The 2D contour plot depicting the interaction between AC, as depicted in Figure 3, holds vital implications for SH crude oil production.

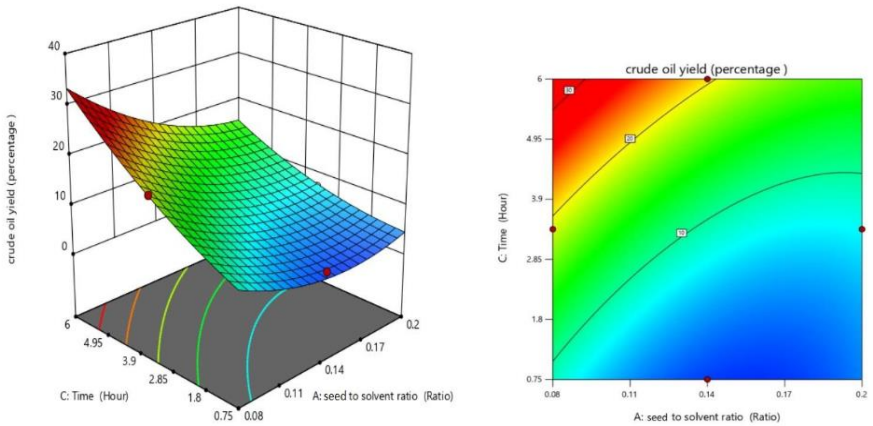

Figure 3. Interaction effect of seed to solvent ratio and time on SH crude oil extraction.

3.1.1.3 Interaction effect of time with temperature:

Figure 4 portrays the interaction effect of temperature and time on Salvia hispanica (SH) crude oil production while maintaining a fixed seed-to-solvent ratio of 0.08. The figure illustrates that the yield increases when both temperature and time are at their optimal levels, whereas it decreases when they deviate from these levels.

This phenomenon occurs because the leaching process only occurs at the boiling point of the solvent, and a minimum temperature is required to achieve a minimum yield of SH oil. However, if the parameters fall below the ideal level, the yield declines. This situation arises because both factors significantly impact the SH oil yield, as indicated by their significant p-value of 0.05 in the ANOVA table. Run orders 12, 13, 14, and 15 exhibit higher yields compared to others due to the optimal range of temperature and timing. Time emerges as more significant, as evidenced by its lower p-value of 0.0001, and contributes significantly to crude oil production. The 2D contour plot depicting the interaction between BC, as shown in Figure 4, significantly impacts the production of SH crude oil.

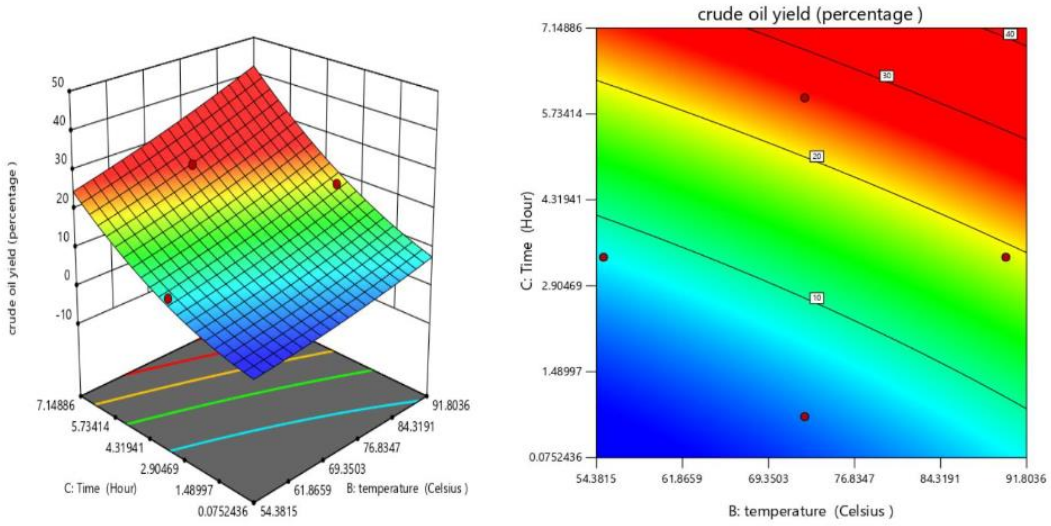

Figure 4. Interaction effect of time and temperature on SH crude oil extraction

3.2 Determination of the fatty acid composition of *Salvia hispanica* oil by GC-MS analysis:

The fatty acid content of Salvia hispanica oil was analyzed using GC-MS, and the results were compared to FAME standards. Table 8 presents the findings, indicating that approximately 66.7% of the oil comprises unsaturated fatty acids of SHO methyl esters, while 30.5% consists of saturated fatty acids of SHO methyl esters. This composition suggests that the double bond composition of SHO unsaturated fatty acids falls within the suitable range as per European biodiesel standards (Reshad et al., 2015).

Table 3: Fatty acid fractions and properties of (SH) oil which is used to produce biodiesel

Fatty acid/Oil properties	**Unit**	**Measured Values (%)**
Ph	-	6.2
Colour	-	Golden yellow
Density at 40^{o} C	Kg/cm^{2}	875
Moisture content	%	11.29
Iodine Value	gI_2 / 100g oil	115
Saponification Value	mg KOH /g oil	130
Peroxide Value	meq/Kg	1.92
Refractive index at 40^{o} C	-	1.355
Acid Value	mg KOH /g oil	8.38
Palmitic acid (C16:0)	%	21.5±0.2
Myristic acid (C14:0)	%	0.2±0.1
Oleic acid (C18:1)	%	10.5 ±0.3
α -Linolenic acid (C18:3)	%	1.4 ±0.2
Eicosanoic acid (C20:0)	%	2.0 ±0.2
Palmitoleic acid (C16:1)	%	0.3 ±0.2
Stearic acid (C18:0)	%	4.5 ±0.3
Gadoleic acid (C20:1)	%	0.6 ±0.2
Linoleic acid (C18:2)	%	56.2 ±0.3
Free Fatty Acid (FFA) value	mg KOH /g oil	4.19
others	%	2.8 ±0.1

3.3 Optimization of Biodiesel yield from SH oil by RSM:

The optimization of biodiesel production from Salvia hispanica (SH) oil was conducted using the Box Behnken Design (BBD) model, with parameters detailed in Table 4. Within the crude oil extraction process, biodiesel yield ranged from 65% to 90%, demonstrating the variability influenced by reaction parameters. The design matrix also includes experimental run order, predicted yields, and actual yields, indicating variation in biodiesel extraction from SH oil based on reaction conditions. All runs were randomized to prevent systematic errors.

Run order	Parameters		Responses			
	E: Methanol to SH Oil ratio	F: Catalyst Load (%)	G: Time (hr)	Actual Value (%) ©	Predicted Value (%)	Residual
1	**12**	**3**	**2**	**90**	**89.75**	**0.25**
2	9	1	1	75	74.25	0.75
3	9	2	2	82	82	0
4	9	2	2	82	82	0
5	9	3	3	85	85.75	-0.75
6	12	1	1	84	84.5	-0.5
7	12	2	2	80	80.25	-0.25
8	9	2	2	82	82	0
9	9	1	1	83	82.75	0.25
10	6	3	3	72	71.5	0.5
11	6	2	2	73	72.75	0.25
12	6	1	1	68	68.5	-0.5
13	6	2	2	65	65.25	-0.25
14	9	2	2	82	82	0
15	9	2	2	82	82	0
16	12	3	3	88	87.5	0.5
17	9	3	3	77	77.25	-0.25

Table 4: Experimental design (BBD) along with Residual, RSM predicted and SH Biodiesel yield (%)

Utilizing Response Surface Methodology (RSM) in Design Expert software version 13.0, predicted biodiesel yields from SH oil were generated through statistical analysis of experimental data. Equation (9) illustrates the relationship between the response parameter and other factors using a quadratic regression model. This model predicts yield (Y) based on Methanol to SH oil ratio (E), Time (G), and Catalyst Load (F) for SH biodiesel production.

$$Y = 82 + 8E + 4.25F + 1.5G + 0.5EF - 3.5E2 - 1.5F2 - 0.5G2 \quad (9)$$

A comparison between experimental and projected biodiesel production is depicted in Figure 5b, showing a well-fitting line with points indicating minimal error. Figure 5a confirms the model's acceptability through ample correlation between actual and predicted values (RSM). The impacts of SH biodiesel production were investigated through interaction, quadratic, and linear effects.

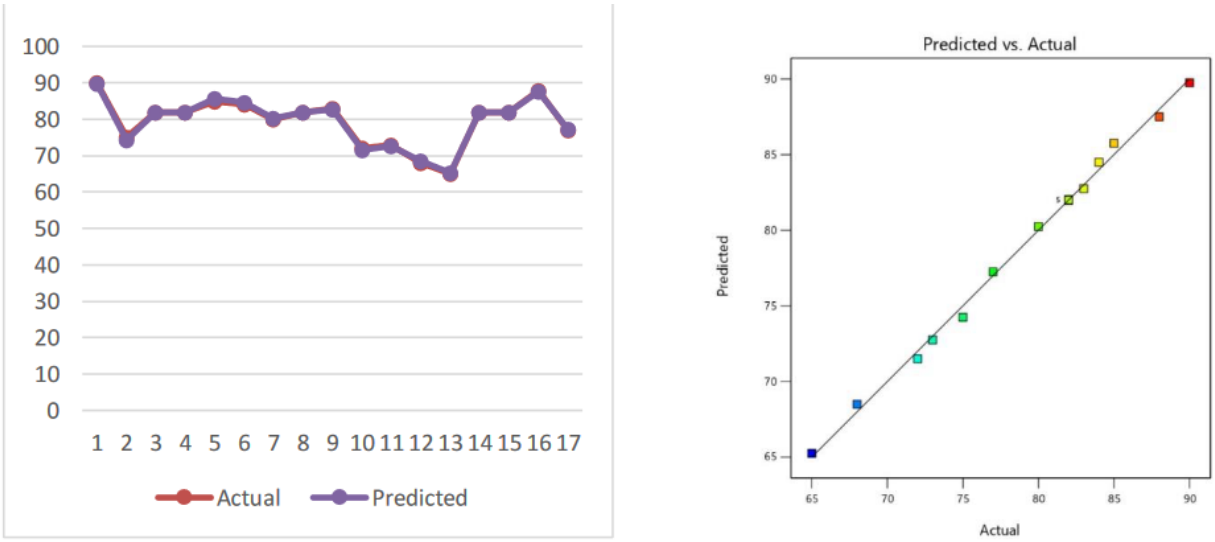

Figure 5 a & b. SH biodiesel yield between Actual and Predicted values.

Table 5 indicates the significance of parameters using P values, with E, F, G, EF, EG, and FG as positive coefficients representing linear effects, while E^2, F^2, and G^2 are negative coefficients with a reducing impact on biodiesel yield. The model's significance is validated with a P value less than 0.05 at a 95% confidence level.

Factor	Coefficient Estimate	Degree of Freedom	Standard Error	F value	P value
Intercept	82	1	0.2673	230.73	<0.0001
E-Methanol to LI oil ratio	8	1	0.2113	1433.6	<0.0001
F-Catalyst load	4.25	1	0.2113	404.6	<0.0001
G-Time	1.5	1	0.2113	50.4	0.0002
EF	0.5	1	0.2988	2.8	0.1382
EG	0	1	0.2988	0	1
FG	0	1	0.2988	0	1
E^2	-3.5	1	0.2912	144.42	<0.0001
F^2	-1.5	1	0.2912	26.53	0.0013
G^2	-0.5	1	0.2912	2.95	0.1297

Table 5: Coefficient of regression for the predicted quadratic polynomial model

Table 6 displays the significance level for each term and its interaction with selected responses. A lower p-value (0.0001) and greater F value (230.73) indicate model significance at a 99% confidence level. Notably, parameters such as E^2, F^2, and G^2, representing quadratic effects, significantly influence SH biodiesel production. Among interaction parameters, the Methanol to SH oil ratio (E) demonstrates the highest significance, with a greater F value (512) and a significance level of P value (0.0001). The regression model's lack of fit was found to be insignificant (0.8333), indicating a strong relationship between dependent and independent parameters such as E, F, and G. With R^2 and adjusted R^2 values of 99.66% and 99.23%, respectively, the regression model effectively explains variations in experimental data,

demonstrating a robust relationship between parameters and response in SH biodiesel production.

Table 6: The analysis of variance (ANOVA) results for SH biodiesel

Source	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F value**	**P value**	**Remarks**
Model	741.62	9	82.4	230.73	< 0.0001	significant
Linear	178.77	3	178.77	629.53	< 0.0001	
E-Methanol to LI oil ratio	512	1	512	1433.6	< 0.0001	
F-Catalyst load	144.5	1	144.5	404.6	< 0.0001	
G-Time	18	1	18	50.4	0.0002	
2-Way interaction	28.63	3	28.63	0.9333	0.7127	
EF	1	1	1	2.8	0.1382	
EG	0.0000	1	0.0000	0.0000	1	
FG	0.0000	1	0.0000	0.0000	1	
Square	29.23	1	29.23	57.96	0.0437	
E^2	51.58	1	51.58	144.42	< 0.0001	
F^2	9.47	1	9.47	26.53	0.0013	
G^2	1.05	1	1.05	2.95	0.1297	
Residual	2.5	7	0.3571			
Lack of Fit	2.5	3	0.8333			
Pure Error	0.0000	4	0.0000			
R^2=0.9966			Adj R^2=0.9923			
Cor Total	744.12	16				

3.3.1 SH biodiesel production's response surface plots:

To assess the influence of each independent variable in the optimization process, it's essential to examine the interaction of two factors on Salvia hispanica (SH) biodiesel production. This evaluation is facilitated by a regression model used to generate contour and surface plots of SH biodiesel yield from the provided equation. Surface plots depict how any two independent variables affect SH biodiesel yield on a three-dimensional (3D) surface curve, while keeping other variables constant at intermediate values. Contour plots, on the other hand, illustrate the interaction of two parameters while holding one constant, highlighting fluctuations in biodiesel production due to changes in experimental parameters.

3.3.1.1 Interaction effect of Methanol to SH oil ratio with catalyst load: The interaction effects of Methanol to Salvia hispanica (SH) oil ratio (E) and catalyst load (F) on SH biodiesel yield, with a constant reaction time of 2 hours, are depicted in Fig. 6. Increasing the methanol to SH oil ratio and catalyst load up to optimal levels boost biodiesel yield. Run order 12 shows a 90% maximum yield with increased catalyst load, while run order 13 demonstrates a 65% minimum yield with decreased catalyst load. These factors significantly influence biodiesel production, as indicated by a lower p-value (0.0001) in the ANOVA table. The 2D contour plot of the EF interaction in Fig. 6 does not significantly affect SH biodiesel production.

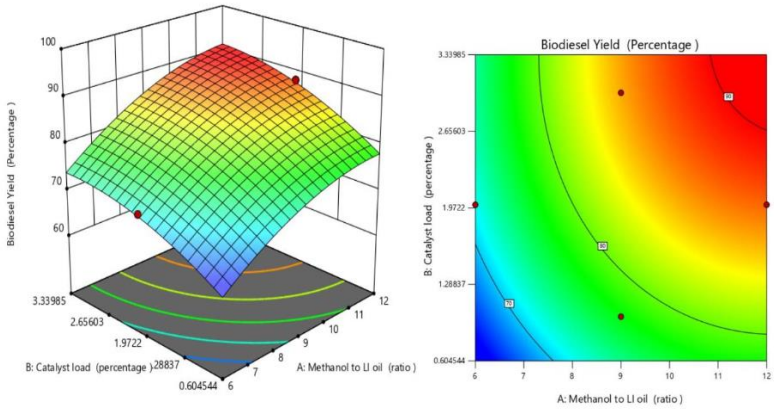

Figure 6. Interaction effect of Methanol to SH oil ratio and catalyst load on SH biodiesel yield.

3.3.1.2 Interaction effect of Methanol to SH oil ratio with time: The interaction effects of Methanol to *Salvia hispanica* (SH) oil ratio (E) and reaction time (F) on SH biodiesel yield, while maintaining a constant catalyst load of 3%, are depicted in Fig. 7 using 3D surface plots. The table indicates that SH biodiesel yield increases with the rise in the methanol to oil ratio. However, after reaching an optimal level, the yield starts to decrease with increasing reaction time. For instance, run order 1 achieves a yield of 90% at an optimal Methanol to SH oil ratio (E) of 12:1 and a reaction time (F) of two hours. Methanol to SH oil ratio (E) emerges as more significant in SH biodiesel production, as indicated by its lower p-value (<0.0001) compared to time (0.0002) in the ANOVA table. The 2D contour plots of the EG interaction displayed in Fig. 7 are not significant for SH biodiesel generation.

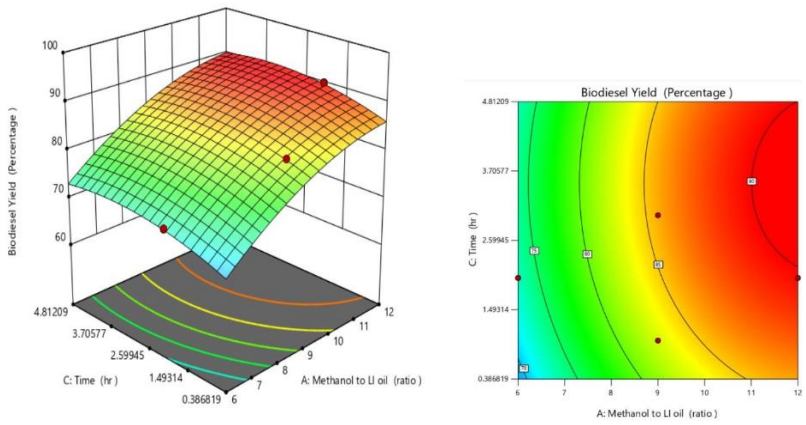

Figure 7. Interaction effect of Methanol to SH oil ratio and time on SH biodiesel yield.

3.3.1.3 Interaction effect of catalyst load with time: For a constant methanol to Salvia hispanica (SH) oil ratio of 12:1, Figure A illustrates the interaction effect of catalyst load (F) and reaction time (G) on SH biodiesel production. The yield of SH biodiesel increases as the catalyst load progressively rises, but decreases with lower catalyst load levels, as depicted in Fig. 10. Conversely, the yield rises up to a certain point in time and decreases beyond that point. Additionally, higher levels of catalyst load (F) and reaction time (G) lead to the saponification of triglycerides, resulting in the production of soap. According to the ANOVA table, both catalyst load (F) and reaction time (G) significantly affect SH biodiesel synthesis, indicated by their p-values being less than the significant value of 0.05. Run orders 3, 4, 5, 14, 15, and 16 demonstrate good yields compared to others, indicating optimal values for these parameters. Figure B displays the 2D contour plots of the FG interaction, which are not significant for SH biodiesel generation. Fig. 8 also depicts the 2D contour plots of the FG interaction, reinforcing their insignificance for SH biodiesel production.

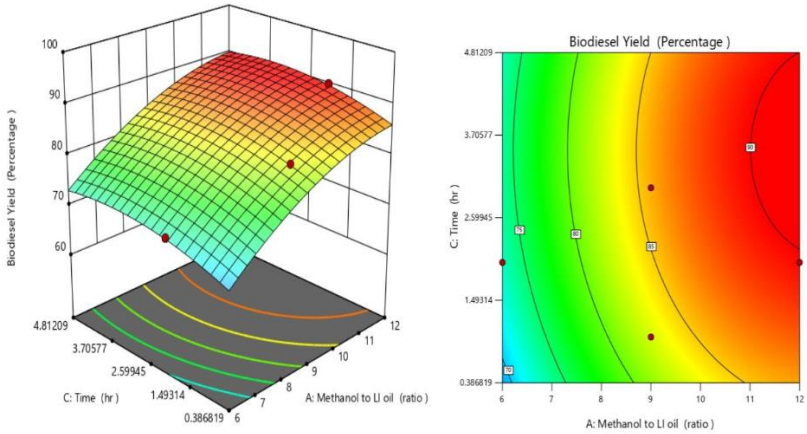

Figure 8. Interaction effect of time and catalyst load on SH biodiesel yield.

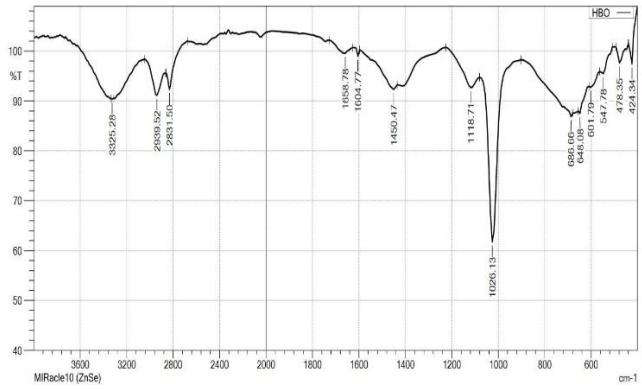

Figure 9. The FTIR spectrum analysis of SHO biodiesel

3.4 Analysis of the functional group of SHO biodiesel:

The FTIR spectral analysis of *Salvia hispanica* (SH) oil (SHO) biodiesel is depicted in Fig. 9. Characteristic peaks at 3325.28 cm^-1 and 2939.52-2851.50 cm^-1 indicate the formation of double bonds and single bond C-H stretching (methylene group). Peaks at 1658.78 cm^-1 and 1604.77 cm^-1 signify the presence of (-C=O ester) in SHO biodiesel. A conventional peak at 1450.47 cm^-1 indicates the presence of (CO)-O-CH3. Strong peaks at 1118.7 cm^-1 and 1026.1 cm^-1 suggest the existence of –C-O- esters,

while peaks ranging from 686.66 to 424.34 cm^-1 confirm the presence of CH2 groups, confirming the conversion of biodiesel in SHO biodiesel. Comparable results were observed in the production of biodiesel from *Argemone mexicana* oil using CaO catalyst (Ashine et al., 2023) and the extraction of oil from rubber seeds.

3.5 Properties of SHO biodiesel:

Table 12 summarizes the physicochemical and fuel parameters of *Salvia hispanica* (SH) oil (SHO) biodiesel synthesized using sodium hydroxide homogeneous catalyst, comparing them with ASTM standards for biodiesel. SHO biodiesel meets ASTM standards for density and kinematic viscosity, crucial for combustion and fuel injection. Cloud point (-4oC) and pour point (-12.5oC) ensure stability in cold climates. Flash point (~150oC) aligns with biodiesel standards, impacting engine parameters like power and stability. Low acid value (0.42%) indicates minimal free fatty acids, ensuring biodiesel quality. Iodine value (80 g I2/100g oil) reflects oxidation stability, while low saponification value (110 mg KOH/g oil) prevents soap formation during the reaction. Overall, SHO biodiesel performance aligns well with ASTM standards for biodiesel.

Properties	**Unit**	**ASTM D6751**	**SHO Biodiesel**
Kinematic viscosity	cSt	1.9-6.0	4.5
Flash Point	^oC	Min 130	150
Fire Point	^oC	-	162
Cloud Point	^oC	-3 to -12	-4
Saponification Value	mg KOH /g oil	312 max	110
Pour Point	^oC	-	-12.5
Iodine Value	g I2 / 100g oil	120 max	80
Acid Value(AV)	mg KOH /g oil	0.8 max	0.42
Density at 40oC	Kg/cm^3	860-900	875

Table 12: Comparison of physicochemical properties of SH biodiesel with fuel standard

4. Conclusion

In this study, biodiesel was produced from *Salvia hispanica* seeds through transesterification employing a homogeneous catalyst. Response surface methodology (RSM) was employed to explore various reaction parameters, including seed-to-solvent ratio, temperature, and time, for the extraction of crude oil from *Salvia hispanica* seeds. The optimal conditions resulted in a maximum crude oil yield of 26%, achieved at a seed-to-solvent ratio of 0.08%, temperature of 72.5°C, and a reaction time of 6 hours. Subsequent optimization studies using RSM focused on enhancing biodiesel synthesis by adjusting factors such as catalyst load, methanol to oil ratio, and reaction time. This led to a significant increase in yield, with 90% SHO (Liquid of Interest) biodiesel obtained under the following optimized conditions: methanol to oil ratio of 12:1, catalyst load of 3%, and a reaction time of 2 hours. Importantly, the produced biodiesel met the ASTM standard requirements for fuel properties.

5. References

[1] Priyam Deka, Mehedi Rahman, Sharmi Dev Sharma., "Biodiesel production from chia seeds and comparison of B5,B10 and B20 blends of conventional diesel", volume:04/Issue:08/August-2022.

[2] Vaibhav V.Mahishi,Sourabh Kulkarni., "Production and optimization of biodiesel transesterification of chia seed oil" Volume:3/issue-1/January-2020.

[3] Izzreen Ishak, Norhayati Hussain, Ranil Coorey, Maaruf Adf Ghani Volumn:45,March 2021.

[4] Kavitha Munisamy sambasivam, praveen kuooan, Lafiya Shanavas Laila, Viswanaathan shashiekha, Krishnamurthi Tamilarasan, Sudharsanam abinandhan, "Kernel-Based Biodiesel Production from Non-Edible Oil Seeds: Techniques, Optimization, and Environmental Implications".

[5] Bhargavi, G., Rao, P.N. and Renganathan, S., "Review on the extraction methods of crude oil from all generation biofuels in last few decades". In IOP conference series: materials science and engineering (Vol. 330, No. 1, p. 012024), 2018. IOP Publishing.

[6] Jalili, F., Jafari, S.M., Emam-Djomeh, Z., Malekjani, N. and Farzaneh, V., "Optimization of ultrasound-assisted extraction of oil from canola seeds with the use of response surface methodology". Food analytical methods, (2018), 11, pp.598-612.

[7] Verma, A., Sharma, A. and Rai, P.K., "Impact of soxhlet extraction method on oil yield and antioxidant potential of Brassica juncea. Journal of Pharmacognosy and Phytochemistry", (2019), 8(4), pp.1134-1137.

[8] Dutta, R., Sarkar, U. and Mukherjee, A., "Extraction of oil from Crotalaria Juncea seeds in a modified Soxhlet apparatus: Physical and chemical characterization of a prospective bio-fuel". Fuel, (2014), 116, pp.794-802.

[9] Hibbert, S., Welham, K. and Zein, S.H., "An innovative method of extraction of coffee oil using an advanced microwave system: in comparison with conventional Soxhlet extraction method". SN Applied Sciences (2019), 1, pp.1-9.

[10] Sulaiman, S., Aziz, A.A. and Aroua, M.K., "Optimization and modeling of extraction of solid coconut waste oil". Journal of Food Engineering, (2013), 114(2), pp.228-234.

[11] Momoh, O.R., Audu, H.I. and Binta, Z.B., "Investigating the production of biodiesel from alphonso mango seed oil". Nigerian Journal of Technology, (2014), 33(4), pp.497-503.

[12] Ashine, F., Kiflie, Z., Prabhu, S.V., Tizazu, B.Z., Varadharajan, V., Rajasimman, M., Joo, S.W., Vasseghian, Y. and Jayakumar, M., "Biodiesel production from Argemone mexicana oil using chicken eggshell derived CaO catalyst". Fuel, (2023), 332, p.126166.

[13] Ahmed, F., Giwa, S.O., Ibrahim, M. and Giwa, A., "Production of biodiesel from Jatropha curcas seed oil using base catalysed transesterification". International Journal of ChemTech Research, (2016).

[14] Martins, P.F., Ito, V.M., Batistella, C.B. and Maciel, M.W., 2006. Free fatty acid separation from vegetable oil deodorizer distillate using molecular distillation process. Separation and Purification Technology, 48(1), pp.78-84.

[15] Bhuiya, M.M.K., Rasul, M.G., Khan, M.M.K. and Ashwath, N., "Biodiesel production and characterisation of poppy (Papaver somniferum L.) seed oil methyl ester as a source of 2nd generation biodiesel feedstock" . Industrial crops and products, (2020), 152, p.112493.

[16] Thiruvengadaravi, K.V., Nandagopal, J., Baskaralingam, P., Bala, V.S.S. and Sivanesan, S., "Acid-catalyzed esterification of karanja (Pongamia pinnata) oil with high free fatty acids for biodiesel production". Fuel, (2012), 98, pp.1-4.

[17] Sutapa, I.W. and Ropa, H., 2019, October. Non-edible Oil of Cerbera manghas L. "Seed From Seram Island-Maluku as Oil Source of Biodiesel Production. In Journal of Physics": Conference Series (Vol. 1341, No. 3, p. 032001). IOP Publishing.

[18] Zhang, J. and Jiang, L., 2008. "Acid-catalyzed esterification of Zanthoxylum bungeanum seed oil with high free fatty acids for biodiesel production". Bioresource technology, 99(18), pp.8995-8998.

[19] Hayyan, A., Alam, M.Z., Mirghani, M.E., Kabbashi, N.A., Hakimi, N.I.N.M., Siran, Y.M. and Tahiruddin, S., 2011. "Reduction of high content of free fatty acid in sludge palm oil via acid catalyst for biodiesel production". Fuel Processing Technology, 92(5), pp.920-924.

[20] Veljković, V.B., Lakićević, S.H., Stamenković, O.S., Todorović, Z.B. and Lazić, M.L., 2006. "Biodiesel production from tobacco (Nicotiana tabacum L.) seed oil with a high content of free fatty acids". Fuel, 85(17-18), pp.2671-2675.

[21] Duz, M.Z., Saydut, A. and Ozturk, G., 2011. "Alkali catalyzed transesterification of safflower seed oil assisted by microwave irradiation". Fuel Processing Technology, 92(3), pp.308-313.

[22] Yesilyurt, M.K., Arslan, M. and Eryilmaz, T., 2019. "Application of response surface methodology for the optimization of biodiesel production from yellow mustard (Sinapis alba L.) seed oil". International journal of green energy, 16(1), pp.60-71.

[23] Kartika, I.A., Yani, M., Ariono, D., Evon, P. and Rigal, L., 2013. "Biodiesel production from jatropha seeds: Solvent extraction and in situ transesterification in a single step". Fuel, 106, pp.111-117.

[24] Nounah, I., Hajib, A., Harhar, H., Madani, N.E., Gharby, S., Guillaume, D. and Charrouf, Z., 2017. "Chemical composition and antioxidant activity of Lawsonia inermis seed extracts from Morocco". Natural Product Communications, 12(4), p.1934578X1701200405.