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Optimization of oil extraction from soybean by Response Surface Methodology utilizing d-Limonene as solvent

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ABSTRACT

Commercial oil extraction from soybeans is commonly achieved using solvent extraction with n-Hexane. The desire for fewer harmful and less combustible solvents has fueled the search for suitable green solvents. In the current investigation, Response Surface Methodology (RSM) was used to optimize oil extraction from soybeans by d-Limonene as a solvent. Temperature was varied from 25 to 170 ºC, solvent volume from 4 to 20 mL, contact time from 30 to 360 min, particle size from 0.25 to 0.50 mm, and agitation rate from 50 to 200 rpm to study their effects on the yield with the aid of a two-level, five-factor central composite design (CCD). The quality of extracted oil using d-Limonene was compared to that extracted with n-Hexane by Fourier transform infrared spectroscopy (FTIR). The second-order model developed accurately predicts the extracted oil yield. A 55 percent maximum oil yield was achieved at 170 °C, 20 mL solvent volume, 360 minutes, 0.5 mm particle size, and 200 rpm. Temperature, solvent volume, contact time, and particle size substantially influence the extracted oil yield. The rate of agitation has minimal effect on the oil yield. The oil extracted at the process condition by d-Limonene or n-Hexane has the same quality.

Keywords: d-Limonene, n-Hexane, solvent extraction, soybean, Optimization

Introduction

The growing environmental concerns about the toxicity and flammability of n-Hexane as extraction solvent have been a drive to search for a green and cost-effective alternative solvent. Besides solvent extraction, mechanical (cold) pressing, and supercritical fluid extraction have been employed as oil extraction techniques.

Cold pressing maintains oil quality, but it produced lower oil yield than solvent and supercritical fluid extraction methods (Pradhan *et al*., 2010). Supercritical fluid oil extraction usually utilizes CO_2 at a critical temperature and pressure to provide moderate oil quality, although at higher capital and operating costs (Yen *et al*., 2015; Kumar *et al*., 2017).

n-Hexane oil extraction is preferable to mechanical pressing because of higher yield and causes low turbidity. n-Hexane efficiently solubilizes oil and may readily recover after extraction since it has low boiling point of 69ºC.

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Though n-Hexane oil extraction has been marketed, a high safety, health and environmental risks associated with its usage. Therefore, it is not an ideal solvent for oil extraction. For instance, to minimize hexane leaks during extraction, expensive equipment is essential to safeguard personnel's health and safety. In addition, n-Hexane is a documented chronic neurotoxicant for humans (Spencer and Schaumburg, 1985; DeCaprio, 1987). In animals and humans, n-Hexane metabolizes into a gamma diketone (2, 5-hexanedione), a more neurotoxic than the n-Hexane itself (Spencer and Schaumburg, 1985). Acute inhalation of n-Hexane induces dizziness, giddiness, nausea, and headaches (Sittig, 1985; U.S. Environmental Protection Agency, 1987; U.S. Department of Health and Human Services, 1993). Acute exposure to n-Hexane may result to dermatitis and irritation of the eyes and throat in humans (Sittig, 1985). n-Hexane is a nonrenewable resource produced from petrochemical that is rapidly depleting.

d-Limonene, a green solvent, is non-hazardous and derived from renewable resources. Given that d-Limonene has a Hasen solubility parameter (HSP) that is similar to n-Hexane (Tanzi *et al*., 2012; Sicaire *et al*., 2015), it is possible that it has a similar affinity for soybean oil as n-Hexane. To the best of the authors' knowledge, optimization of oil yield from soybean using d-Limonene as a solvent, specifically the effects of five independent variables on oil yield, namely solvent volume, contact time, temperature, particle size, and agitation rate, has not been reported in the literature. This study investigates this gap by statistically altering those independent variables and assessing the effect on oil yield using Response Surface Methodology (RSM).

Materials and Methods

Materials

Clean and dried soybean was obtained from a local seed merchant. Analytical grade solvents purchased from Sigma Aldrich (UK) was used as received. d-Limonene has a purity of 98 percent, whereas n-hexane has a purity of 95 percent. Other physical properties of d-Limonene and n-Hexane are listed in Table 1.

Table 1 : Properties of d-Limonene and n-Hexane

Property	Hexane	d-Limonene
Molecular weight	86.17	136.23
Specific gravity (25^{o}C)	0.65	0.84
Viscosity, cp (25^{o}C)	0.32	0.92
Boiling point, ^{o}C	68.74	162.78
Latent heat of vaporization (cal./g)	79.9	84.4
Specific heat (cal./g^{o}C)	0.53	0.44
Solubility in water, wt. (25^{o}C)	0.00123	0.00138
Dielectric constant, 20^{o}C	1.89	2.37
Flash point, ^{o}C	-23	48
Surface tension, dyne/cm (25^{o}C)	18.4	27
Hansen solubility parameters $\delta_{total}(cal^{1/2}.cm^{-3/2})$	7.3	8
δ_d	7.3	8
δ_p	0	0.1
δ_h	0	0.1
Renewable	No	Yes
Toxic	Yes	No

Riddick *et al.* (1986); Braddock (1999); Tanzi *et al*., 2012; Sicaire *et al*., 2015

Sample preparation

The soybean seed's husk was manually removed. The dehulled soybean was roasted for 35 minutes at 100 ^{o}C in a stainless steel container to increase oil yield (Tulashie *et al*., 2018). After that, it was ground into a powder and sieved into various particle sizes.

Extraction Procedure

The central composite design described in Table 2 was used to determine the various combinations for each oil extraction experiment. Each row corresponds to a different experiment. A defined volume of d-Limonene was poured over 250 mg of pulverized soybean weighed into a 50 mL beaker. A magnetic stirrer (RE 400) was used to agitate the mixture for the duration of the extraction time. For a list of other experimental combinations, see Table 2. The extracted oil yield was calculated as a percentage (Equation 1), with the mass of oil extracted divided by the mass of soy bean weighed into the beaker. Every experiment was carried out twice.

$$Extraction\ yield = \frac{Mass\ of\ Extracted\ oil}{Mass\ of\ Biomass} \times 100\%$$

Equation 1

Experimental Design and Statistical Analysis

Minitab 18 was used to design the trials and statistically analyze the results in order to determine how each of the independent variables, as well as their interactions, affects oil extraction yields. The experimental design was then applied to the response surface methodology's central composite design (CCD).

The optimization of the extraction oil yield involves three main steps: (i) conducting statistically designed tests by CCD, (ii) estimating the coefficients in the mathematical model, and (iii) predicting the response and verifying model suitability (Myers *et al*., 2009).

The response (yield) was expressed as a function of the selected independent variables using a second-order quadratic model. It usually takes the form illustrated in Equation 2 (Myers *et al*., 2009).

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k}\sum_{j=1}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \varepsilon \qquad Equation\ 2$$

Where Y is the response variables, β_o is the intercept, β_i , β_{ij} and β_{ii} are the coefficients of the linear effect, double interactions, X_i , X_j are the independent variables or factors and ε is error.

Table 2 lists the lower and upper-level values of each variable used in the CCD design. Due to the usage of a replica, 64 experimental runs were created from CCD and utilized to assess the effect of the five independent factors on the oil extraction yield. Each column represents an independent variable, whereas each row represents an experiment.

Table 2: Lower and upper values of independent variables for design of experiment

S/N	Independent variables	Lower level	Upper level
1	Temperature (ºC)	25	170
2	Volume(ml)	4	20
3	Time (mins)	30	360
4	Particle size (mm)	0.25	0.50
5	Agitation rate (rpm)	50	200

FT-IR characterization of the extracted oil

The functional groups present in soybean oil extracted with hexane and limonene were detected using an FT-IR spectroscope (FTIR-8400S, Shimadzu) in the range of 4,000–500 cm^{-1}. The oil samples were combined with spectroscopic grade KBr that had been oven dried and pressed into a disk (Bakar *et al*., 2013).

Table 3: Central composite design for the experimental runs

StdOrder	RunOrder	Blocks	Temp	Vol	Time	Particle size	Agitation rate
42	1	1	170.0	4	30	0.500	200
58	2	1	97.5	12	195	0.375	200
9	3	1	25.0	4	30	0.500	50
39	4	1	25.0	20	360	0.250	200
48	5	1	170.0	20	360	0.500	200
63	6	1	97.5	12	195	0.375	125
60	7	1	97.5	12	195	0.375	125
23	8	1	97.5	12	195	0.250	125
6	9	1	170.0	4	360	0.250	200
10	10	1	170.0	4	30	0.500	200
61	11	1	97.5	12	195	0.375	125
7	12	1	25.0	20	360	0.250	200
24	13	1	97.5	12	195	0.500	125
4	14	1	170.0	20	30	0.250	200
47	15	1	25.0	20	360	0.500	50
34	16	1	170.0	4	30	0.250	50
17	17	1	25.0	12	195	0.375	125
18	18	1	170.0	12	195	0.375	125
64	19	1	97.5	12	195	0.375	125
54	20	1	97.5	12	360	0.375	125
37	21	1	25.0	4	360	0.250	50
1	22	1	25.0	4	30	0.250	200
13	23	1	25.0	4	360	0.500	200
14	24	1	170.0	4	360	0.500	50
16	25	1	170.0	20	360	0.500	200
25	26	1	97.5	12	195	0.375	50
22	27	1	97.5	12	360	0.375	125
35	28	1	25.0	20	30	0.250	50
59	29	1	97.5	12	195	0.375	125
62	30	1	97.5	12	195	0.375	125
44	31	1	170.0	20	30	0.500	50
51	32	1	97.5	4	195	0.375	125
52	33	1	97.5	20	195	0.375	125
28	34	1	97.5	12	195	0.375	125
26	35	1	97.5	12	195	0.375	200
2	36	1	170.0	4	30	0.250	50
32	37	1	97.5	12	195	0.375	125
12	38	1	170.0	20	30	0.500	50
8	39	1	170.0	20	360	0.250	50
3	40	1	25.0	20	30	0.250	50
33	41	1	25.0	4	30	0.250	200
50	42	1	170.0	12	195	0.375	125
45	43	1	25.0	4	360	0.500	200
40	44	1	170.0	20	360	0.250	50
55	45	1	97.5	12	195	0.250	125
43	46	1	25.0	20	30	0.500	200
31	47	1	97.5	12	195	0.375	125
53	48	1	97.5	12	30	0.375	125
56	49	1	97.5	12	195	0.500	125
57	50	1	97.5	12	195	0.375	50
15	51	1	25.0	20	360	0.500	50
27	52	1	97.5	12	195	0.375	125
36	53	1	170.0	20	30	0.250	200
19	54	1	97.5	4	195	0.375	125
46	55	1	170.0	4	360	0.500	50
20	56	1	97.5	20	195	0.375	125
11	57	1	25.0	20	30	0.500	200
49	58	1	25.0	12	195	0.375	125
30	59	1	97.5	12	195	0.375	125
21	60	1	97.5	12	30	0.375	125
29	61	1	97.5	12	195	0.375	125
41	62	1	25.0	4	30	0.500	50
38	63	1	170.0	4	360	0.250	200
5	64	1	25.0	4	360	0.250	50

Results and Discussion

Solvent extraction of soybean oil is a mass transfer operation that requires a driving force for excellent performance. The level of oil/solvent interaction (which is usually localized between cells) determines the efficacy of such operation. Temperature (X_1), d-Limonene volume (X_2), extraction time (X_3), particle size (X_4), and agitation rate (X_5) are the selected five factors (independent variables), whereas oil yield is the response factor (dependent variable) (Y).

The maximum and the least oil yield obtained

The effects of the five independent factors on the oil yield using d-limonene as a solvent were studied. In Response Surface Methodology, their impacts were statistically investigated using central composite design (CCD) (RSM). Table 3 shows the CCD matrix utilized in the experiment. Each row indicates a set of parameters that were employed in each experiment, as well as the observed and projected oil yields. The oil yields range from 29 percent to 55 percent, according to the data in Table 4. This fluctuation demonstrates why it is critical to explore the process variables that result in the maximum possible oil yield.

At 170 °C, 20 mL limonene, 360 minutes of contact time, 0.5 mm particle size, and 200 rpm agitation, 55 percent maximum oil was achieved. With 170 °C, 20mL of limonene, 360 minutes of contact time, and 0.5 mm particle size, a predicted yield of 54.9 percent was obtained by using the regression model, Equation 1, obtained from the software after the analysis of result. At 25 ºC, 4mL limonene, 30 min contact time, 0.5 mm particle size, and 50 rpm agitation, a minimum of 29 percent oil yield was obtained, while a minimum of 29 percent predicted oil yield was obtained when these variables were placed into Equation 3. This demonstrates that for both low and high levels of the independent variables, the second-order model (Equation 3) effectively predicted the oil production. Other experimental settings studied were accurately predicted by the model equation, as shown in the table 4. When d-Limonene was employed to extract rice bran oil, Sean and Mamidipally (2005) reported a highest oil yield of 23 percent, which is less than the 54.9 percent in the current study. It's worth noting that they employed rice bran, and the extraction conditions were 30 minutes, 5:1 d-Limonene/biomass ratio, equivalent to 42 mL/g of biomass, which was substantially less than the 6 hours and 80mL/g biomass used here.

$$Y = 22.68 - 0.0169\,X_1 + 0.660\,X_2 + 0.0399\,X_3 - 1.1X_4 + 0.0172\,X_5 + 0.000254\,X_1^2 - 0.0104X_2^2 - 0.000044\,X_3^2 + 10.3\,X_4^2 + 0.000038\,X_5^2 + 0.003270\,X_1X_2 - 0.000027\,X_1X_3 + 0.0334\,X_1X_4 + 0.000136\,X_1X_5 - 0.000845X_2X_3 - 0.053X_2X_4 - 0.001234\,X_2X_5 + 0.0202\,X_3X_4 - 0.000042\,X_3X_5 - 0.0290\,X_4X_5 \quad \text{Equation 3}$$

Where:

$Y = Oil\ Yield$

$X_1 = Temperature$

$X_2 = Volume$

$X_3 = Contact\ Time$

$X_4 = Particle\ size$

$X_5 = Agitation\ rate$

Oil yield versus independent variables relationship

CCD was used to examine oil yield versus independent factor's relationship, and the results are displayed in Table 5 and Fig. 1. Each factor's coefficient indicates the magnitude of the factor's impact on percentage oil yield. All five components have positive coefficients, as seen in the table. This suggests that they all have a beneficial impact on oil yield. This explains why the highest oil yield (55%) was obtained when all of the variables were at their maximum values. When all of the variables were at their lowest values, however, a minimum of 29% oil yield was obtained.

Temperature has the highest positive effect on the yield, with a regression coefficient of 6.981, while agitation rate has the least positive effect on the yield, with a regression coefficient of 0.450. Decreasing order of effect of the factor on the yield is $Temp. > Vol. > Contact\ time > Particle\ size > Agitation\ rate$ as shown clearly by their regression coefficient in Table 5.

Table 4: Dependent variables versus actual and predicted yield

Std Order	Run Order	Blocks	Temp	Vol.	Time	particle size	Agitation	Actual yield	Predicted Yield
42	1	1	170.0	4	30	0.500	200	47.5	43.3
58	2	1	97.5	12	195	0.375	200	41.0	41.6
9	3	1	25.0	4	30	0.500	50	29.0	29.0
39	4	1	25.0	20	360	0.250	200	34.0	33.8
48	5	1	170.0	20	360	0.500	200	55.0	54.9
63	6	1	97.5	12	195	0.375	125	40.8	41.0
60	7	1	97.5	12	195	0.375	125	40.8	41.0
23	8	1	97.5	12	195	0.250	125	39.0	39.9
6	9	1	170.0	4	360	0.250	200	45.0	45.3
10	10	1	170.0	4	30	0.500	200	39.0	43.3
61	11	1	97.5	12	195	0.375	125	40.8	41.0
7	12	1	25.0	20	360	0.250	200	34.0	33.8
24	13	1	97.5	12	195	0.500	125	41.8	42.3
4	14	1	170.0	20	30	0.250	200	55.5	52.4
47	15	1	25.0	20	360	0.500	50	39.0	39.4
34	16	1	170.0	4	30	0.250	50	31.0	36.1
17	17	1	25.0	12	195	0.375	125	32.5	35.3
18	18	1	170.0	12	195	0.375	125	50.0	49.3
64	19	1	97.5	12	195	0.375	125	42.5	41.0
54	20	1	97.5	12	360	0.375	125	45.9	41.8
37	21	1	25.0	4	360	0.250	50	34.0	34.5
1	22	1	25.0	4	30	0.250	200	30.0	29.9
13	23	1	25.0	4	360	0.500	200	34.0	37.1
14	24	1	170.0	4	360	0.500	50	47.0	46.4
16	25	1	170.0	20	360	0.500	200	55.0	54.9
25	26	1	97.5	12	195	0.375	50	42.5	40.7
22	27	1	97.5	12	360	0.375	125	40.0	41.8
35	28	1	25.0	20	30	0.250	50	34.0	33.7
59	29	1	97.5	12	195	0.375	125	40.8	41.0
62	30	1	97.5	12	195	0.375	125	40.8	41.0
44	31	1	170.0	20	30	0.500	50	55.5	52.5
51	32	1	97.5	4	195	0.375	125	38.5	37.2
52	33	1	97.5	20	195	0.375	125	41.5	43.4
28	34	1	97.5	12	195	0.375	125	40.8	41.0
26	35	1	97.5	12	195	0.375	200	41.0	41.6
2	36	1	170.0	4	30	0.250	50	40.8	36.1
32	37	1	97.5	12	195	0.375	125	40.8	41.0
12	38	1	170.0	20	30	0.500	50	50.0	52.5
8	39	1	170.0	20	360	0.250	50	52.5	51.3
3	40	1	25.0	20	30	0.250	50	34.0	33.7
33	41	1	25.0	4	30	0.250	200	30.0	29.9
50	42	1	170.0	12	195	0.375	125	50.0	49.3
45	43	1	25.0	4	360	0.500	200	40.0	37.1
40	44	1	170.0	20	360	0.250	50	50.0	51.3
55	45	1	97.5	12	195	0.250	125	41.0	39.9
43	46	1	25.0	20	30	0.500	200	34.0	33.5
31	47	1	97.5	12	195	0.375	125	42.5	41.0
53	48	1	97.5	12	30	0.375	125	38.0	37.7
56	49	1	97.5	12	195	0.500	125	42.5	42.3
57	50	1	97.5	12	195	0.375	50	40.0	40.7
15	51	1	25.0	20	360	0.500	50	40.0	39.4
27	52	1	97.5	12	195	0.375	125	40.8	41.0
36	53	1	170.0	20	30	0.250	200	50.0	52.4
19	54	1	97.5	4	195	0.375	125	38.5	37.2
46	55	1	170.0	4	360	0.500	50	45.0	46.4
20	56	1	97.5	20	195	0.375	125	42.5	43.4
11	57	1	25.0	20	30	0.500	200	34.0	33.5
49	58	1	25.0	12	195	0.375	125	36.5	35.3
30	59	1	97.5	12	195	0.375	125	40.8	41.0
21	60	1	97.5	12	30	0.375	125	35.0	37.7
29	61	1	97.5	12	195	0.375	125	40.8	41.0
41	62	1	25.0	4	30	0.500	50	29.0	29.0
38	63	1	170.0	4	360	0.250	200	45.0	45.3
5	64	1	25.0	4	360	0.250	50	34.5	34.5

Table 5: Coded Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	40.986	0.434	94.51	0.000	
Temp (X_1)	6.981	0.358	19.52	0.000	1.00
Vol. (X_2)	3.131	0.358	8.75	0.000	1.00
Time (X_3)	2.044	0.358	5.72	0.000	1.00
Particle size (X_4)	1.194	0.358	3.34	0.002	1.00
Agitation (X_5)	0.450	0.358	1.26	0.215	1.00
Temp*Temp (X_1^2)	1.337	0.967	1.38	0.174	3.20
Vol*Vol (X_2^2)	-0.663	0.967	-0.69	0.496	3.20
time*time (X_3^2)	-1.188	0.967	-1.23	0.226	3.20
Particle size*Particle size (X_4^2)	0.162	0.967	0.17	0.868	3.20
Agitation*Agitation (X_5^2)	0.212	0.967	0.22	0.828	3.20
Temp*Vol (X_1X_2)	1.897	0.379	5.00	0.000	1.00
Temp*Time (X_1X_3)	-0.322	0.379	-0.85	0.401	1.00
Temp*Particle size (X_1X_4)	0.303	0.379	0.80	0.429	1.00
Temp*Agitation (X_1X_5)	0.741	0.379	1.95	0.057	1.00
Vol*Time (X_2X_3)	-1.116	0.379	-2.94	0.005	1.00
Vol*Particle size (X_2X_4)	-0.053	0.379	-0.14	0.889	1.00
Vol*Agitation (X_2X_5)	-0.741	0.379	-1.95	0.057	1.00
Time*Particle size (X_3X_4)	0.416	0.379	1.10	0.279	1.00
Time*Agitation (X_3X_5)	-0.522	0.379	-1.38	0.176	1.00
Particle size*Agitation (X_4X_5)	-0.272	0.379	-0.72	0.477	1.00

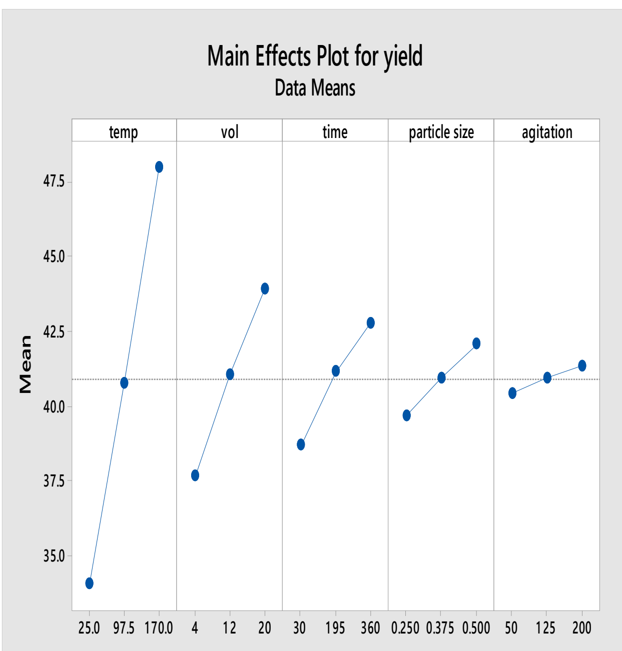

Fig. 1: Main Effects Plot for oil yield

The main effect plot of oil yield against the independent factor (i.e., temperature, volume, time, particle size, and agitation) is shown in Fig. 1. The graph depicts the intensity and direction of each independent variable's relationship with the oil yield. All of the independent factors have a positive effect on the oil yield, as seen in the graph. However, the temperature had the biggest impact, whereas the agitation rate had the smallest. The strength of the effect on oil yield is ranked in this order: Temperature > volume > time > particle size > agitation rate. The oil yield increased from 30% to 49% when the temperature was raised from 25 to 170 ºC. The oil output increased from 37.5 to 44 percent when the solvent volume was increased from 4 to 20 ml. The increase in contact time from 30 minutes to 360 minutes caused yield to increase from 38 to 43 percent. The oil yield increased from 39 to 42.5 percent when the particle size was altered from 0.25 to 0.5 mm. The oil yield increased from 40.5 to 42 percent when the agitation rate was increased from 50 to 200 rpm.

RSM Diagnostic plots

The four RSM diagnostic graphs are shown in Fig. 2. They are the residual probability plot, the frequency versus residual plot, the residual versus fitted plot, and the residual vs. observation order plot. Each plot is used to assess the model's suitability for predicting the response (Myers *et al*., 2009). The disparity between the actual response and the predicted response from the theoretical model is known as residuals (Myers *et al*., 2009).

The Residual Probability Plot is the normal probability plot of the residuals. The data points on the plots are near to the diagonal line, indicating that the residuals follow a normal distribution and that the model prediction is adequate (Myers *et al*., 2009).

As seen in Fig. 2, the Frequency vs. Residual plot is somewhat bell-shaped and symmetric around the mean. This also suggests that the residuals have a normal distribution, indicating the model's accuracy in predicting the oil output (Myers *et al*., 2009).

The residuals are presented against the fitted (predicted) values of the selected response in the Residual vs. Fitted graph. The data points are randomly scattered around the "0" line in the plot, as can be observed. This shows that the model is well-suited to the data (Myers *et al*., 2009).

The residuals are plotted against the order of runs utilized in the design in the residual vs. observation order graph. The data points are randomly scattered around the "0" line in the plot, as illustrated. This means that the experiment's test sequence had no effect. As a result, the experiment is unaffected by time-related variables (Myers *et al*., 2009).

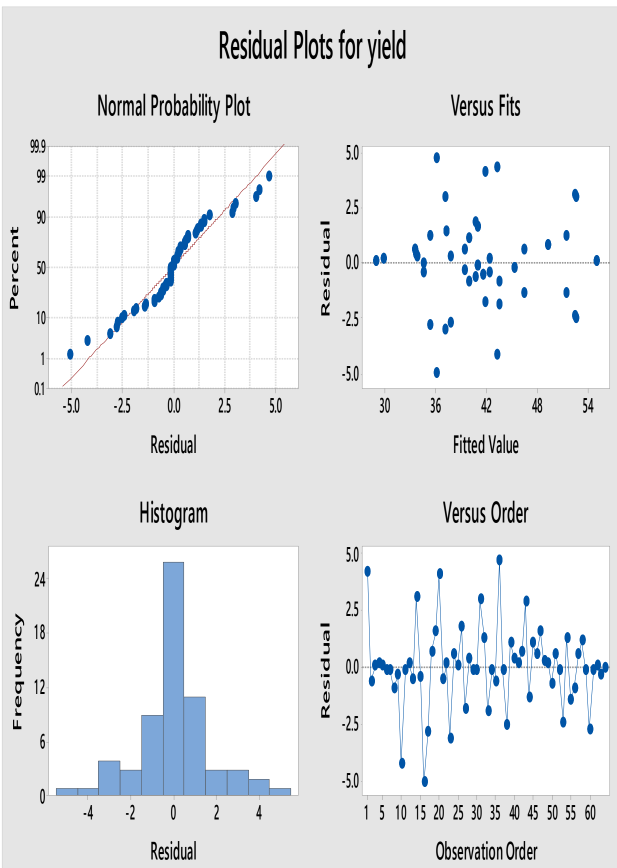

Fig. 2: Four in one RSM diagnostic plots

Analysis of variance (ANOVA) and significant factors

The model was very significant, as evidenced by the low P-value (0.000) in the CCD ANOVA, as shown in Table 6. At the 95 percent confidence level, a factor with a P-value of less than 0.05 is considered a significant factor that affects oil yield. Lack of fit has a P-value of 0.678, which is higher than the level of significance (0.05), indicating that the lack of fit is not significant. It indicates that the model equation did not suffer from lack of fit. The whole quadratic model for the five independent parameters has a considerable impact on oil yield and can accurately predict it.

Table 6: Analysis of Variance (ANOVA) and significant factors

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	20	2544.25	127.21	27.62	0.000
Linear	5	2316.15	463.23	100.59	0.000
Temp (X_1)	1	1754.21	1754.21	380.92	0.000
Vol. (X_2)	1	352.81	352.81	76.61	0.000
Time (X_3)	1	150.47	150.47	32.67	0.000
Particle size (X_4)	1	51.36	51.36	11.15	0.002
Agitation (X_5)	1	7.29	7.29	1.58	0.215
Square	5	15.08	3.02	0.65	0.659
Temp*Temp (X_1^2)	1	8.79	8.79	1.91	0.174
Vol*Vol (X_2^2)	1	2.17	2.17	0.47	0.496
time*time (X_3^2)	1	6.95	6.95	1.51	0.226
Particle size*Particle size (X_4^2)	1	0.13	0.13	0.03	0.868
Agitation*Agitation (X_5^2)	1	0.22	0.22	0.05	0.828
2-Way Interaction	10	213.03	21.30	4.63	0.000
Temp*Vol (X_1X_2)	1	115.14	115.14	25.00	0.000
Temp*Time (X_1X_3)	1	3.32	3.32	0.72	0.401
Temp*Particle size (X_1X_4)	1	2.94	2.94	0.64	0.429
Temp*Agitation (X_1X_5)	1	17.55	17.55	3.81	0.057
Vol*Time (X_2X_3)	1	39.83	39.83	8.65	0.005
Vol*Particle size (X_2X_4)	1	0.09	0.09	0.02	0.889
Vol*Agitation (X_2X_5)	1	17.55	17.55	3.81	0.057
Time*Particle size (X_3X_4)	1	5.53	5.53	1.20	0.279
Time*Agitation (X_3X_5)	1	8.72	8.72	1.89	0.176
Particle size*Agitation (X_4X_5)	1	2.37	2.37	0.51	0.477
Error	43	198.02	4.61		
Lack-of-Fit	6	19.29	3.21	0.67	0.678
Pure Error	37	178.74	4.83		
Total	63	2742.28			

Contour plot for yield versus two independent variables

Simultaneous effect of two independent factors versus response oil yield is investigated here. Effects of temperature and volume, as well as volume and time on the oil yield were only considered, because the P-values for the two-way interactions of temperature and volume (0.0) and volume and time (0.005) were both lower than the level of significance (0.05), indicating that they had a significant impact on oil yield.

Contour plot for yield versus temperature and solvent volume

Fig. 3 shows how temperature and solvent volume simultaneously affected the extracted oil yield. The oil yield is positively affected by both independent factors. An increase in temperature from 50 to 170 ºC enhanced the oil output from 35 to 55 percent when the solvent volume was 20 ml. The viscosity of d-Limonene reduced as the temperature increased, resulting in an increase in the oil's solubility in solvent. This explains why as the solvent temperature rises, the oil yield rises as well. As the volume of d-Limonene grew from 5 to 20 ml at 170 ºC, the oil output increased from 45 to 55 percent. This increase in oil yield was expected since the solvent volume was increased, allowing the soybean to soak up more solvent and improve oil solubility and yield. Fixing the temperature at 50 ºC while increasing the volume from 5 to 20 mL.

Contour plot of yield versus volume and contact time

Fig. 4 shows how solvent volume and contact duration simultaneously impacted the extracted oil yield. The oil yield is positively influenced by both the solvent volume and the contact time. An increase in contact time from 50 to 360 minutes enhanced the oil output from 35 to 45 percent when the solvent amount was 5 mL. This is because the oil needed more contact to be solubilized in the solvent at low solvent volumes. When the solvent amount was 20 mL, however, changing the contact duration from 50 to 360 minutes had no significant effect on the extracted oil yield, which remained at 45 percent. This was expected because the solvent volume (20 mL) was large enough to easily dissolve the oil, resulting in a 50-minute equilibrium contact period. The oil production increased from 35 to 45 percent at 50 minutes when the solvent volume was raised from 5 to 20 mL. A large volume of solvent was required to obtain a high oil yield at a short contact time.

Characterization the extracted oil

Fig. 5 shows the overlaid FT-IR spectra of the hexane extracted oil and d-Limonene extracted oil. The graph indicates no significant difference between the overlay spectra, implying that d-Limonene had no impact on the extracted oil's quality. This finding backs up research by Sean and Mamidipally (2005), who reported that rice bran oil derived from d-Limonene and n-Hexane has similar quality.

Table 7 depicts the primary peaks as well as the spectra's allocations. Methylene C-H stretches are ascribed to the peaks at 2916 and 2847 cm^{-1}. Carbonyl functional groups are attributed to the peaks at 1736 and 1737 cm^{-1}. The C-O stretch, which arose from the C-O-C of the carboxylic acid linked to the glycerol backbone of the triglyceride, is responsible for the peak at 1149 cm^{-1}. All of the functional groups are those that would be expected from triglyceride, which is the extracted oil's main component.

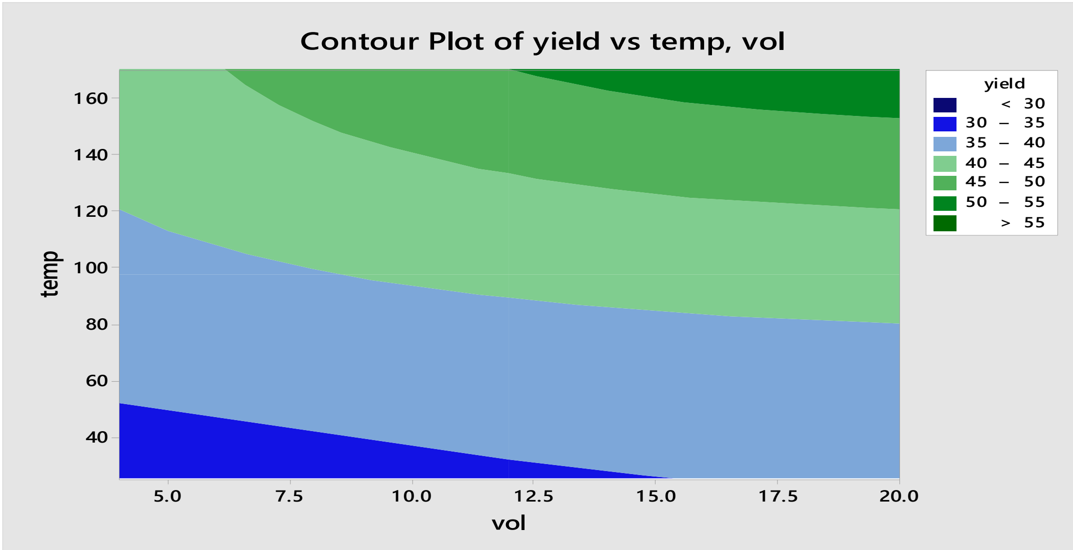

Fig. 3: Contour plot of yield versus temperature and volume

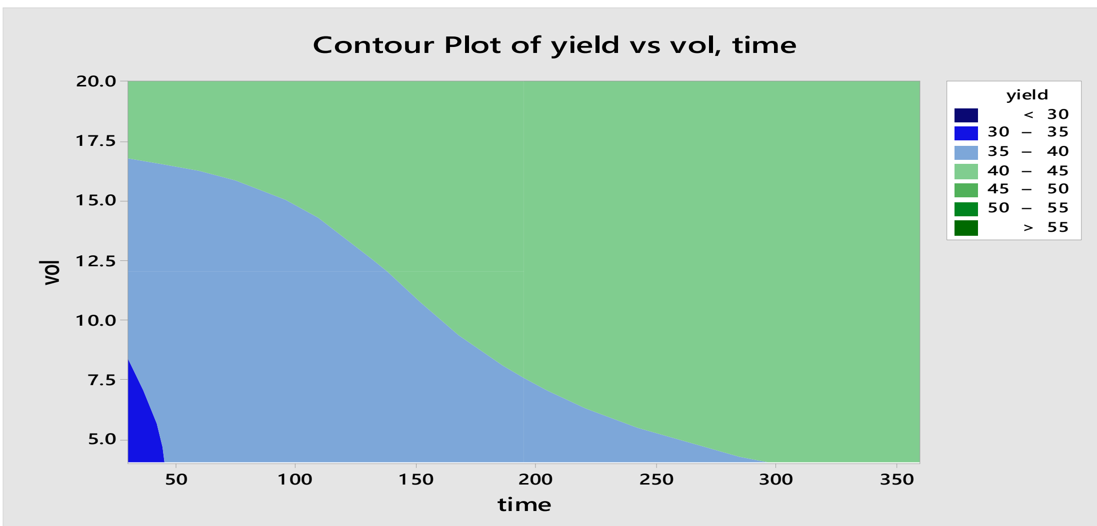

Fig. 4: Contour plot of yield versus volume and contact time

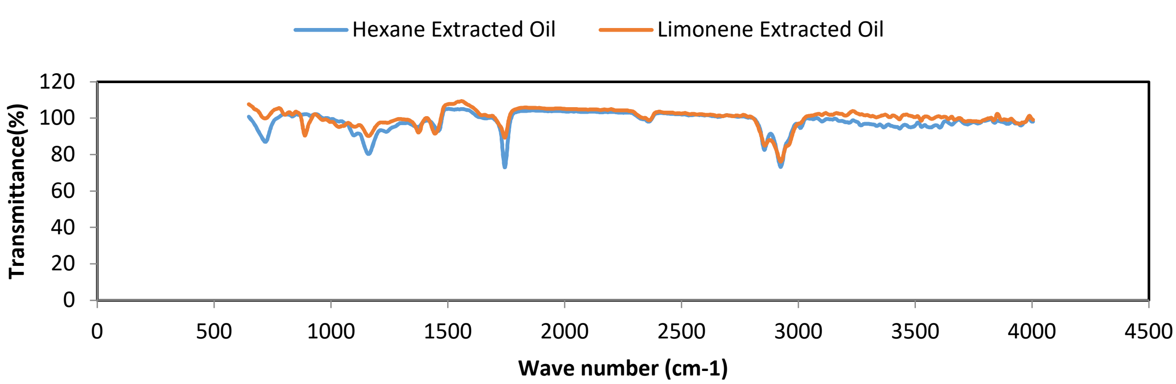

Fig.5: Overlaid spectra of the oil extracted by d-Limonene and n-Hexane

Table 7: Major peaks of the FT-IR Spectrum of the oil extract

Wave number (cm-1)	Assignment	Reference
2916	Methylene C-H stretch	Coates (2000)
2847	Methylene C-H stretch	Coates (2000)
1736	C=O carbonyl functional group	Coates (2000)
1149	C-O stretch	Coates (2000)

Conclusions

Soybean seeds were successfully extracted using a d-Limonene solvent. The quality of the n-Hexane extracted oil extracted and d-Limonene extracted at the same process conditions is the same.. The second-order regression model created from the response surface methodology accurately predicts the extracted oil yield. A 55 percent maximum oil yield was achieved at 170 °C, 20 mL solvent volume, 360 minutes, 0.5 mm particle size, and 200 rpm. The most important factors regulating the extracted oil yield are temperature, solvent volume, contact duration, and particle size. The agitation rate had a less effect on the oil yield.

Conflict of interest disclosure

I hereby declare that the author has no affiliations or financial involvement with anybody or organization with a financial interest or conflict with the subject or material discussed in this manuscript.

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