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Optimization of Oil from *Moringa oleifera* seed using Soxhlet Extraction method

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Abstract

Extraction of oil from *Moringa oleifera* seed using Response Surface Methodology (RSM) was investigated. Effects of three factors namely: sample mass, particle size and extraction time on the response, *Moringa oleifera a* volume extracted, were determined. The Box-Behnken design of RSM was employed which resulted in 15 experimental runs. Extraction was carried out in a 250 ml Soxhlet extractor with Hexane and Ethanol as solvent. The *Moringa oleifera* seed powder was packed inside a muslin cloth placed in a thimble of the Soxhlet extractor. The extraction was carried out at 60ºC using thermostatic heating mantle. The solvent in the extracted oil was evaporated and the resulting oil further dried to constant weight in the oven. This study demonstrates that Moringa oleifera oil can be extracted from its seed using ethanol and acetone as extraction solvent. The optimum process variables for both solvent (ethanol and acetone) was determined at sample weight of 40 g, particle size of 325 µm and extraction time of 8 hours. It can be deduced that using acetone as solvent produces a higher yield of oil at the same optimum variable conditions compared to when ethanol was used.

Keywords: *Moringa oleifera,* RSM, Design of Experiment, Oil yield, Solvent, Soxhlet extraction

Major classification: Health Science.

1. Introduction

Moringa oleifera is a native of north western part of India, it can also be located in several other tropical areas (Martins, 2016; Ojewumi, Olikeze, Babatunde, & Emetere, 2018a). Other countries where it is well-known include Philippines, Nigeria, Malaysia, Bangladesh, Pakistan and Kenya (Yusoff, 2016). Moringa is cultivated for its numerous functional values as it provides various practical application in developing countries due to its economic, nutritional and medicinal resource over the past few years (Anwar, Latif, Ashraf, & Gilani, 2007; Fakayode, 2016). Every part of the plant has been found useful, extracts from the roots have been reported to possess antimicrobial functions (Busani, 2012), Moringa flower extracts have been noted to have hepatoprotective effect (Upadhyay, 2015). In counties like Sudan, *Moringa oleifera* seeds have been used instead of alum to treat a high turbid Nile water by the rural dwellers (Muyibi, 1995). The seed cake has been noted as one of the most suitable natural coagulants and can be applied in the purification and treatment of water with high turbidity (Bhutada, 2016; Zhao, 2013). The seed oil (known as Ben oil) has been reported to be edible (Bhutada, 2016), it was observed to be suitable for frying purposes when compared to palm olein, soybean oil and canola oil (Abdulkarim, Long, Lai, Muhammad, Ghazali, 2007). Also, the oxidative stability of other oils such as the sunflower and soybean oil has been improved by blending Moringa oil with the mixture (Anwar, Hussain, Iqbal, Bhanger, 2007).

Moringa oleifera seed comprise of 19-47 % of oil. It is commercially referred to as Ben oil, its rich in oleic, behenic, steric and palmitic acids (Ojiako, 2013). It has been noted to be composed of 70 % oleic acid, and it has a nice fragrance (Bhutada, 2016). The presence of this high content of oleic acid makes it suitable for frying (Zhao, 2013). It has been used as lubricants, to produce air care products, air perfume, and other edible functions. It has similar composition of fatty acids as the olive oil (Zhao, 2013) apart from the presence of linoleic acid (Tsaknis, 1998). The oil is applied in hair and body care products as a skin conditioner and air moisturizer; it has an incredible cosmetic value. It has been used in the past for skin ointments and preparations by Egyptians (Mahmood, 2010). Moringa seed oil produces less dense smoke when it's ignited for lighting. The edible oil has the potential of adsorbing and retaining its flowery fragrance, thus it's much beneficial in the production of perfumes. The flavour has been reported to be comparable to peanut oil (Ghazali, 2011). Another major application of the oil is as a feedstock for biodiesel production (Mofijur, Masjuki, Kalam, Atabani, Arbab, Cheng, & Gouk, 2014; Mofijur, Masjuki, Kalam, Atabani, Fattah, & Mobarak, 2014; Rashid, 2008), the most notable characteristic of biodiesel produced from *M. oleifera* oil is the high value of cetane number (above 60) reported by Kafuku (2010).

Various techniques have been used to extract oil from seed kernels of various sources, these include aqueous enzymatic and Soxhlet extraction (Ojewumi, Adeyemi, & Ojewumi, 2018b; Ojewumi, Eluagwule, Ayoola, et al., 2017a; Yusoff, 2016), supercritical fluid extraction (Martins, 2016; Nguyen, 2011), cold mechanical pressing and solvent extraction methods (Bhutada, 2016). Aqueous enzymatic extraction entail the use of water which is economical, portable, benign, and safer compared to the solvent extraction process (Yusoff, 2016), it has been reported to produce oil with better oxidative properties and low free fatty acids, although it has low oil yield when compared with solvent extraction (Abdulkarim, Lai, Muhammad, Long, & Ghazali, 2006; Abdulkarim, Long, Lai, Muhammad, & Ghazali, 2005; Latif, Anwar, Hussain, & Shahid, 2011). Another major drawback of this process is that oil-in-water emulsions are formed that require further separation to retrieve the oil (Chabrand, 2008; Latif, & Anwar, 2011; Long, 2011). The use of cold mechanical pressing has resulted into low oil yield while the traditional use of organic solvents in solid-liquid extraction has resulted into a strenuous removal of the solvent residue with a major impact on the environment (Zhao, 2013). Solvents such as petroleum ether and n-hexane have been demonstrated to produce high yield of oil, although a major demerit of this process is the incomplete removal of the toxic solvents in the oil and a high chance of thermal degradation of the light components in the oil (Nguyen, 2011). But it's cheaper since it requires minimal solvent and minimal effort (Bhutada, 2016). In this study optimization of oil extraction from *M. oleifera* seeds is carried out in order to determine the best combination of variables required for obtaining the optimum oil yield.

Design of experiments (DOE) can be defined as the systematical method of determining the relationship between factors affecting a process and the output of that process (Ojewumi, Emetere, Babatunde, & Okeniyi, 2017b). This investigates the effects of input variables (factors) on output variable (response) simultaneously. It is majorly used to find the cause-and-effect relationships, which is needed to manage process inputs in order to optimize the experimental outputs. In an experiment, one or more process factors or variables are deliberately changed in order to observe the effect the changes have on one or more response variables (Ojewumi, 2016, Ojewumi, Oyeyemi, Emetere, & Okeniyi, 2018c; Ojewumi, Oyeyemi, Omoleye, & Oyekunle, 2019).

2. Materials and method

Moringa Oleifera seed was obtained from the open market.

2.1 Seed preparation

This was carried out using (Ojewumi, 2018d) method.

2.2 Extraction process

This was carried out using (Ojewumi, Adedokun, Omodara, et al., (2017c); Ojewumi, Banjo, Oresegun, et al., 2017d;Ojewumi, Adedokun, Ayoola, & Taiwo, 2018d) method. The experimental set up for the oil extract from *M. oleifera* was shown in figure 1.

Figure 1. Solvent Extraction Setup

3. Result and Discussion of Result

3.1 Oil extraction from Moringa seeds experimental design

Experiments were carried out using Box-Behnken response surface design. This require 3 factors with 3 different levels, it resulted into 15 experimental runs. Experimental factors and their range of levels applied in this study are shown in Table 1. Minitab 17 statistical software was used to randomize the experimental runs as displayed in Table 2. The experimental data was analyzed using a polynomial of the second-order to determine the response surface regression method as displayed in Eqn. 1 (Oyekunle, 2018; Temitayo, 2017, Ojewumi, Omoleye, & Ajayi, 2017e).

$$R_F = \mu_0 + \mu_1 Y_1 + \mu_2 Y_2 + \mu_3 Y_3 + \mu_{1,1} Y_1^2 + \mu_{2,2} Y_2^2 + \mu_{3,3} Y_3^2 + \mu_{1,2} Y_1 Y_2 + \mu_{1,3} Y_1 Y_3 + \mu_{2,3} Y_2 Y_3 \quad (1)$$

Where R_F represents the predicted response (ethanol yield), the intercept term is denoted by μ_0, the linear coefficients are represented by μ_1, μ_2, μ_3, the interactive coefficients are $\mu_{1,2}$, $\mu_{1,3}$, $\mu_{2,3}$, while the quadratic coefficients are denoted by $\mu_{1,1}$, $\mu_{2,2}$, $\mu_{3,3}$. Table 1 shows the experimental factors and their various levels for the extraction, while table also shows the experimental conditions and analyzed result for the extraction process using acetone and ethanol as solvent.

Table 1: Experimental factors and their levels for extraction of oil.

			Levels		
Parameter	Unit	Symbol	-1	0	1
Weight	g	Y_1	10	25	40

Particle size	µm	Y_2	150	325	500
Time of extraction	Hour	Y_3	2	5	8

Table 2: Experimental conditions and results for oil extraction from Moringa seeds using Ethanol and Acetone as extraction solvent.

				Ethanol			Acetone		
Run	Y_1	Y_2	Y_3	Experimental results	Predicted values	Residual values	Experimental yield	Predicted values	Residual values
1	10	150	5	25.60	25.56	0.04	33.00	32.62	0.38
2	40	150	5	28.50	28.99	-0.49	37.50	38.09	-0.59
3	40	325	2	23.50	22.97	0.53	25.25	24.79	0.46
4	25	150	2	24.40	24.42	-0.02	30.00	29.75	0.25
5	25	500	2	32.20	32.63	-0.43	24.00	23.80	0.20
6	25	500	8	27.40	27.35	0.05	34.40	34.29	0.11
7	25	325	5	26.60	26.58	0.02	31.80	31.97	-0.17
8	10	325	8	24.00	24.56	-0.56	28.00	28.20	-0.20
9	25	325	5	26.60	26.58	0.02	31.80	31.97	-0.17
10	10	500	5	24.00	23.47	0.53	25.00	24.79	0.21
11	25	325	5	26.60	26.58	0.02	31.80	31.97	-0.17
12	40	500	5	26.75	26.90	-0.15	33.50	34.02	-0.52
13	40	325	8	43.25	43.21	0.04	47.60	46.95	0.65
14	10	325	2	34.70	34.77	-0.07	29.00	29.39	-0.39
15	25	150	8	40.20	39.73	0.47	40.20	40.24	-0.04

3.2 Optimization of oil extraction using Ethanol

Table 2 displays the randomized experimental factors by Minitab 17 with their results. From the table, the optimum yield observed was 43.25 % at 40 g weight of seeds at 325 µm particle size and extraction time of 8 hours. The predicted responses at the optimum value was 43.21 %. This shows that the model was suitable for prediction of the response oil yield. The lowest oil yield of 23.5 % was obtained at 40 g of seeds, 325 µm particle size and 2 hours of extraction time. Corresponding predicted responses at this point was 23.0 %, the predicted response was similar to the experimental values. This further shows that this model was efficient in predicting this experiment.

Results of oil produced using Minitab 17 Box-Behnken design are illustrated in Table 2. Table 2 shows factors examined in this work alongside experimental results, predicted and residual values derived from RSM. The regression equation that defines the experimental correlation given by Minitab 17 statistical software is demonstrated in Eqn. 2.

$$R_F = 38.83 - 0.7317Y_1 + 0.05045\,Y_2 - 5.542Y_3 + 0.000011\,Y_2^2 + 0.5337\,Y_3^2 + 0.16917Y_1Y_3 - 0.009810Y_2Y_3 \quad (2)$$

R_F represents the oil yield, Y_1 is the weight, Y_2 is the particle size and Y_3 is the time of extraction.

Table 3 outlines analysis of significance for every coefficient of regression and its ANOVA as produced by the statistical software, Minitab 17. Each coefficient level of importance was determined in term of P-value. From the table, the model terms demonstrates a high level of P-value significance, i.e. $p < 0.05$ (Temitayo, 2017). All the three linear terms were significant (Y_1, Y_2 and Y_3), the interaction effect (Y_2Y_3, Y_1Y_3) and the quadratic term (Y_3^2) were all notably significant. Although, the quadratic term (Y_2^2) is not significant since its P value > 0.05. The analyzed significance for the coefficient of regression and ANOVA was shown in table 3.

Table 3: Analysis of Significance for Coefficient of Regression and ANOVA

Source	Deg	SOS	Contribution (%)	SOS Adj	MS Adj	F-Value	P-Value
Y_1	1	23.461	4.61	23.461	23.461	106.2	0.000
Y_2	1	8.715	1.71	8.715	8.715	39.45	0.000
Y_3	1	50.250	9.87	50.250	50.250	227.46	0.000
Y_2^2	1	1.778	0.35	0.448	0.448	2.03	0.198
Y_3^2	1	85.680	16.82	85.680	85.680	387.83	0.000
$Y_1\,Y_3$	1	231.801	45.51	231.801	231.801	1049.25	0.000
$Y_2\,Y_3$	1	106.090	20.83	106.090	106.090	480.22	0.000
ANOVA							
Model	7	507.776	99.70	507.776	72.539	328.35	0.000
Lack-of-Fit	5	1.546	0.30	1.546	0.309	*	*
Pure Error	2	0.000	0.00	0.000	0.000		
Total	14	509.322	100.00				
R^2 = 99.70 %, (Adjusted) R^2 = 99.39 %, (Predicted) R^2 = 97.57 %							

Where Deg represents Degree of freedom, SOS - Sequential Sum of Square, SOS Adj –Sum of Square Adjusted, MS Adj –Mean Square Adjusted, F and P represents Fisher and Probability respectively.

The essence of the regression model was determined by F and P values using test for Fischer's and null-hypothesis. The quality of the whole model is predicted by the F-value while taking note of all the factors used to design the model at the same time. Probability of obtaining small or insignificant outcome on the response can be described as the P-value. Preferable model fitness to the data obtained experimentally was signified by larger F-values (Panwal, 2011). Datta (2012) states that a small P-value with a large F-value suggest a high significant level of the regression model. In addition, for a statistically important model the P-value should be below 0.05 (Patel, 2011). Based on previous studies by Datta (2012); Oyekunle (2018); Panwal (2011); Patel (2011); Temitayo (2017), the regression model observed was remarkably significant as illustrated by high F-value and small P-value of 328.35 and 0.00

respectively. The “lack of fit” has no values for P and F-values, therefore the level of significance of the model with respect to the pure error could not be determined.

To determine the fitness of the model equation, R^2 (determination coefficient) was used to determine the regression model. This measures the level of variability in the values of the experimental response which can be analysed by the variables and their relations (Sudamalla, 2012). The value of R^2 is within the range of 0 and 1 (Haider, 2007; Liu, 2007; Temitayo, 2017), but in order to attain better fitness of the model, it was stated that it should be above 0.8 (Joglekar, 1987). From Table 3, R^2 values is 99.70 % signifying that 99.70 % variations in the response observed can be described by the model while 0.30 % cannot be determined by the model. 0.30 % of the R^2 values of the overall variations would be as a result of other factors not considered in this model.

The R^2 adjusted is an amended R^2 value obtained after the removal of all the other model terms that are not required. The R^2 adjusted was noted to be lower than R^2, this signify that a lot of insignificant terms were considered in the model (Fang, 2010). In this study, R^2 adjusted observed was lower and near the R^2 value. The values of R^2 and R^2 adjusted are 99.70 % and 99.39 % respectively. The R^2 predicted was 99.57 % which was higher but conforms to the adjusted R^2 values. The R^2 and R^2 adjusted values shows an elevated level of correlation and dependence on the experimental and predicted values. Table 4 shows the coefficient of regression and level of the significance for the response surface relationship

Table 4: Coefficient of Regression and the level of significance for response surface

Term	Effect	Coefficient	Standard Error Coefficient	95% CI Low	95% CI High	T-Value	P-Value	VIF
Constant		26.577	0.226	26.043	27.111	117.71	0.000	
Y_1	3.425	1.712	0.166	1.320	2.105	10.31	0.000	1.00
Y_2	-2.088	-1.044	0.166	-1.437	-0.651	-6.28	0.000	1.00
Y_3	5.013	2.506	0.166	2.113	2.899	15.08	0.000	1.00
Y_2^2	-0.694	-0.347	0.244	-0.924	0.23	-1.42	0.198	1.01
Y_3^2	9.606	4.803	0.244	4.226	5.38	19.69	0.000	1.01
Y_1Y_3	15.225	7.612	0.235	7.057	8.168	32.39	0.000	1.00
Y_2Y_3	-10.3	-5.15	0.235	-5.706	-4.594	-21.91	0.000	1.00

Table 4 illustrates the test of statistical analysis which comprise of T and P values for linear, quadratic and integrated effects on the variables. The experimental model shows the importance of each coefficient in the experimental model as dictated by the Minitab 17 based on T and P values. A small P-value and a large T-test value displayed by the model illustrate that the linear terms (Y_1, Y_3), quadratic terms (Y_3^2) and the integrated or combined terms (Y_1Y_3) have significant effect on the oil yield (Baoxin, 2011; Temitayo, 2017). All other terms (Y_2, Y_2^2, and Y_2Y_3) are unimportant. From the table, standard error coefficient low values observed for each term shows that there was a strong agreement between the data and the regression model and the prediction was appropriate. The F ratio was determined at a 95% confidence interval (CI), the VIF (variance inflation factor) observed in this study demonstrated that the center points were orthogonal to other terms in this model (Temitayo, 2017).

Three dimensional response surface and contour plots of the variables were plotted against the oil yield as displayed in Figure 2. Figure 2(a) illustrates the effect of particle size and sample weight on oil yield at a constant time. It was observed that at a constant particle size of 150 µm and time increasing the weight of the seeds from 10 – 40 g led to a 10 % increase in the oil yield. This was also noted at a constant particle size of 500 µm. However at 325 µm, there was a 33.7 % decrease in the oil yield when the weight was increased from 10 – 40 g. This proves that at a constant extraction time higher sample weight of Moringa at 150 µm and 500 µm particle sizes were required in order to maximize oil yield. While 325 µm particle size require a smaller sample weight in order to achieve maximum oil yield.

Figure 2(b) illustrates the effect of weight and time of extraction on oil yield while the particle size was kept constant. As can be observed on the plots, at a constant time of extraction the oil yield increases as the sample weight increases. At 40 g of the sample, it was noted that the oil yield was increased by 45.7 % as the extraction time rises from 2 – 8 hours. While at 10 g of sample it can be observed that oil yield reduced as time increases, this can be as a result of loss in the oil yield during solvent recovery.

Figure 2(c) illustrates the effect of particle size and time of extraction on oil yield at a constant sample weight. The contour and surface plots indicate that at constant particle size of 150 µm, the oil yield was increased by 39.3 % when the time of extraction was increased from 2 – 8 hours. Although at 500 µm particle size, the oil yield decreased by 15 % when the time of extraction increased from 2 – 8 hours. This can be as a result of loss in the oil yield during solvent recovery. However at 325 µm at an extraction time of 8 hours the maximum oil yield of 43.25 % was attained, which was a 46 % increase from when the extraction time was 2 hours at the same particle sizes.

3.3 Optimization of oil extraction using Acetone

Table 2 displays the randomized experimental factors by Minitab 17 with their results. From the Table, the optimum yield observed was 47.60 % at 40 g weight of seeds at 300 µm particle size and extraction time of 8 hours. The predicted responses at the optimum value was 46.95 %. This shows that the model was suitable for prediction of the response oil yield. The lowest oil yield of 24.0 % was obtained at 25 g of seeds, 500 µm particle size and 2 hours of extraction time. Corresponding predicted responses at this point was 23.8 %, the predicted response was similar to the experimental values. This further shows that this model was efficient in predicting this experiment.

Results of oil produced using Minitab 17 Box-Behnken design are illustrated in Table 2. Table 2 shows factors examined in this work alongside experimental results, predicted and residual values derived from RSM. The regression equation that defines the experimental correlation given by Minitab 17 statistical software is demonstrated in Eqn. 3.

$$R_F = 43.99 - 0.6000Y_1 - 0.03618Y_2 - 1.495Y_3 + 0.00160Y_1^2 + 0.000016Y_2^2 + 0.000358Y_1Y_2 + 0.12972Y_1Y_3 \quad (3)$$

R_F represents the oil yield, Y_1 is the weight, Y_2 is the particle size and Y_3 is the time of extraction.

Table 5: Analysis of Significance for Coefficient of Regression and ANOVA

Source	Deg	SOS	Contribution (%)	SOS Adj	MS Adj	F-Value	P-Value
Y_1	1	104.040	19.37	106.869	106.869	409.71	0.000
Y_2	1	70.070	13.05	70.805	70.805	271.45	0.000
Y_3	1	219.975	40.96	219.975	219.975	843.34	0.000
Y_1^2	1	0.397	0.07	0.480	0.480	1.84	0.217
Y_2^2	1	0.820	0.15	0.820	0.820	3.14	0.119
$Y_1 Y_2$	1	3.567	0.66	3.567	3.567	13.67	0.008
$Y_1 Y_3$	1	136.306	25.38	136.306	136.306	522.57	0.000
ANOVA							
Model	7	535.175	99.66	535.175	76.454	293.11	0.000

Error	7	1.826	0.34	1.826	0.261		
Lack-of-Fit	5	1.826	0.34	1.826	0.365	*	*
Pure Error	2	0.000	0.00	0.000	0.000		
Total	14	537.001	100.00				
R^2 = 99.66%, (Adjusted) R^2 = 99.32%, (Predicted) R^2 = 96.73%							

Where Deg represents Degree of freedom, SOS - Sequential Sum of Square, SOS Adj –Sum of Square Adjusted, MS Adj –Mean Square Adjusted, F and P represents Fisher and Probability respectively.

Table 5 outlines analysis of significance for every coefficient of regression and its ANOVA as produced by the statistical software, Minitab 17. Each coefficient level of importance was determined in term of P-value. From the table, the model terms demonstrates a high level of P-value significance, i.e. $p < 0.05$ (Temitayo, 2017). All the three linear terms were significant (Y_1, Y_2 and Y_3), and the interaction effect (Y_1Y_2, Y_1Y_3) were all notably significant. Although, the two quadratic term (Y_1^2, Y_2^2) are not significant since their P values are > 0.05.

The essence of the regression model was determined by F and P values using test for Fischer's and null-hypothesis. The quality of the whole model is predicted by the F-value while taking note of all the factors used to design the model at the same time. Probability of obtaining small or insignificant outcome on the response can be described as the P-value. Preferable model fitness to the data obtained experimentally was signified by larger F-values (Panwal, 2011). Datta (2012) states that a small P-value with a large F-value suggest a high significant level of the regression model. In addition, for a statistically important model the P-value should be below 0.05 (Patel, 2011). Based on previous studies by Datta (2012); Oyekunle (2018); Panwal (2011); Patel (2011); Temitayo (2017), the regression model observed was remarkably significant as illustrated by high F-value and small P-value of 293.11 and 0.00 respectively. The "lack of fit" has no values for P and F-values, therefore the level of significance of the model with respect to the pure error could not be determined.

To determine the fitness of the model equation, R^2 (determination coefficient) was used to determine the regression model. This measures the level of variability in the values of the experimental response which can be analysed by the variables and their relations (Sudamalla, 2012). The value of R^2 is within the range of 0 and 1 (Haider, 2007; Liu, 2007; Temitayo, 2017), but in order to attain better fitness of the model, it was stated that it should be above 0.8 (Joglekar, 1987). From Table 5, R^2 values was 99.66 % signifying that 99.66 % variations in the response observed can be described by the model while 0.34 % cannot be determined by the model. 0.34 % of the R^2 values of the overall variations would be as a result of other factors not considered in this model.

The R^2 adjusted is an amended R^2 value obtained after the removal of all the other model terms that are not required. The R^2 adjusted was noted to be lower than R^2, this signify that a lot of insignificant terms were considered in the model (Fang, 2010). In this study, R^2 adjusted observed was lower and near the R^2 value. The values of R^2 and R^2 adjusted are 99.66 % and 99.32 % respectively. The R^2 predicted was 99.73 % which was higher but conforms to the adjusted R^2 values. The R^2 and R^2 adjusted values shows an elevated level of correlation and dependence on the experimental and predicted values.

Table 6: Coefficient of Regression and the level of significance for response surface

Term	Effect	Coefficient	Standard Error Coefficient	95% CI Low	95% CI High	T-Value	P-Value	VIF
Constant		31.538	0.25	30.947	32.13	126.06	0.000	
Y_1	7.347	3.673	0.181	3.244	4.102	20.24	0.000	1.01

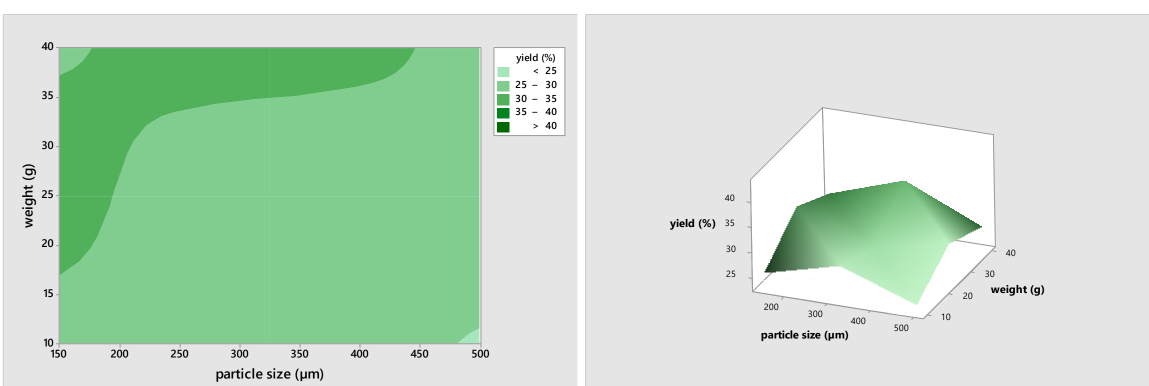
Y_2	-5.950	-2.975	0.181	-3.402	-2.548	-16.48	0.000	1.01
Y_3	10.488	5.244	0.181	4.817	5.671	29.04	0.000	1.00
Y_1^2	0.719	0.360	0.265	-0.267	0.986	1.36	0.217	1.01
Y_2^2	0.964	0.482	0.272	-0.161	1.125	1.77	0.119	1.01
$Y_1 Y_2$	1.879	0.940	0.254	0.339	1.54	3.70	0.008	1.01
$Y_1 Y_3$	11.675	5.837	0.255	5.234	6.441	22.86	0.000	1.00

Table 6 illustrates the test of statistical analysis which comprise of T and P values for linear, quadratic and integrated effects on the variables. The experimental model shows the importance of each coefficient in the experimental model as dictated by the Minitab 17 based on T and P values. A small P-value and a large T-test value displayed by the model illustrate that the linear terms (Y_1, Y_3), quadratic terms (Y_1^2, Y_2^2) and the integrated or combined terms (Y_1Y_2, Y_1Y_3) have significant effect on the oil yield (Baoxin, 2011; Temitayo, 2017). The other term (Y_2) was unimportant. From the table, standard error coefficient low values observed for each term shows that there was a strong agreement between the data and the regression model and the prediction was appropriate. The F ratio was determined at a 95% confidence interval (CI), the VIF (variance inflation factor) observed in this study demonstrated that the center points were orthogonal to other terms in this model (Temitayo, 2017).

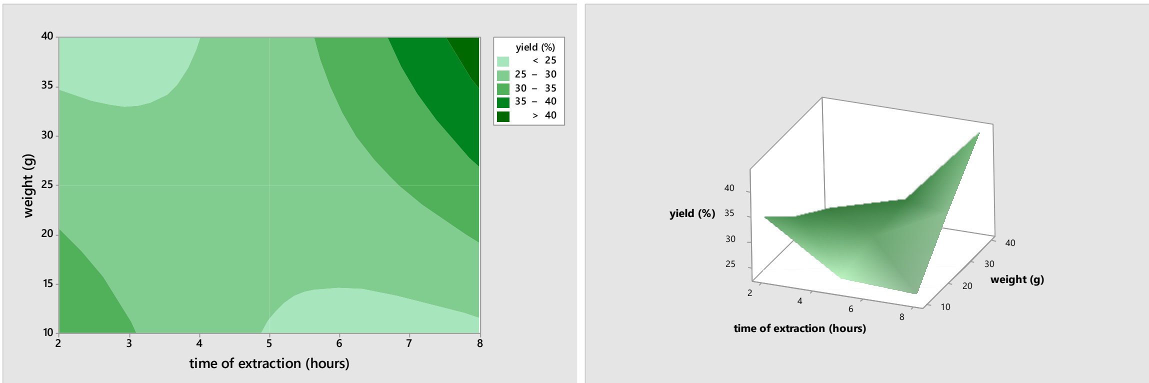
Three dimensional response surface and contour plots of the variables were plotted against the oil yield as displayed in Figure 3. Figure 3(a) illustrates the effect of particle size and sample weight on oil yield at a constant time. It was reported that at constant particle size of 150 µm, increasing the sample weight from 10 – 40 g shows a 14% increase in the amount of oil extracted. Also at 500 µm particle size, it was observed that the oil yield increased by 25% when the sample weight was increased from 10 – 40 g. This illustrates that at a constant extraction time higher sample weight of Moringa at 150 µm and 500 µm particle sizes were required in order to maximize oil yield.

Figure 3(b) illustrates the effect of weight and time of extraction on oil yield while the particle size was kept constant. As can be observed on the plots, at a constant time of extraction the oil yield increases as the sample weight increases. This was demonstrated at 500 µm particle size, there was a 25% increase when the sample weight was increased from 10 – 40 g. Also, the optimum oil yield was attained at the maximum extraction time of 8 hours while the lowest oil yield was obtained at the lowest extraction time of 2 hours.

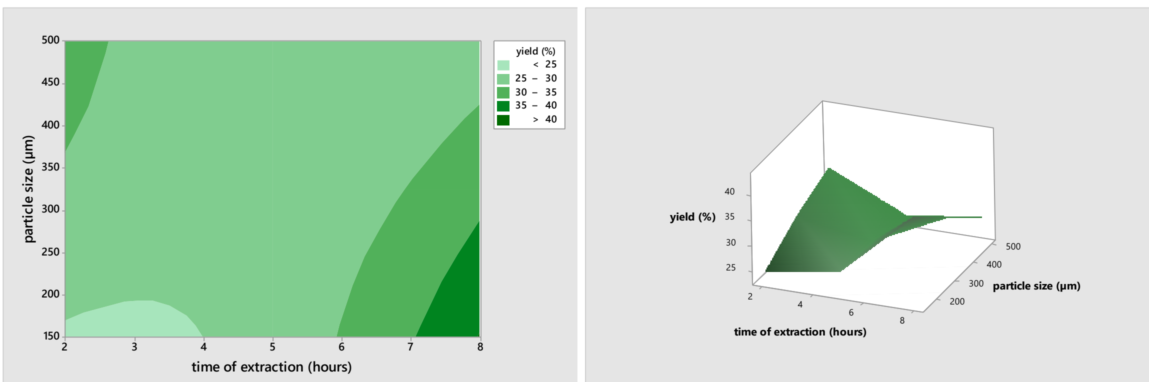
Figure 3(c) illustrates the effect of particle size and time of extraction on oil yield at a constant sample weight. At a constant reaction time of 5 hours and reducing the particle size from 500 – 150 µm increased the oil yield by 11% while maintaining a constant particle size of 150 µm and increasing the reaction time from 2 – 8 hours the oil yield increased by 25%. This proves that a higher reaction time favors high amount of oil extracted.



(a)



(b)

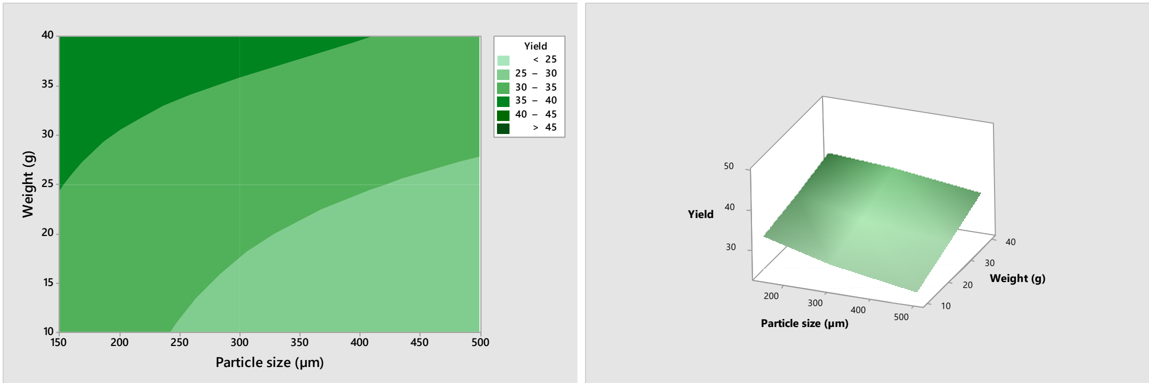


(c)

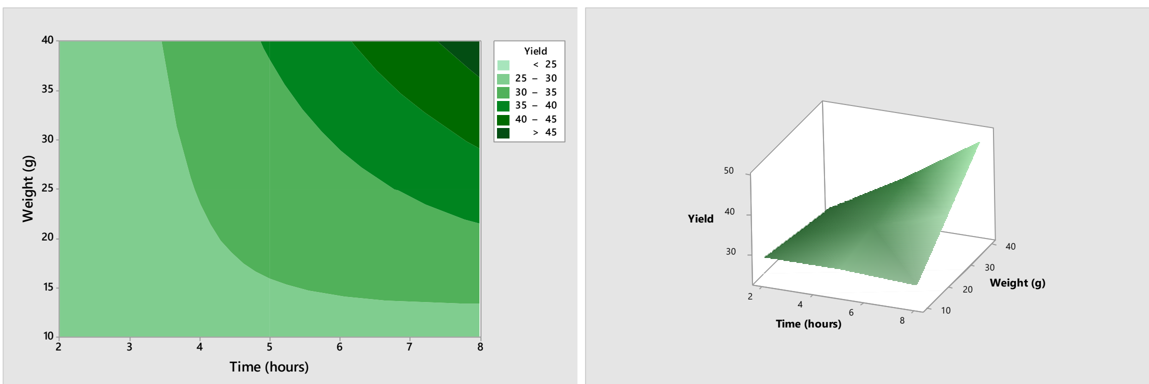
Figure 2: contour and surface plots of the oil yield against (a) weight (g) and particle size (µm), (b) weight (g) and time of reaction (hour) and (c) particle size (µm) and time of reaction (hour).

3.4 Comparing the oil yield using Acetone and Ethanol as solvent

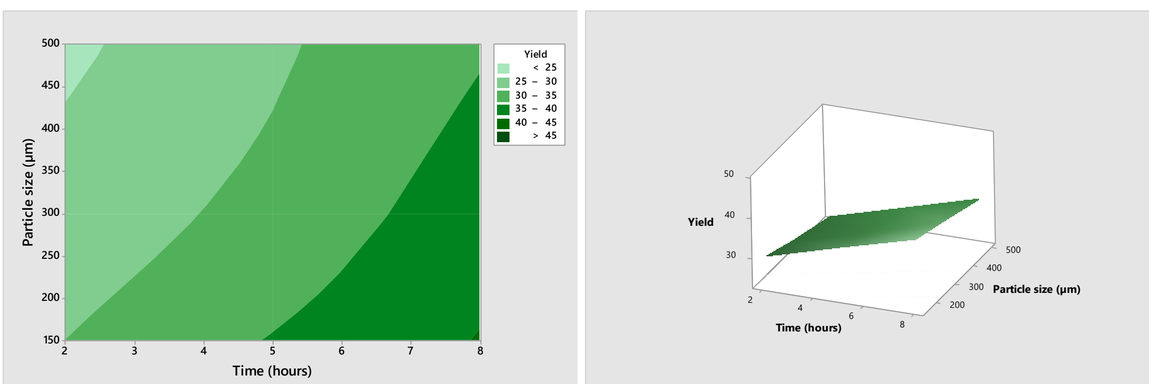
Validity of the model predicted by regression analyses was confirmed by a linear correlation of the predicted and experimental responses (Figure 3). As displayed in the figure, using both solvent as for the extraction process gave a good R^2 value for each model. Using ethanol as solvent gave a R^2 value of 0.997 while acetone solvent gave a R^2 value of 0.9966 which are both comparable and demonstrate the effectiveness of the model in predicting the oil yield. Although, it can be argued that using acetone as solvent gave a higher yield of 47.6%, while using ethanol as solvent gave 43.25% has its highest oil yield. This takes place at the same variable conditions. Therefore higher oil yield was obtained when acetone was used as the extraction solvent in contrast to using ethanol. Based on previous studies by Temitayo (2017) and Oyekunle (2018) the plot (Figure 3) show that the model was appropriate without defying independence or consistent assumption.



(a)



(b)



(c)

Figure 3: contour and surface plots of the oil yield against (a) weight (g) and particle size (μm), (b) weight (g) and time of reaction (hour) and (c) particle size (μm) and time of reaction (hour).

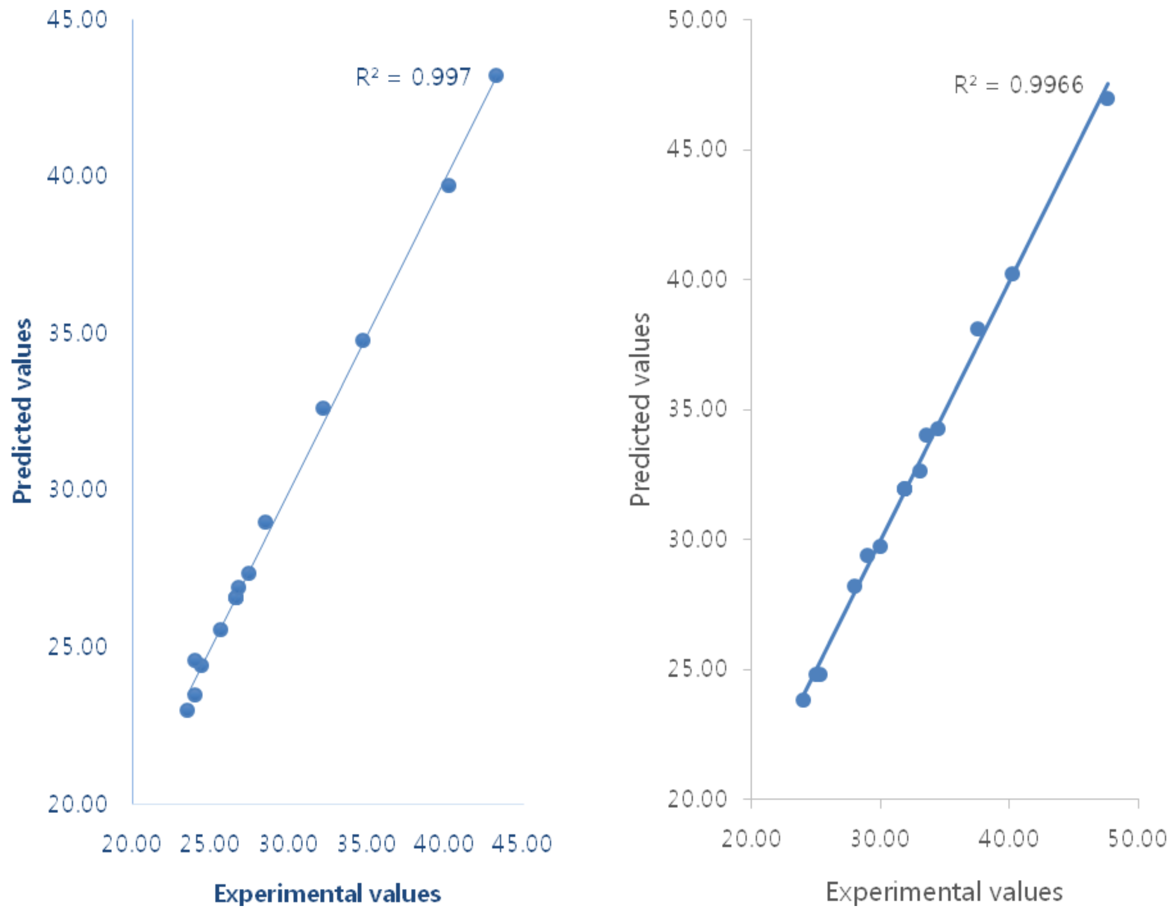


Figure 4: Plot of predicted and experimental response values using ethanol as solvent (Left), using Acetone as solvent (Right)

4. Conclusion

This study demonstrates that *Moringa oleifera* oil can be extracted from its seed using ethanol and acetone as extraction solvent. The optimum process variables for both solvent (ethanol and acetone) was determined at sample weight of 40 g, particle size of 325 µm and extraction time of 8 hours. It can be deduced that using acetone as solvent produces a higher yield of oil at the same optimum variable conditions compared to when ethanol was used. However, using ethanol as extraction solvent produces a lower oil yield (24.00%) compared to when acetone (25.00 %) was used at the same variable conditions (sample weight of 10 g, particle size of 500 µm and extraction time of 5 hours). Although using both solvents gave a very good R^2 value. R^2 value of 0.997 was determined using ethanol as extraction solvent, while R^2 value of 0.9966 was determined using acetone as extraction solvent. These study validate the effectiveness of using RSM model as a good predictive optimization tool for the extraction of oil from *Moringa oleifera* seeds using ethanol and acetone as solvent.

Conflict of interest: Authors declares no conflict of interest.

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