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Optimization of Solvent Extraction of Oil from Watermelon (Citrullus lanata) and its Fatty Acid Characterization

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Abstract
Watermelon also known as ***Citrullus lanata*** is one of the major underutilized fruits grown in the warmer part of the world. Over the years, with respect to the high rate of production of watermelon along with lack of consciousness of the benefits, its seed has suffered negligence and consequently become waste. Due to the growing awareness of the usage of seed oil; there is need to maximize the use and identify the appropriate sets of parameters necessary for the optimum extraction of the oil. This research looked at the parametric effect of hot continuous extraction process at 60-70 ^{0}Cfor watermelon seed using response surface methodology, Box Behnken design with 3 variables which are time of extraction, solvent volume and size particle of 30-50 mins, 100-200 ml and 4-10 µm respectively. The optimized solution indicates that the time of extraction of 50 mins, 199.99 ml of solvent with 10 µm of size particle give the maximum oil yield of 68% compared to the maximum experimental oil yield of 52.5%. Free fatty acid (FFA) content in the oil was determined using (Analysis of the Association of Official Analytical Chemists), 2000 method which gives 17.35%, (higher than 1.0% standard) indicating the need to reduce the FFA content in the oil before the production of biodiesel to avoid low fatty methyl ester (FAME) production against high soap formation.

Keywords: Optimization, Watermelon, Solvent extraction, Box Behnken and Free fatty acid.

Introduction
A global depletion of energy resources basically of non-renewable fuels, records of high gasoline prices, degradation of environment due to release of CO_2 in burning of such fuels, decreasing dependency on foreign energy supply and biofuel production along with bio-products which provide new income and employment opportunities in rural areas have shifted the attention to the production of biofuels to renewable sources. (Naik *et al.,* 2010). This has inspired recent interest in alternative sources to replace petroleum-based fuels. Among the alternative fuels bio-diesel obtained from vegetable oil holds good promises as an eco-friendly alternative to diesel and because it has chemical composition similar to that of conventional diesel. In addition, it reduces $C0_2$ emissions, has higher oxygen content with respect to petroleum diesel which ensures a more complete combustion, with lower quantity of particulate matter. It is found to be non-toxic and biodegradable in nature. Several tradition oil seed crops had been in place for biodiesel production which could either be edible or non-edible, but in order to forestall shortage of food (Palligarnai and Vasudevan, 2008) waste cooking oil, tallow animal fat hence replaced the edible oils in biodiesel production, due to their fact that they constitute the major disposal problems and they can be obtained at lowest price (Singh and Singh, 2010) Non edible oils like bran (Ramadhas *et*

al., 2005), rubber seed (Melvin, 2011), pongamia (Atabani, 2013) rape seed (Gryglewicz, 1999, Leclercq *et al.,* 2001, and Yuan, 2008), sunflower (Ramos, 2008, Albuquerque *et. al.,* 2008, and Arzamendi , 2008), algae (Encinar, 1999 and Miao,2006), tobacco seed (Usta, 2005), konaya (Meher *et al.,* 2006) babassu (Dos Reis 2005, Abreu, 2004) all these one found to be one of the cheapest feedstock for production of biodiesel.

Watermelon is a kind of crop that tolerates drought and belongs to the cucurbitaceae family of flowering plants. It is generally considered to be of the citrullus species and has been referred to as citrullus vulgaris (Naseri and Tehrani, 1995). Watermelon is originally found in southern Africa and reaches maximum inherited diversity there, with sweet, bland and bitter forms (Dane and Liu, 2007). It has been reported that seeds of some species of cucurbitaceae can be the edible oil sources to meet the increasing demands for vegetable oil (Esuoso *et al.,* 1998). The watermelon seed, despite its high nutritional content and medicinal value still remain under-utilized in Nigeria. Over the years, due to the high rate of production of watermelon and lack of awareness of the benefits its seed, the seed has suffered negligence which results in wastage. The role of production technology cannot be underrated. In treating multiple feedstock with low-cost such as waste oil, a specific technological approaches are required along with further growth of scientific and technological revolution (L. C. Meher, D Vidya Sagar, S. N. Naik, P. T. Vasudevan, M. Briggs) Study of the direct use of straight vegetable oils as fuel in diesel engine were carried out before 1970s energy crises (G. Knothe, J. Van Gerpen, J. Krahl,). However, the high viscosity of straight vegetable oil (A. H. Demirbas, A. Srivastava, R. Prasad,) causes a range of difficulties and leads to poor fuel characteristics. Among possible results such as engine modification, bleaching straight oils with fossil diesel, micro-emulsification, transesterification or thermal cracking were suggested. Due to its easiness, cost-effective technology as well as favourable physico-chemical parameters of the fuel. Transesterification of oils to its equivalent fatty acid methyl esters (FAMEs) has seen wider solicitation (Meher *et al.,* 2006).

Response Surface Methodology (RSM) is defined as an assembly of mathematical and statistical procedures useful for modeling, analyzing and simultaneously solving problems in which a response of concern is influenced by several variables and the objectives is to enhance this response (Ravikumar *et al.,* 2005). It also reckons the relationship between the controllable input parameters and the obtained response surfaces. It is distinguished for constructing guesstimate models based on physical investigated observations. The main advantage of RSM is the reduction of number of experimental runs desirable to deliver satisfactory information for statistically acceptable outcomes. The conventional approach for the optimization of a multivariable system is usually one variable at a time. Therefore, there is a need to carry out numerous sequential experimental runs, recently many statistical experimental design methods have been employed in bioprocess optimization. Response surface methodology is one of the statistical experimental design method employed in bioprocess optimization approach that is useful for developing and improving the process in order to analyze the effect of several independent variables on the system response for optimum determination of operational condition within the process (Jo *et al.,* 2008; Lee man . *et al.,* 2010).

The aim of this present work is to investigate the performance of extraction process in order to maximize the castor oil yield using Design Expert software version 10.0.5.0 for sustainable biodiesel production. The variables influencing the process were systematically investigated and both the acid values and free fatty acid were determined towards pure biodiesel.

Materials and Method

Sample preparation

Waste watermelon fruit was bought from Odo Oba market in Ogbomoso and the seeds were removed, washed, sorted, drained and sundried. The dried seeds were blended and screened to give different range of size particles.

The extraction processes

This was carried out in a 250 ml Soxhlet extractor on a heating mantle to extract the oil using n-hexane as the solvent for the different experimental runs. The grinded seed was packed inside a muslin cloth placed in a thimble of soxhlet extractor. A round bottom flask containing n-hexane was fixed to the end of the extractor and a condenser was tightly fixed at the bottom end of the extractor. The flask was heated at 60 ^{0}C using electric mantle. The solvent then vapourised and condensed into evaporator. The mixture obtained (oil and solvent) poured directly into round bottom flask. The process continued for 30 minutes. Recovery of the oil was done by distillation process using the same apparatus, while the oil obtained was stored at room temperature in a bottle for further use

Percentage oil yield was calculated for each experimental runs using the equation below

$$\%\ extracted\ oil\ yield\ = \left(\frac{W_1 - W_2}{W_2}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

where W_1= Weight of watermelon seed before extraction and W_2=Weight of cake after extraction and drying to constant weight. The acid value in the oil was calculated in order to determine the free fatty acid (FFA) content of the oil by pouring 2.5 g of watermelon seed oil in a dried conical flask, then 50 ml of absolute ethanol and adding 1ml of phenolphthalein, heated for 3 minutes then titrated against 0.1 N KOH until the end point was reached.

The results obtained were recorded and the acid value (AV) and free fatty acid (%FFA) were calculated using equations 2 and 3 respectively.

$$Acid\ value\ = \left(\frac{ml\ of\ KOH * N * 56}{Weight\ of\ the\ sample}\right) mg\ of\ KOH \ldots\ldots\ldots\ldots\ldots\ldots 2$$

$$\%\ FFA\ = Acid\ value * 0.503 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Where mg of KOH is the titration value of the KOH, N = is the normality of KOH = 0.1 for 0.1M of KOH

Study Design

A three-variable (two levels of each variable) Box Behnken experimental design was employed which generated 17 experimental runs (Babatunde *et al.,* 2016). The factors and their levels were chosen based on the practical understanding and related process optimization to maximize the quantity of oil extracted. The independent variables included time (30-50minutes), solvent volume

(100 – 200ml), and particle size (20-60 µm) each at three levels. The experimental design was as shown in the Table 1 below

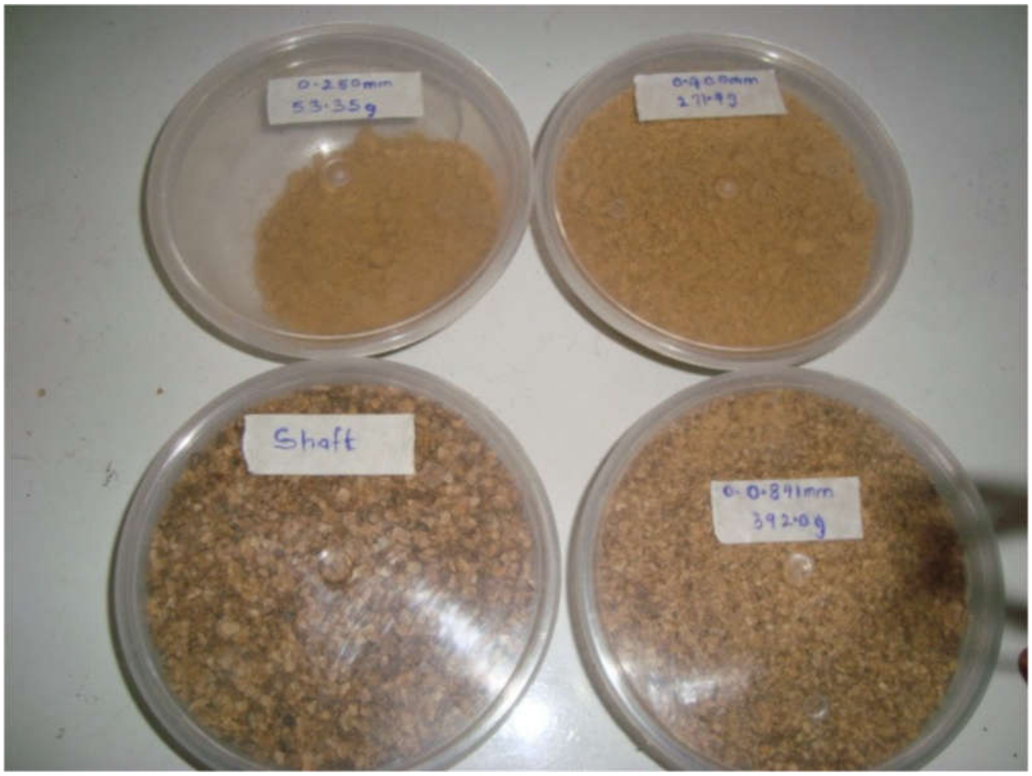

Plate1: Blended Watermelon Seed

Table 1: Design entries determining factors for extraction process and their units

Factors	Units	Low Level	High level
Extraction period	minutes	30	50
Solvent volume	milliliter	100	200
Particles size	µm	4	10

Data analysis

The obtained data for the response was introduced into the software (Design Expert software version 10.0.5.0 (STAT-EASE Inc., Minneapolis, USA)), the ANOVA for a multiple linear regression model was performed to fit the coefficient of the polynomial model of the response variable and to correlate it with the independent variables. The fitted quadratic response model is described by:

$$Y = \beta_0 + q \sum j_{=1}\ \beta_j x_{ij} + q \sum j_{=1} \beta_{jj} \left(x_j\right)^2 + \sum \sum i_{<j}\ \beta_{ij} x_i x_j + \ \epsilon \ldots \ldots \ldots \ldots 4$$
$$= \beta_0 + \ x_j'\ \beta + \ x_j'\ \beta x_i + \epsilon_j$$

Where

$$x_i = (x_{1j}, x_{2i}, \dots \dots \dots \dots x_{qi}), \qquad \beta = (\beta_1, \beta_{2, \dots \dots \dots \dots} \beta_q).$$

Y is the response factor (% of extracted oil), i and j denote linear and quadratic coefficient respectively β_0 is the intercept, β_1 is the first order model coefficient, q is the number of factors and Є is the random error.

The model was adopted because of its flexibility, for it can take a variety of functional forms and approximate the response surface locally. Therefore, it is usually a good estimation of true response surface. When a model was significant ($p \leq 0.05$) its coefficients along with their significance were determined and the non-significant coefficients were erased from the model and the effects, sum of squares and percent influence were calculated from the significant ones. The weighted sum of the squared deviation between the mean response at each parameter level and the corresponding fitted value (lack of fit) shown whether there were no significance relative to a pure error. Low value of the coefficient of variation (1.73) shows that the fitted model is reliable.
The quality of model developed was estimated using correlation coefficient that is the coefficient of determination (R^2) which established the compatibility of the experiment data with the predicted data. The effect of different treatment parameters on purity drop at boiling, to aid visualization of variation in responses with respect to processing variables, interaction plot for the response surfaces were obtained in order to determine the effect of two other variables on the response. The non-variant parameter was set at the optimum point and the relationships developed between dependent variable and independent variables were used to plot response surfaces.
The software also permits numerical optimization of the models. An anticipated goal for each variable and response is selected from the menu. The probable goals are: maximize, minimize, target, in range and equal to (for responses only). With this a maximum and a minimum level must be provided for each factor involved.

Results and discussion

Extraction experiment with the designed experimental conditions shows a great variation in the final yield of extracted oil. The relationship between response (watermelon oil yield) and other three independent factors (extraction period, solvent and particle size) were studied through the Box-Behnken design with three-level-two factors (Table 1). Table 2 shows the matrix of the experimental design and the results

The Design Expert 10.0.5.0 was employed to estimate and determine the coefficient of the full regression model equation and their statistical significance, the model equation in term of coded factors for the Box-Behnken response surface quadratic model is

Table 2: Box Behnken experimental design matrix for three-level- two -factors response surface study

Standard	Runs	Extraction period (min)	Solvent volume (ml)	Particle size (µm)	Yield (%)
3	1	30.0	200.0	8.0	42.0
4	2	50.0	200.0	8.0	59.0
12	3	40.0	200.0	12.0	52.5
9	4	40.0	100.0	4.0	7.0

15	5	40.0	150.0	8.0	31.5
13	6	40.0	150.0	8.0	31.5
5	7	30.0	150.0	4.0	20.0
7	8	30.0	150.0	12.0	39.0
14	9	40.0	150.0	8.0	31.5
1	10	30.0	100.0	8.0	21.0
17	11	40.0	150.0	8.0	31.5
8	12	50.0	150.0	12.0	46.5
11	13	40.0	100.0	12.0	28.0
10	14	40.0	200.0	4.0	310
2	15	50.0	100.0	8.0	14.0
17	16	40.0	150.0	8.0	31.5
6	17	50.0	150.0	4.0	19.5

Table 3: ANOVA test for watermelon seed extraction

Source	Sum of Squares	DF	Mean Square	F value	Prob.>F	
Model	4.26	9	4.30	140.74	< 0.0001	Significant
A	9.026E-004	1	9.03E-004	0.27	0.6206	Not significant
B	2.29	1	2.30	670.16	< 0.0001	
C	1.49	1	1.50	441.95	< 0.0001	
A^2	0.016	1	0.02	4.79	0.0648	
B^2	0.079	1	0.08	23.54	0.0019	
C^2	0.088	1	0.09	26.15	0.0014	
AB	0.140	1	0.14	41.25	0.0004	
AC	0.010	1	0.01	3.01	0.1265	
BC	0.18	1	0.20	54.86	0.0001	
Residual	0,024	7	3.366E-003			
Lack of Fit	0.024	3	7.855E-003			
Pure Error	0.000	4				
Cor. Total	4.29	16				

$$\ln(\text{Yield}) = +3.45 + (0.011*A) + (0.53*B) + (0.43*C) + (0.062*A^2) - (0.14*B^2) - (0.14*C^2) + (0.19*A*B) + (0.050*A*C) - (0.21*B*C) \quad \text{......5}$$

Quadratic model was suggested by the design program for this response to test for its adequacy and to describe its variation with independent variables. From ANOVA test in table 3, the Model

F-value of 140.74 implies the model is significant. And there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, C, B^2, C^2, AB, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Pred R-Squared" of 0.9121 is in reasonable agreement with the "Adj R-Squared" of 0.9874. "Adeq Precision" measures the signal to noise ratio. For a ratio greater than 4 is desirable. The ratio of 47.452 indicates an adequate signal. Which means the model can be used to navigate the design space (table 4).

Table 4: Post ANOVA statistics Watermelon seed oil extraction

Std. Dev.	0.058	R-Squared	0.9945
Mean	3.35	Adj. R-Squared	0.9874
C.V.	1.73	Pred. R-Squared	0.9121
PRESS	0.38	Adeq. Precision	47.452

The strength of a linear relationship between the variables was measured by correlation coefficient, (table 5). Here, almost all the factor- to-factor correlation is zero. The factors- to- responses correlation are different from zero, because for the factors- to - factors correlation, off-diagonal values close to zero are better.

Table 5: Correlation Matrix of Factors [Pearson's r] for oil extract yield

	A	B	C	A^2	B^2	C^2	AB	AC	BC
A	!.000								
B	0.000	1.000							
C	0.000	0.000	1.000						
A^2	0.000	0.000	0.000	1.000					
B^2	0.000	0.000	0.000	0.000	1.000				
C^2	0.000	0.000	0.000	0.000	0.000	1.000			
AB	0.000	0.000	0.000	0.000	0.000	0.000	1.000		
AC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	
BC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000

Diagnostic Test for the Extracted oil yield

All diagnostic plots were tested for adequacy of the models in this study, figure 2a shows the normality of residual while figure 2b shows the predicted versus the actual where precisely the oil yield is modeled, it is realized that all the points line up and the deviation of points for oil yield from normality is insignificant.

Effect of Individual Process Variables

Time as a variable has no influence on extraction process, as shown in the figure 3a below. Although with actual factors of 150 ml of solvent volume and 8 µm particle size, the yield increases with increase in time (30- 50 mins.) which is liable to the volume of oil contain in the watermelon seed. When the process reached the maximum extracting point, the process still goes on, the yield remains constant.

Solvent volume is one of the main variables that affect the extraction rate. Figure 3b shows the natural logarithm yield increased with increase in solvent volume (100 – 200 ml) for actual factors of 8 µm size particle and time of 40 mins. Therefore, at high solvent volume there is high yield irrespective of the other two variables because with much volume of solvent their high adsorption rate which enhance extraction rate as well. Likewise, Figure.3c shows the effect of particle size (4, 8 and 12 µm) on the natural logarithm yield of the extraction reaction

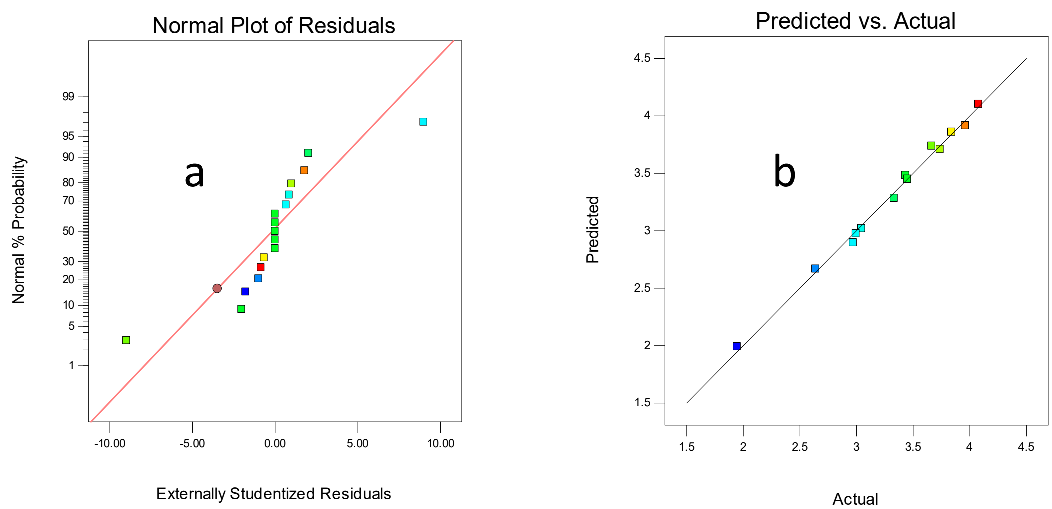

Figure 2 Showing **normal probability curve and predicted vs actual**

It was found that with increasing particle size, the natural logarithm yield extracted oil increased significantly with actual factors of 150ml of solvent and 40 min. The increase in the yield of extracted oil at higher particle size (12 µm) is due to higher penetration rate, therefore higher wide surface area which led to higher rate of extraction and eventually higher oil yield and the lower yield of extracted oil at lower particle size (4 µm) could be due to the coarseness of the surface area which leads to slow extraction rate This is in agreement with Ioannis *et al.* 2019 who achieved the highest oil recoveries from an intermediate particle diameter between 0.5 and 0.85 mm when considering effect of solvent extraction parameters on the recovery of oil from spent coffee grounds for biofuel production.

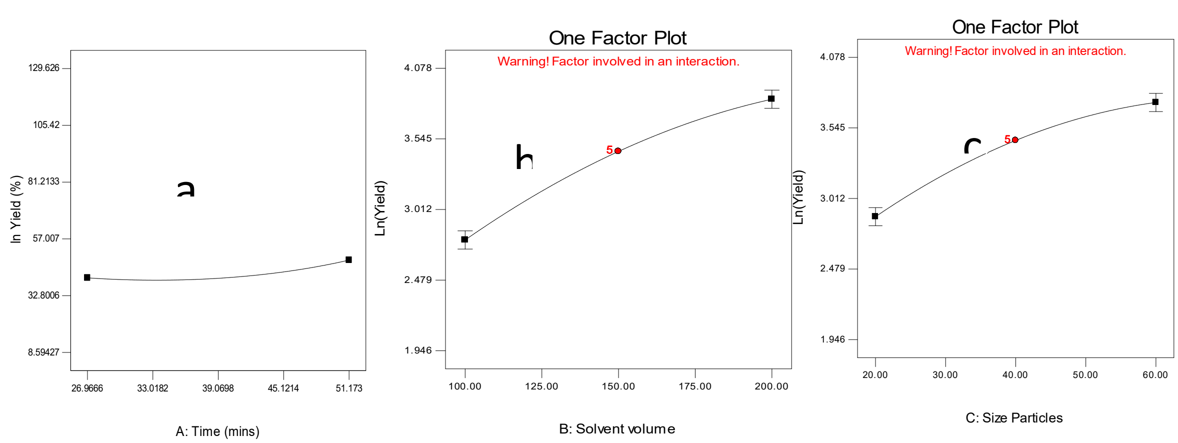

Fig. 3: Natural Logarithm Plot of yields (%)

Effect of Interactions between the Process Variables

Figure 4a below shows the interactions between time and size particles with natural logarithm yield and it was deduced that the time and size particle are also inversely proportional with little variance, while the time and solvent volume are inversely proportional to each other with respect to the natural logarithm of the yield (figure 4b) because as the natural logarithm of oil yield increases the solvent volume increases but time increases with decrease in natural logarithm of oil yield, while figure 4c shows that solvent volume and size particle are directly proportional to natural logarithm of yield because as both increases the yield also increases. This is in line with the research works of Akhihiero *et al.,* 2013 and Jesús *et al.,* 2014 where oil extraction yield was higher as particle size decreased and contact time increased.

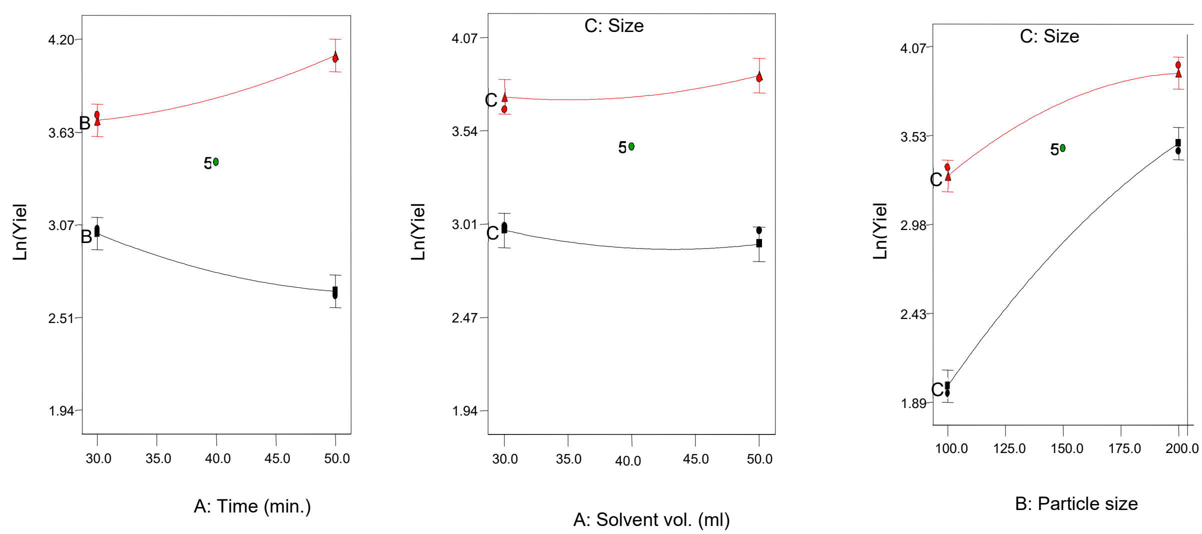

Figure 4: Interaction between Process Variables.

Table 6: Optimization solutions for extracted oil

Number	Time	Solvent volume	Particle size	Ln (Yield)	Desirability	
1	50.00	199.99	8.00	4.225	1.000	Selected
2	49.85	200.00	8.00	4.219	0.998	
3	50.00	195.12	8.00	4.201	0.998	
4	50.00	188.57	8.00	4.165	0.970	
5	40.14	200.00	7.46	3.921	0.825	

Optimization Solution for extracted oil yield

The optimum parameter values for oil extract; time of extraction, solvent volume and size particles were calculated. The optimization was to get maximum oil extract as much as possible. Five (5) solutions were obtained and the solution with maximum desirability is selected as an optimum solution (table 6). Time of 50min, solvent volume of 199.99ml and particle size of 12 µm were selected to obtain optimum oil yield of 68% (ln yield of 4.225) using equation 6. This shows that there is better yield when compared with 26 % of oil obtained by Ioannis *et al.* (2019) when considering the "Effect of Solvent Extraction Parameters on the Recovery of Oil from Spent Coffee Grounds for Biofuel Production".

$$\ln yield = +1.47835 - (0.11441 * 50) + (0.020770 * 200) + (0.072649 * 60) + (6.18853e^{-004} * 50^2) - (5.48705e^{-005} * 200^2) - (3{,}61457e^{-004} * 60^2) + (3.72666e^{-004} * 50 * 200) + (2.51511e^{-004} * 50 * 60) - (2.14867e^{-004} * 200 * 60) = 4.225 \quad \ldots\ldots 6$$

$$\boldsymbol{ln}\ \textbf{yield} = \mathbf{4.225}$$
$$\textbf{Yield} = \mathbf{68}\ \%$$

FFA Analysis

After the percentage conversion of the extract at each run follows by determination of the FFA content in the oil. It was found that 15.4ml of KOH was used in titrating against the mixture of 2.5grams of oil, 50ml of absolute ethanol and 1ml of phenolphthalein to give a pink color after which the equations 2 and 3 were used to calculate the acid value and % FFA respectively.

$$Acid\ Value = \frac{ml\ of\ KOH \times N \times 5.6}{Weight\ of\ the\ oil\ extract}$$

$$\%FFA = acid\ value * 0.503$$

$$= 34.50 * 0.503$$

$$= 17.35\%$$

Conclusion

In the current research work, extraction process was carried out by means of Soxhlet extractor on a heating mantle to extract the oil using n-hexane as the solvent for the different experimental runs. A three-variable (two levels of each variable) Box Behnken experimental design was employed which generated seventeen experimental runs. The independent variables included time (minutes), solvent volume (ml), and particle size (μm) each at three levels. The outcomes revealed that oil yield was significantly altered by all the investigated parameters. The optimal extraction efficiency with respect to the oil yield of 68% and free fatty acid content of 17.35% were achieved at 50.00 minutes of experimental duration, 199.99 mil solvent volume and 8.00 μm of particle size. This research serves as the basis for an in-depth investigation on the optimization of extraction procedure of oil yield and determination of free fatty acid using response surface methodology approach.

Recommendation

Central composite design (CCD) may be adopted apart from Box Behnken experimental design hat was employed, with CCD which is well suited for fitting a quadratic surface which usually consider a three- factor layout found to be working well for process optimization.

References

Akhihiero, E.T. Ayodele, B.V. and Akpojotor, G. E. (2013) Effect of particle size and temperature variation on the yield of essential oil from lemon grass using steam distillation. African Journal of Physics 6, pp. 105-112.

AOAC, Official methods of analysis of the association of o*fficial analytical chemists,* (2000): 17th Edition, Washington DC.

Atabani, E. (2013) Non-edible vegetable oils: A critical evolution of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emission production. Renewable and Sustainable Energy Reviews 18, 211-245.

Babatunde, K.A., Ogunleye, O.O., Osuolale, F.N. & Agbede, O.O. (2016) Comparative Study of Different Concentrations of NaOH and KOH Embedded Bentonite Clay as Heterogeneous Catalyst on Transesterification Variables of the Methyl Esters of Castor Seed Oils in Nigeria. Advances in Multidisciplinary & Scientific Research. 2, No. 1, March: 135-146.

Dane F. Liu, J. (2007) Diversity and origin of cultivated and citron type watermelon *(citrilluslanatus*). *Genetic Resources and Crop Evolution*. *Australian journal of crop science* 5(13), 1832-1836

Esuoso, K., Lutz, H., Kutubuddin, M. Bayer, E. (1998) Chemical composition and potential of some underutilized tropical biomass. *Fluted Pumpkin* (*Telfairia Occidentalis*). *Food Chemistry*. *61: 487-492.*

Ioannis E, Paul H, Nicos L, Arthur K, and Ben M. (2019) Effect of Solvent Extraction Parameters on the Recovery of Oil from Spent Coffee Grounds for Biofuel Production. Waste Biomass Valorization 10(2): 253–264.

Jesús R-M, Betsabé H-S, Erasmo H-L, Carlos A. Gómez-Aldapa, H. S. G. & Cecilia E. M-S. (2014) Effect of some variables on oil extraction yield from Mexican pumpkin seeds, CyTA - Journal of Food, 12:1, 9-15, DOI: 10.1080/19476337.2013.777123

Jo M S., Rene, E. R., Kim, S. H. Park, H. S., (2008). An analysis of synergistic and antagonistic behavior during BTEX removal in batch system response surface methodology. J. Hazard Mater, 152(3), 1276-1284.

Lee man, H., Behera, S. K., Park H. S. (2010) Optimization of operational parameter for ethanol production from Korean food waste leachate. Int. J. Environ. Sci. Tech, 7(1), 157-164.

Naik, S. N. Vaibhav V. Goud P. Rout, K. and Ajay K. D. (2010) Production of first- and second-generation biofuels: A comprehensive review" Renewable and Sustainable Energy Reviews. 14: 578-597.

Naseri, P.M.T., Tehrani, F. A. (1995) Production of Vegetables Seeds, Dejahad Denshgahi Press of Mashhad (in Persian language), Iran. Pages 50- 69.

Palligarnai T. Vasudevan, (2008) Bioiesel production-current state of the art and challenges. Springer-J Ind Microbiol Biotechnol 35:421-430.

Ramadhas, S. Jayaraj, S. Muraleedharan, C. (2005) Biodiesel production from high FFA rubber seed oil, Fuel 84; 335–340.

Ravindra P., Jegannathan K., Raman I, Gujjula R. (2012) Evaluation of activation energy and thermodynamic properties of enzyme-catalysed transesterification reactions. *Advances in Chemical Engineering and Science*, 2, 150-154

Ravikumar, K., Swaminathan, T., Balu, K, (2005) Optimization of batch process parameter using response methodology for dye removal by a novel absorbent. Chem. Eng. J., 105 (3) 131-138.

Singh D, Singh S.P. (2008) Low cost production of ester from non-edible oil of *Argemone mexicana*. Biomass Bioenergy 34:545–9.

Stevens C V. and Verhe R. (2004) Renewable bio resources scope and modification for Non-food application. England: John Wiley and Sons Ltd.

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