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Sorrel (*Hibiscus sabdariffa*) Seed Oil Extraction Optimization and Quality Characterization

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Authors' contributions

Both authors EB and TFA write, read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This study was aimed at separating seed oil from sorrel (*Hibiscus sabdariffa*) oilseeds using application of solvent extraction method. The process was optimized using response surface methodology and the quality of the seed oil was determined.
Methodology: Optimization of oil extraction from the oilseeds using response surface methodology was carried out. The effects of three independent factors (extraction time, solvent volume and sample weight) and their respective interactions on the response, oil yield, were investigated. A total of 17 experimental runs were generated using Box-Behnken design. The extracted seed oil was characterized to determine its quality.
Results: A quadratic polynomial was obtained to predict the oil yield and the ANOVA test showed the developed model to be significant ($P < 0.05$). A statistical model predicted the maximum seed oil yield to be 18.25% at the optimal condition of sample weight, 22g, solvent volume, 157 ml and time, 2 h. The optimized condition was validated with the actual oil yield of 17.85%, which was well within the range predicted. The seed oil analysis showed the physical state of the oil to be liquid/yellow-greenish in colour, specific gravity 0.886 ± 0.026, viscosity (at 40ºC) 15.40 cP, p-anisidine value 6.31, Totox number 16.31, %FFA 0.40 ± 0.01, acid value 0.80 ± 0.01 mg KOH.g oil^{-1}, saponification value 197.75 ± 0.05 mg KOH.g oil^{-1}, iodine value 97.77 ± 0.02 g I_2.100 g oil^{-1}, peroxide value 5.00 ± 0.01 meq O_2.kg oil^{-1} and cetane number 51.90 ± 0.1. The fatty acid profile of the oil revealed

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that it is highly unsaturated (76.45%) with linoleic acid the highest (44.39%).
Conclusion: The physicochemical analysis of the sorrel seed oil indicated it is edible and could serve as feedstock for many industrial applications.

Keywords: Fatty acid; physicochemical properties; optimization; response surface methodology; sorrel seed oil.

1. INTRODUCTION

Oilseed crops are vital sources of oils of nutritional, pharmaceutical and industrial importance. The characteristics of oils from different sources depend mainly on their compositions and no oil from single source can be suitable for all purposes [1]. Presently, the quest for traditional vegetable oils has increased immensely because of the ever-growing World population and their use for industrial purposes. Several oils such as moringa oil, sunflower oil, rapeseed oil, palm oil, soybean oil, corn oil and pumpkin oil have been used for industrial purposes. New low-cost oilseed crops are needed to produce inexpensive oils suitable for food, pharmaceutical and industrial applications. One of the possible alternative crops is *Hibiscus sabdariffa*, also known as sorrel or Roselle. It is an herb belonging to the malvaceae family, which is grown in Nigeria, India and West Indies, and to some extent in tropical America. The sorrel seed oil is rich in both linoleic (39.4 - 40.1%) and oleic (26.2 - 28%) fatty acids [2,3]. In Sudan, the seeds are used for edible oil production and the by-products of this process are used for poultry feeding [4].

Numerous methods exist in oil separation from oilseeds and these include mechanical pressing, pressurized solvent extraction, Soxhlet extraction, ultra-sonic extraction and Aqueous Enzymatic Oil Extraction (AEOE). Mechanical pressing is the most widely used but the oils produced with this method usually have low quality. With extraction method using supercritical fluid such as CO_2, the oil produced has very high purity at high operating and investment cost. Extraction with solvent has a number of advantages, which include higher yield and less turbidity as well as relatively low operating cost. Previous studies showed that extraction with organic solvents have been one of the major approaches employed. Some of the recent work on oil extraction using solvent extraction technique include oils from *Washingtonia filifera* [5], *Moringa oleifera* [6], bitter seed, pumpkin (*Cucurbita pepo* L.), Kalahari melon seed, kenaf and sorrel [7].

Response surface methodology (RSM) is a useful optimization tool, which has been applied in research to study the effect of individual variables and their interactions on response variables. It has been used extensively on the optimization of extractions of edible and non edible oils from different oil sources such as pumpkin, palm oil, silkworm pupae, *Vetiveria zizanioides*, locust bean, to mention but a few [8,9,10,11]. The major benefit of RSM is the ability to reduced number of experimental runs needed to arrive at optimized and statistically acceptable results. Thus, it saves time and less difficult compared with full-factorial design [10].

This study aimed at oil separation from sorrel (*Hibiscus sabdariffa* Linn.) oilseeds through application of solvent extraction method. To optimize the extraction conditions for the process, RSM was applied to determine the effects of three-level-three factor and their reciprocal interactions on the oil extracted. In addition, the quality of the oil extracted was evaluated by carrying out physicochemical analysis with a view to determine its potential use.

2. MATERIALS AND METHODS

2.1 Materials and Chemicals

Sorrel (*Hibiscus sabdariffa*) oilseed samples were collected from Gaya Hong Local Government Area in Adamawa State, Nigeria. The oilseeds had some foreign materials and dirt, which were removed by thorough washing followed by sun-drying for 5 days. The oilseeds were winnowed to remove the chaffs. Finally, the cleaned oilseeds were made into powder by grinding with a milling machine. All chemicals and reagents used for this work were of analytical grades.

2.2 Methods

2.2.1 Experimental design

In this study, the Box-Behnken experimental design was employed in order to optimize the sorrel oil extraction. The coded independent factors levels are presented in Table 1. Selected extraction parameters for the separation of oil from the sorrel oilseeds were extraction time (X_1), solvent volume (X_2) and sample weight (X_3).

Table 1. Factors and their levels for Box-Behnken design

Factor	Symbol	Coded factor levels		
		-1	0	+1
Extraction time (h)	X_1	2	3	4
Solvent volume (ml)	X_2	150	275	400
Sample weight (g)	X_3	20	40	60

A three-level-three-factor design was applied, which generated 17 experimental runs (Table 2). This included 6 factorial points, 6 axial points and 5 central points to provide information regarding the interior of the experimental region, making it possible to evaluate the curvature effect. Depicted in Table 2 also are the observed yields, the predicted yields and the residual values. The effects of unexplained variability in the observed response due to extraneous factors were minimized by randomizing the order of experiments.

2.2.2 Oil extraction procedure

A 500-ml Soxhlet apparatus and *n*-hexane as solvent were used for this study. Initially, the apparatus was charged with a known weight of sorrel oilseeds powder in a muslin cloth placed in a thimble of Soxhlet apparatus. A round bottom flask containing known volume of *n*-hexane was fixed to the end of the apparatus and a condenser was tightly fixed at the bottom end of the extractor. The whole set up was heated up in a water bath (Lamfield Medicals, Model DK-420, UK) at temperature of 70ºC. The excess solvent in the oil was recycled by heating in a heating mantle at temperature of 70ºC after the extraction. Quantity of oil extracted was determined gravimetrically. The oil yield was evaluated as the ratio of the weight of the extracted seed oil to the weight of the sorrel oilseed powder sample as described in Eq. (1). The oil obtained was stored appropriately for further processing.

$$\%\ Oil\ yield\ (w/w) = \frac{Weight\ in\ gram\ of\ extracted\ oil}{Weight\ in\ gram\ of\ oilseed\ powder\ sample} \quad (1)$$

Table 2. Experimental design matrix by Box-Behnken for three-level-three-factor response surface study

Std run	X_1	X_2	X_3	Observed oil yield % (w/w)	Predicted oil yield % (w/w)	Residual
1	-1	-1	0	17.37	17.39	-0.02
2	1	-1	0	14.50	14.45	0.05
3	-1	1	0	12.38	12.43	-0.05
4	1	1	0	10.86	10.84	0.02
5	-1	0	-1	15.65	15.63	0.02
6	1	0	-1	14.37	14.42	-0.05
7	-1	0	1	13.97	13.92	0.05
8	1	0	1	10.56	10.58	-0.02
9	0	-1	-1	13.53	13.53	0.00
10	0	1	-1	9.09	9.06	0.03
11	0	-1	1	10.54	10.57	-0.03
12	0	1	1	6.48	6.48	0.00
13	0	0	0	8.42	8.50	-0.08
14	0	0	0	8.62	8.50	0.12
15	0	0	0	8.42	8.50	-0.08
16	0	0	0	8.62	8.50	0.12
17	0	0	0	8.62	8.50	0.12

2.2.3 Statistical data analysis

The data obtained from the sorrel seed oil extraction experiments were analyzed statistically using response surface methodology, so as to fit the second-order mathematical model generated by the Design-Expert software version 8.0.3.1 (Stat-Ease Inc., Minneapolis, USA). To correlate the response variable to the independent variables, multiple regressions was used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using test of significance and analysis of variance (ANOVA). The fitted second-order mathematical model is described in Eq. (2).

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + e \quad (2)$$

Where, Y is response variable (sorrel oil yield), b_o is the intercept value, b_i (i = 1, 2, …, k) is the first order model coefficient, b_{ij} is the interaction effect, and b_{ii} represents the quadratic coefficients of X_i, and e is the random error.

2.2.4 Physicochemical analysis of the extracted sorrel seed oil

The evaluation of the following physicochemical properties of the extracted seed oil were determined by the AOAC methods: refractive index, moisture content, viscosity, acid value, saponification value, peroxide value, specific gravity, % FFA, *p*-anisidine and Totox number while the higher heating value was determined using the method of Demirbas [12] and iodine value was obtained by Wijs method.

2.2.5 Analysis of fatty acid compositions of sorrel seed oil

Fatty acid profile of the extracted sorrel seed oil was determined using gas chromatography (HP 6890 powered with HP ChemStation Rev. A 09.01 [1206] Software) equipped with a flame ionization detector (FID) and a polar capillary column (HP-Innowax polyethylene glycol, 0.25 mm internal diameter, 30 m length and 0.25 µm film in thickness). Oil sample (50 mg) was esterified for 5 min at 95ºC with 3.4 ml of the 0.5 M KOH in dry methanol. The fatty acids were thrice extracted from the mixture with redistilled *n*-hexane. The carrier gas was nitrogen and the oven initial temperature was at 60ºC. While the first ramping was at 12ºC/min for 20 min and maintained for 2 min, the second ramping was at 15ºC/min for 3 min and maintained for 8 min. The inlet temperature and detector temperature were 250ºC and 320ºC, respectively.

3. RESULTS AND DISCUSSION

3.1 Optimization of Sorrel Seed Oil Extraction

Table 3 described the results of test of significance for every regression coefficient. Considering the large F-values and low corresponding P-values, all the model terms have very strong effects on the oil yield except the cross product of solvent volume and sample weight (X_2X_3) with P>0.05 (Table 3). However, the quadratic term of extraction time (X_1^2) with F-value of 9503.07 and P<0.0001, was the most significant model term. In order to minimize error, all the coefficients were considered in the design.

Table 3. Test of significance for all regression coefficient terms

Source	Sum of squares	df	Mean square	F-value	P-value
X_1	10.31	1	10.31	1147.37	<0.0001
X_2	36.68	1	36.68	4083.61	<0.0001
X_3	15.37	1	15.37	1711.56	<0.0001
X_1X_2	0.46	1	0.46	50.73	0.0002
X_1X_3	1.13	1	1.13	126.28	<0.0001
X_2X_3	0.036	1	0.036	4.02	0.0850
X_1^2	85.36	1	85.36	9503.07	<0.0001
X_2^2	2.53	1	2.53	281.55	<0.0001
X_3^2	1.70	1	1.70	189.02	<0.0001

The results of the second-order response surface model fitting in the form of ANOVA are presented in Table 4. The model F-value of 1946.03 with low P-value (P<0.0001) implied a high significance for the regression model [13]. The goodness of fit of the model was checked by the coefficient of determination (R^2), which should be at least 0.80 for the good fit of a model [14]. In this case, the R^2 value of 0.9996 indicated that the sample variation of 99.96% for the oil extraction is attributed to the independent variables (extraction time, solvent volume and sample weight) and only 0.04% of the total variations are not explained by the model. All the p-values were less than 0.05 except X_2X_3 (solvent volume-sample weight), implying that the model proved suitable for the adequate representation of the actual relationship among the selected factors. The lack-of-fit term of 0.7532 was not significant relative to the pure error. In this case, a non-significant lack of fit is good. Hence,

the model could be used in theoretical prediction of the oil extraction. The developed regression model describing the relationship between the oil yield (Y) and the coded values of independent factors of extraction time (X_1), solvent volume (X_2) and sample weight (X_3) and their respective interactions is described in Eq. (3).

$$Y = 8.50 - 1.14X_1 - 2.14X_2 - 1.39X_3 + 0.34X_1X_2 - 0.53X_1X_3 + 0.095X_2X_3 + 4.50X_1^2 + 0.77X_2^2 + 0.64X_3^2 \quad (3)$$

Table 4. Analysis of variance (ANOVA) of regression equation

Source	Sum of squares	df	Mean square	F-value	P-value
Model	157.32	9	17.48	1946.03	<0.0001
Residual	0.063	7	0.00898		
Lack of fit	0.015	3	0.00495	0.41	0.7532
Pure error	0.048	4	0.012		
Cor total	157.38	16			
R^2 = 99.96%,			R^2 (adj) = 99.91%		

Fig. 1a shows the response surface plot representing the effect of extraction time, solvent volume and their reciprocal interaction on oil yield while keeping sample weight constant at zero level. Response surface plot describing the effect of extraction time, sample weight and their reciprocal interaction on oil yield while keeping solvent volume constant at zero level is depicted in (Fig. 1b). The curvatures nature of the surface plots in Fig. 1(a & b) indicates mutual interactions between extraction time and solvent volume and, between sample weight and extraction time, respectively [6]. Fig. 1c shows the response surface plot of the effect of solvent volume, sample weight and their reciprocal interaction on oil yield while extraction time constant at zero level. The interaction between solvent volume and sample weight was not mutual.

The optimal values of the independent factors selected for the extraction process were obtained by solving the regression Eq. (3) using the Design-Expert software package. The optimal condition was established as sample weight of 22 g, solvent volume of 157 ml and extraction time of 2 h. The predicted sorrel oil yield under the optimal condition was Y=18.25% (w/w). To verify the prediction of the model, the optimal condition values were applied to three independent replicates and the average sorrel oil yield obtained was 17.85% (w/w), which was well within the predicted value of the model equation. The results of this study demonstrate that RSM with appropriate experimental design can be effectively applied to the optimization of the process factors in oil extraction work.

3.2 Quality Characterization of Sorrel Seed Oil

3.2.1 Physical properties of the seed oil

To characterize the quality of the sorrel seed oil extracted in this work, the oil was subjected to physicochemical analysis and the results obtained are presented in Table 5. At room temperature, the seed oil was liquid yellow-greenish in colour with refractive index and moisture content of 1.4603 and 0.065%, respectively. Observations on the colour and the refractive index of the oil agreed with previous published reports [2,3,15]. The specific gravity of the seed oil was determined as 0.886 ± 0.026 and the viscosity, which is a measure of the

resistance of oil to shear, was 15.4 cP. These values are within the range earlier reported for sorrel seed oil [15].

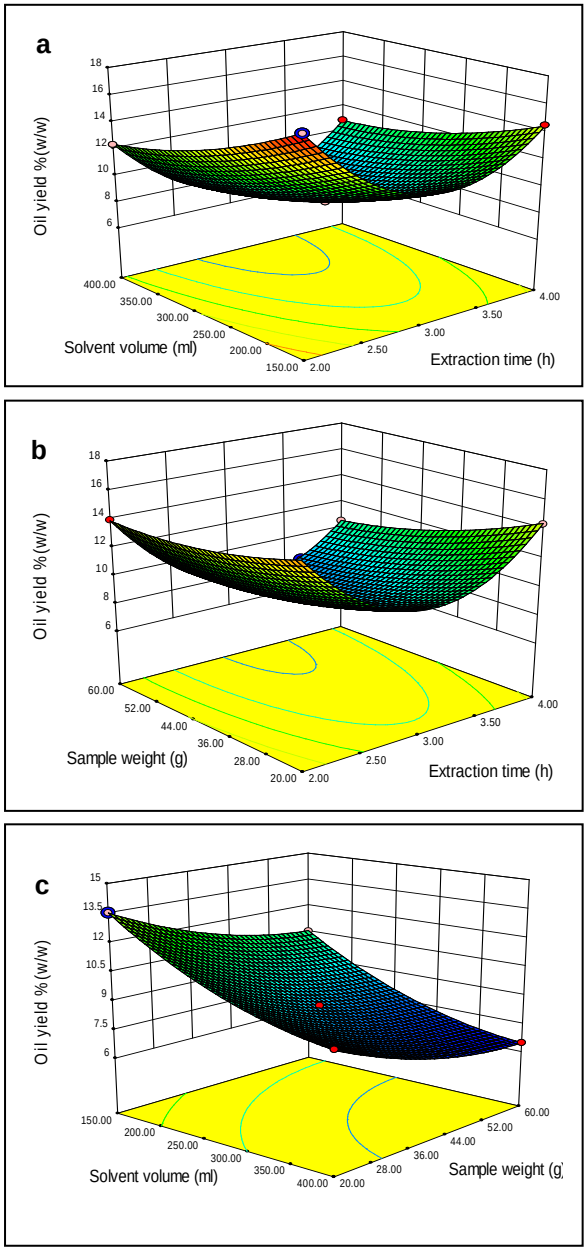

Fig. 1. Surface plots for solvent extraction of sorrel seed oil

3.2.2 Chemical properties of sorrel seed oil

Among the most important characteristics used to determine the present condition and quality of oil samples are their chemical properties. Table 5 contains results obtained for the

chemical properties of the sorrel seed oil. Low FFA content (0.40 ± 0.01) of sorrel seed oil obtained in this study is indicative of the good resistance of this oil to hydrolysis. The low acid value (0.80 ± 0.01 mg KOH/g oil) of this oil showed that it is not only edible but could also have a long shelf life. A high saponification value of 197.77 ± 0.05 (mg KOH/g oil) was obtained for the sorrel seed oil, indicating high concentration of triglycerides. The iodine value of the sorrel seed oil was high (97.77 ± 0.02 g I_2/100 g oil), which showed the oil contained a substantial level of unsaturation. The peroxide and *p*-anisidine values measure hydroperoxides and secondary oxidation products, i.e. aldehydes, of oils, respectively [16]. The peroxide value obtained for the seed oil in this study was 5.00 ± 0.01 meq O_2/kg oil, which is a low value. A *p*-anisidine value of 6.31 of the seed oil suggested the presence of significant amounts of secondary oxidation products in the seed oil. The combination of high iodine value and low peroxide value indicated the sorrel seed oil could be stored for a long period without deterioration. These also demonstrated the oil possessed the desirable qualities of edible oils. The Totox value of the sorrel seed oil was 16.31. Totox values reported for cottonseed, canola and soybean oils are 18.58, 9.41, and 12.49, respectively [17] and the lower the value, the better the quality of the oil. The Higher Heating Value (HHV) determined for the sorrel seed oil was 39.86 ± 0.02 MJ/kg. The value was within the range earlier reported for vegetable oils [12].

Table 5. Physicochemical and other characteristics of sorrel seed oil

Parameters	Mean values
Physical properties	
Physical state at 28ºC	Liquid/Yellow-greenish in colour
Refractive index at 25ºC	1.4603
Moisture content (%)	0.065
Specific gravity	0.886 ± 0.026
Viscosity (cP) at 40ºC	15.40
Mean Molecular mass	283.30
p-anisidine value	6.31
Totox number	16.31
Chemical properties	
%FFA (as oleic acid)	0.40 ± 0.01
Acid value (mg KOH/g oil)	0.80 ± 0.01
Saponification value (mg KOH/g oil)	197.75 ± 0.05
Iodine value (g I_2/100g oil)	97.77 ± 0.02
Peroxide value (meq O_2/kg oil)	5.00 ± 0.01
Higher heating value (MJ/kg)	39.86 ± 0.02

Hence, the physicochemical characteristics of the oil showed that the sorrel seed oil is a good candidate for use as edible oil and as an industrial feedstock.

3.2.3 Fatty acid profile of sorrel seed oil

Gas chromatography analysis of fatty acids present in the seed oil is shown in Table 6. The results indicated that the oil was highly unsaturated. The major fatty acids present in the seed oil were linoleic (44.39%), oleic (32.06%), palmitic (12.68%) and stearic (10.87%). The total unsaturated fatty acid composition of the oil was 76.45%. Although this result followed the trend of reported fatty acid compositions for sorrel seed oil, it has been observed that the

quantity of each acid present in this seed oil varies considerably among the different cultivars studied [2,15].

Table 6. Fatty acids compositions of the sorrel seed oil

Parameters	Compositions %
Palmitic Acids (C16:0)	12.68
Stearic Acids (C18:0)	10.87
Oleic Acids (C18:1)	32.06
Linoleic Acids (C18:2)	44.39

4. CONCLUSION

In this work, experiments were conducted using response surface methodology (RSM) to determine the optimal condition for the solvent extraction of oil from sorrel (*Hibiscus sabdariffa* Linn.) oilseed. From the Box-Behnken design, a statistical model predicted the highest oil yield to be 18.25% (w/w), at the optimal condition of sample weight of 22 g, solvent volume of 157 ml and extraction time of 2 h. Using these optimal condition values in three independent replicates, an average oil content of 17.85% (w/w) was achieved, which was well within the range predicted by the model. The fatty acid profile of the seed oil revealed that the oil was highly unsaturated. In addition, the quality of oil extracted under the optimal condition showed that the oil is edible and could serve as feedstock for many industrial applications.

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COMPETING INTERESTS

Authors declare no conflict of interest.

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