

Statistical Approach to Oils Extraction from Sorrel Seed (*Hibiscus sabdariffa L.*) and Its Quality Characterization

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Abstract - This work focused on optimization of oil extraction from Sorrel oilseeds using Response Surface Methodology (RSM). Based on Box-Behnken design, 17 experimental runs were conducted to investigate the effects of extraction time, solvent volume and sample weight, and their reciprocal interactions on the oil yield. A quadratic polynomial was obtained to predict the oil yield and the ANOVA test showed the 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, 22 g, 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 fatty acid profile of the oil revealed is highly unsaturated (74.45%) with linoleic acid the highest (44.39%). The physicochemical analysis of oil suggested the oil could have important food and industrial applications.

Keywords: Sorrel oil, optimization, response surface methodology, fatty acid, physicochemical properties.

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 a single source can be suitable for all purposes (Ramadan and Mörsel, 2003). 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 (Alcantara et al., 2000; Dorado et al., 2004; Mitra et al., 2009). 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 widely 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 (Mohamed et al., 2007; Nakpong and Wootthikanokkhan, 2010). Al-Wandawi et al. (1984) reported that in Sudan, the seeds are used for edible oil manufacture and the by-products of this process are used for poultry feeding.

Numerous methods exist in oil separation from oilseeds and these include mechanical pressing, pressurized solvent extraction, Soxhlet extraction, ultra-sonic extraction, Aqueous Enzymatic Oil Extraction (AEOE), among others. Mechanical pressing is the most widely used but the oils produced with this method usually have low value. With extraction method using supercritical fluid such as CO₂, the oil produced has very high purity but for the 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* (Nehdi, 2011), *Moringa oleifera* (Rashid et al., 2011), bitter seed, pumpkin (*Cucurbita pepo* L.), Kalahari melon seed, kenaf and Sorrel (Nyam et al., 2009).

Response surface methodology (RSM) originally described by Box and Wilson (1951) 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 (Mitra et al., 2009; Danh et al., 2009; Tan et al., 2009; Akinoso and Raji, 2011). The major benefit of RSM is the ability to reduced number of experimental runs needed to arrive at optimized and statistically acceptable results (Akinoso and Raji, 2011). Thus, it saves time and less difficult compared with full-factorial design (Tan et al., 2009).

This study was aimed at oil separation from Sorrel (*Hibiscus sabdariffa* Linn.) oilseeds via application of solvent extraction method. To optimize the extraction conditions for the process, RSM was applied to determine the effects of three-level-three factors 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 determining its potential use.

2. Materials and methods

2.1 Materials

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 with water (4 to 5 times) followed by sun-drying for 5 days. Separation of the chaffs from the oilseeds was carried out by winnowing. Finally, the cleaned oilseeds were milled 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 seeds 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-factors 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 70°C. The excess solvent in the oil was recycled by heating in a heating mantle at temperature of 70 °C after the extraction. Quantity of oil extracted was determined gravimetrically. The oil yield was evaluated as the ratio of the weight of the extracted oil to the weight of the Sorrel oilseed powder sample (Eq.1). The oil obtained was stored appropriately for further processing.

Table 2: Experimental design matrix by Box-Behnken for three-level-three-factors response surface study

Std. run	X ₁	X ₂	X ₃	OOY% (w/w)	POY % (w/w)	R
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

**OOY = Observed oil Yield, POY = Predicted oil Yield, R =

Residual

$$\% \text{ Oil yield} = \frac{\text{weight in gram of oil extracted}}{\text{weight in gram of oil sample}} \quad (1)$$

2.2.3 Statistical data analysis

The data obtained from the Sorrel seed oil extraction experiments were analysed statistically using RSM 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.

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

Where, Y is response factor (Sorrel oil yield), b₀ 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 mean molecular mass was obtained by the method of Akintayo and Bayer (2002), whereas the higher heating value was determined using the method of Demirbas (1998) 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 Sorrel seed oil was determined using gas chromatography (GC) (HP 6890 powered with HP ChemStation Rev. A 09.01 [1206] Software). Oil sample (50 mg) was esterified for five minute at 95 °C with 3.4 ml of the 0.5 M KOH in dry methanol. The mixture was neutralized using 0.7 M HCl and 3 ml of 14% boron trifluoride in methanol was added. The mixture was heated for 5 min at the temperature of 90°C to achieve complete methylation process. The fatty acids were thrice extracted from the

mixture with redistilled n-hexane. The content was concentrated to 1 µl for GC analysis and 1 µl was injected into the injection port of GC.

3. Results and discussion

3.1 Optimization of Sorrel seed oil extraction

As a result of the global gap between demand and production of vegetable oils, investigations focusing on the use of unconventional oilseeds as sources of oils have greatly increased. In this study, an investigation was conducted into the optimization of oil extraction factors of Sorrel (*H. sabdariffa*) oilseed. Table 2 shows the coded factors considered in this study with observed values, predicted values as well as the residual values obtained. Design Expert 8.0.3.1 software was employed to evaluate and determine the coefficients of the full regression model equation and their statistical significance. 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 X_2X_3 with $p > 0.05$ (Table 3). However, second-order term 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. 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 (Yuan et al., 2008).

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 goodness of fit of the model was checked by the coefficient of determination (R^2). Guan and Yao (2008) reported that an R^2 should be at least 0.80 for the good fit of a model. 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 factors (extraction time, solvent volume and sample weight) and only 0.04% of the total variations are not explained by the model. The value of the adjusted determination coefficient (Adj. R^2 of 0.9991) was also very high, supporting a high significance of the model (Akhazarova and Kefarov, 1982; Khuri and Cornell, 1987) and all 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 (X₁), solvent volume (X₂) and sample weight (X₃) and their respective interactions is described in Eq. (3).

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

The model coefficients and probability values i.e. coded value are shown in Table 5. The low values of standard error observed in the intercept and all the model terms showed that the regression model fits the data well, and the prediction is good (Table 5). The variance inflation factor (VIF) obtained in this study showed that the centre points are orthogonal to all other factors in the model (Table 5). The model also proved suitable for the adequate representation of the real relationship among the selected independent factors.

Figure 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. The results revealed that low

extraction time and low solvent volume favoured Sorrel oil yield while increasing both variables led to low oil yield while working at the highest extraction time, whereas decreasing the solvent volume gave a better yield. 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 Figure 1b. Low oil yield was observed at the high sample weight and high extraction time; the reverse resulted into only marginal increase of the oil. The combination of high sample weight and high extraction time did not significantly increase the oil yield. However, the high oil yield was observed at low sample weight and low extraction time. The curvatures nature of the surface plots in Figure 1(a and b) indicate mutual interactions between extraction time and solvent volume and, between sample weight and extraction time, respectively (Rashid et al., 2011).

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%	

Table 5: Regression coefficients and significance of response

surface quadratic.

Fact.	Coefficient estimate	df	Standard error	95% CI low	95% CI high	VIF
Intercept	9.85	1	0.076	9.67	10.03	-
X ₁	-1.19	1	0.060	-1.33	-1.05	1.00
X ₂	-2.17	1	0.060	-2.32	-2.03	1.00
X ₃	-1.37	1	0.060	-1.51	-1.22	1.00
X ₁ X ₂	0.33	1	0.085	0.13	0.53	1.00
X ₁ X ₃	-0.53	1	0.085	-0.73	-0.33	1.00
X ₂ X ₃	1.84	1	0.085	1.64	2.04	1.00
X ₁ ²	2.95	1	0.083	2.75	3.14	1.01
X ₂ ²	0.97	1	0.083	0.77	1.16	1.01
X ₃ ²	0.84	1	0.083	0.64	1.03	1.01

Figure 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. Low oil yield was recorded at the high sample weight and high solvent volume. An increase in level of oil yield was observed at low sample weight and high solvent volume. High oil yield was also achieved at low sample weight and low solvent volume.

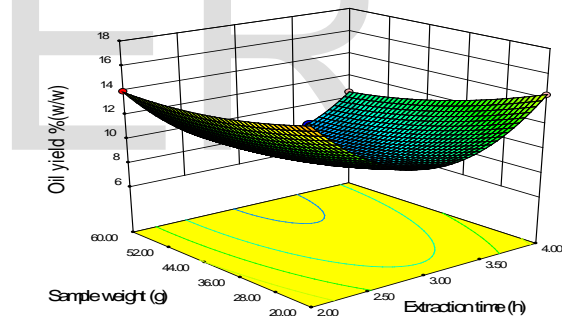
The optimal values of the independent factors selected for the extraction process were obtained by solving the regression equation (Eq. 3) using the Design-Expert software package. The optimal condition was established as sample weight of 22 g, solvent volume of 157 ml and extraction time of 2 h. The predicted Sorrel oil yield under the optimal condition was $Y = 18.25\%$ (w/w). To verify the prediction of the model, the optimal condition was applied to three independent replicates and the average Sorrel oil yield obtained was 17.85% (w/w), which was well within the

estimated 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.

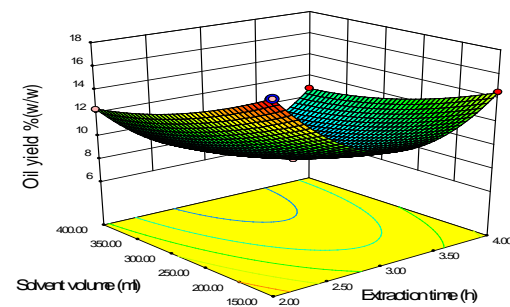
3.2 Quality characterization of Sorrel seed oil

3.2.1 Physical properties of the seed oil

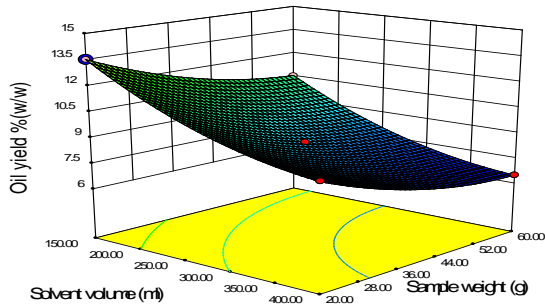
To characterize the quality of the Sorrel seed oil extracted in this work, the oil was subjected to physicochemical analysis and the results obtained are presented in Table 6. At room temperature, the seed oil was liquid yellow-greenish in colour with refractive index and moisture content of 1.4603 and 0.065%, respectively.



(a)



(b)



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Fig. 1(a-c). Surface plots for solvent extraction of Sorrel seed oil.

Observations on the colour and the refractive index of the oil agreed with previous published reports (Mohamed et al., 2007; Mahmoud et al., 2008; Nakpong and Wootthikanokkhan, 2010). 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 ranges reported for Sorrel seed oil (Mohamed et al., 2007). The mean molecular weight of the crude Sorrel oil was determined as 283.30.

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 6 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. Nakpong and Wootthikanokkhan (2010) observed 0.67% FFA for the seed oil in their work. The low acid value ($0.80 \pm$

0.01 mg KOH/g oil) of this oil showed that it is not only edible but could also have a long shelf life. Mahmoud et al. (2008) reported seed oil acidity of 0.78% and acidity level of the oil as determined by Mohamed et al. (2007) was 2.24% while Nyam et al. (2009) observed a very high acid value of 12.9 ± 0.6 mg KOH/g oil for the same oil. These variations may be due to different cultivars used. A high saponification value of 197.77 ± 0.05 (mg of KOH/g of oil) was obtained for the Sorrel seed oil, indicating high concentration of triglycerides. This value is closed to the result (196.82 mg KOH/g oil) reported by Mahmoud et al. (2008). The iodine value of the Sorrel seed oil was high (97.77 ± 0.02 g of $I_2/100$ g of oil), showing that 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 (Bockisch, 1998). The peroxide value obtained for the seed oil in this study was 5.00 ± 0.01 milli-equivalent of peroxide/kg of oil, which is a low value. A peroxide value range of 4.82 – 8.63 milli-equivalent of peroxide/kg of oil has been earlier reported for Sorrel seed oil (Mohamed et al., 2007; Mahmoud et al., 2008; Nyam et al., 2009). A p-anisidine value of 6.31 of the seed oil suggests the presence of significant amounts of secondary oxidation products in the seed oil. The combination of high iodine value and low peroxide value suggested 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 (Daniel-O'Dwyer et al., 2007) and the lower the value, the better the quality of the oil.

Table 6: Physicochemical and other characteristics of Sorrel seed oil.

Parameters	Mean values
<i>Physical properties</i>	
Physical state at 28 °C	Liquid/Yellow-greenish in colour
Refractive index at 25 °C	1.4603
Moisture content (%)	0.065
Specific gravity	0.886 ± 0.026
Viscosity (cP) at 40 °C	15.40
Mean Molecular mass	283.30
p-anisidine value	6.31
Totox number	16.31
<i>Chemical properties</i>	
%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
<i>Other properties</i>	
Cetane number	51.90 ± 0.1
API	28.21
Diesel index	58.19
Aniline point (°F)	121.11

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 by Demirbas (1998) for vegetable oils (37.47 – 40.62 MJ/kg). 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

GC analysis of fatty acids present in the seed oil is shown in Table 7. The results indicated that the oil was highly unsaturated. The major fatty acids present in the seed oil were linoleic (44.39%), oleic (30.06%), palmitic (12.68%); stearic (10.87%) and other trace fatty acids (2.00%). The total unsaturated fatty acid composition of the oil was 74.45%. 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 (Mohamed et al., 2007; Mahmoud et al., 2008; Nyam et al., 2009).

Table 7: Fatty acids compositions of the Sorrel seed oil.

Parameters	Compositions %
Palmitic acids (C16:0)	12.684
Stearic acids (C18:0)	10.865
Oleic acids (C18:1)	30.061
Linoleic acids (C18:2)	44.390
Others	2.000

4. Conclusions

RSM was used 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 22 g, solvent volume 157 ml and extraction time 2 h. Using these optimal factor 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 revealed that the oil is edible and could serve as feedstock for many industrial applications.

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FIGURE LEGENDS

Fig. 1(a-c). Surface plots for solvent extraction of Sorrel seed oil. (a) effect of solvent volume, extraction time and their reciprocal interaction on oil yield. (b) effect of sample weight, extraction time and their reciprocal interaction on oil yield. (c) effect of solvent volume, sample weight and their reciprocal interaction on oil yield.

HIGHLIGHTS

- First report on Sorrel oil extraction optimization using response surface method.
- Average oil content of 17.85% was achieved using the optimal factor values obtained
- Fatty acid profile showed that the oil was highly unsaturated (74.45%).
- The oil quality revealed that it is edible and could have industrial applications.