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Characterization and Optimization of Solvent and Enzymatic Extraction of Okra (*Abelmoschus esculentus*) Oil

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Authors' contributions

Author OOA designed the experiment, supervise the work, carried out statistical evaluations and wrote the manuscript. Author FOI work in the laboratory together with the author ABS. Author ABS also carried out literature reviews.

Original Research Article

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ABSTRACT

Aims: The work seeks to extract oil from Okra (*Abelmoschus esculentus*) using both solvent and enzymatic processes, and to find out which of the processes will be better in terms of quantity and quality
Study Design: Box-Behken model of response surface methodology with three-levels-three-factors was used which produced seventeen experimental runs.
Place and Duration of Study: Experment was carried out in the Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria between March and December, 2012.
Methodology: The solvent extraction was optimized using Box-Behnken model of response surface methodology; while the enzyme assisted aqueous extraction was carried out using ground okra mixed with distilled water at a ratio of 1:6 w/v. The oil obtained from both extraction methods were subjected to gas chromatography for characterization of okra oil.
Results: The results showed that the enzymatic extracted okra oil contained higher

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percentage of unsaturated fatty acid when compared to the solvent-extracted oil, hence better oil quality. Optimized oil yield of 30.4% was obtained at 2.25h, 45g sample mass and 175 mL solvent volume for the solvent extraction process.
Conclusion: Qualitatively, enzymatic extraction was the preferred method while quantitatively, solvent extraction was better.

Keywords: Okra; enzymatic extraction; response surface methodology; characterization; solvent extraction.

1. INTRODUCTION

Okra (Abelmoschus esculentus) is an annual, often cross pollinated important vegetable of the tropical and subtropical areas. It is grown in many parts of world including the Middle East, Africa, Brazil, Turkey and southern states of USA [1]. Okra seeds containing good quality edible oil and high protein content used to complement other protein sources [2].

Several works on enzyme assisted aqueous extraction of oil from oil seeds have been carried out [3,4]. Moreau et al. [5] carried out aqueous enzymatic extraction of corn oil from dried milled corn germ and enzymatic wet milled corn (E-Germ). They were able to achieve aqueous enzymatic oil extraction process that can achieve oil yields of 50–65% from dry milled corn germ and 80–90% from E-Germ. Cooking or drying of the germ is not required in this process, thus, saving energy costs [5]. Also, Abdulkarim et al. [6] extracted oil from the seeds of *Moringa oleifera* using both solvent and enzymatic extraction processes. The solvent-extracted oil has 67.9% oleic acid while the enzyme-extracted oil contained 70.0 %. Further analyses revealed that the extracted oil is highly unsaturated because of the high percentage of oleic acid [6]. Apart from oleic acid, other prominent fatty acids were palmitic (7.8% and 6.8%), stearic (7.6% and 6.5%), and behenic (6.2% and 5.8%) acids for solvent and enzyme-extracted oils, respectively.

Response surface methodology (RSM) is a powerful tool for optimization of chemical reactions or industrial processes [7]. It also help in evaluating the effective factors and in building models to study interaction, and select optimum conditions of variables for a desired response [8].

In this study, okra oil was extracted from okra seed using n-hexane as solvent. The solvent extraction process was optimized using Response Surface Methodology. The enzymatic aqueous extraction was carried out using Cellulase as enzyme. The efficiency of both methods was compared in terms of quality and yield.

2. MATERIALS AND METHODS

2.1 Material Preparation

Okra seeds were purchased from a popular market in Akure, Nigeria. It was washed, sundried, and cleaned to remove foreign materials. The cleaning was done by winnowing and was later oven dried at 50 ^{o}C until constant moisture content was achieved. The size reduction was carried out using an attrition mill.

2.2 Experimental Design of Solvent Extraction Process

In order to optimize the Box-Behnken experimental design, a three-level-three-factors Box-Behnken was employed for this study, which generated 17 experimental runs. This included 6 factorial, 6 axial and 3 central points to provide information regarding the interior of the experimental region, making it possible to evaluate the curvature effect. The factors investigated in this study were mass of the sample (g), solvent volume (mL) and extraction time (h). Factors and their levels for Box-Behnken design are shown in Table 1.

Table 1. Factors and their Levels for Box-Behnken Design

Variable	Symbol	Code levels		
		-1	0	1
Extraction time (h)	A	0.50	2.25	4.0
Mass of sample (g)	B	20	45	70
Solvent volume (ml)	C	50	175	300

2.3 Solvent Extraction of Okra oil

This was carried using a 250 mL soxhlet apparatus on a heating mantle. The solvent used was Hexane. The milled okra seeds were packed in a muslin cloth placed in a thimble of soxhlet extractor. A round bottom flask containing hexane was fixed to the end of the extractor and the condenser was tightly fixed to the bottom end of the extractor. The extractor was heated at 60ºC with the use of an electric mantle. The solvent was vapourised and condensed into the evaporator. The mixture obtained (solvent and oil) moves directly into the round bottom flask. The process continued for the specified time as shown in Table 1. Oil was recovered by distillation process using the same apparatus. The obtained oil was stored in a bottle for further analyses. The extraction parameters were designed as shown in Table 1.

2.4 Statistical Analysis for the Solvent Extraction Process

The data obtained in Table 2 were analyzed using Response Surface Methodology, so as to fit the quadratic polynomial equation generated by the Design-Expert software version 8.0.3.1 (Stat-Ease Inc., Minneapolis, USA).

2.5 Enzyme-assisted Aqueous Extraction

The okra seeds (75 g) were ground and mixed with distilled water at a ratio of 1:6 (w/v). The mixture was then gently boiled for 5 min and allowed to cool down to room temperature. The pH was adjusted to 7.0 (optimal pH for Cellulase) with 0.5 N NaOH. Two percent w/v of Cellulase (9 g) was added and the mixture incubated at 45°C for 24 h in a water bath with constant shaking at 120 rpm. Oil from enzymatic extraction was recovered by centrifugation of the aqueous mixture using a Beckman centrifuge model J2-21M/E at 5000 rpm and 20°C for 20 min to separate the emulsion and the residue. The emulsion was decanted into a separating funnel and allowed to separate into the oil and water layer. The water layer was then drained off to obtain the oil. Residual moisture in the oil was removed by gentle heating according to the work of Aparna et al. [9]. The oil obtained from the extractions was stored at −20°C until it was analyzed.

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

Std	Run	A	B	C	Oil yield (ml)		Residual
					Actual value	Predicted value	
1	14	+1	+1	0	7.00	7.32	-0.32
2	4	+1	0	-1	5.00	5.83	-0.83
3	12	0	-1	-1	11.50	10.68	0.82
4	2	0	0	0	13.70	13.38	0.32
5	17	0	+1	-1	4.50	4.05	0.45
6	7	0	0	0	8.70	7.75	0.95
7	11	-1	-1	0	11.50	12.45	-0.95
8	9	-1	0	-1	9.50	9.95	-0.45
9	5	+1	-1	0	2.00	2.12	-0.12
10	16	0	0	0	2.60	3.87	-1.27
11	1	-1	+1	0	5.00	3.72	1.28
12	3	+1	0	+1	13.00	12.88	0.13
13	8	0	0	0	6.00	6.00	0.000
14	6	0	-1	+1	6.00	6.00	0.000
15	13	0	+1	+1	6.00	6.00	0.000
16	15	-1	0	+1	6.00	6.00	0.000
17	10	0	0	0	6.00	6.00	0.000

Std = standard

In order to correlate the response variable to the independent variable, 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 analysis of variance (ANOVA). The fitted quadratic response model is described by:

$$Y = b_o + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{iJ} X_i^2 + \sum_{i_1<j}^{k} \sum_{j}^{k} b_i X_i X_j + e \quad (1)$$

Where:

Y is the response factor (volume of okra oil), í and j denote linear and quadratic coefficients, respectively. bo is the intercept, bí is the first order model coefficient, k is the number of factors and e is the random error.

2.6 Fatty Acid Profile Determination of Okra oil From Both Extraction Processes

Fatty acid composition was determined using Gas Chromatography (HP 6890 powered with HP Chem Station Rev.A 09.01 [1206] Software). 50 mg of the oil sample was hydrolysed for five minute at 95 oC with 3.4 mL of 0.5 M KOH in dry methanol. The mixture was neutralized using titrimetric method by using 0.7 M HCI and then methylated using 3 mL of 14 % boron triflouride in methanol. The mixture was heated for 5 minutes at 90ºC to achieve complete methylation process. The Fatty Acid Methyl Esters were extracted three times from the mixture with redistilled n- hexane. The hexane extract was concentrated to 1 µl for gas chromatography analysis and 1 µl was injected into the injection port of GC. Carrier gas was Nitrogen, Inlet temperature was 250ºC, column dimension 30m × 0.25mm × 0.25 µm.

Initial temperature is 60ºC, first ramping at 12ºC for 2 min, while second ramping at 15ºC for 8 min. Detector temperature was 320ºC.

3. RESULTS AND DISCUSSION

3.1 Optimization of Okra Oil Extraction via Response Surface Methodology

In this work, the relationship between the response (okra oil yield) and three independent factors (mass of sample, solvent volume and extraction period) was studied in order to optimize the oil yield conditions via the Box-Behnken design with three-level-three-factors (Table 1). Table 2 shows the coded factors considered in this study and the experimental results as well as the predicted values. Design Expert 8.0.3 Trial software was employed to evaluate and determine the coefficients of the full regression model equation and their statistical significance. The final equation in terms of coded factors for the Box-Behnken response surface quadratic model is in equation (2)

$$Y = +47.30 + 10.7A + 1.58B + 3.50C + 2.50AB + 2.00AC - 0.50BC - 14.4712A^2 - 12.33B^2 - 11.32\,C^2 \quad (2)$$

The results of the ANOVA analysis for the response surface model (Eqn. 2) are shown in Table 3. The p-values, p less than 0.05 indicate model terms are significant. In this case, B, C, AC, BC and A^2 are significant model terms (Table 3).

Table 3. ANOVA for Response Surface Quadratic Model

Source	Sum of squares	df	Mean Squares	F-value	P-value (Prob> F)
Model	185.60	9	20.62	20.43	0.0003
A	0.72	1	0.72	0.71	0.4262
B	59.41	1	59.41	58.86	0.0001
C	56.18	1	56.18	55.66	0.0001
AB	4.41	1	4.41	4.37	0.0749
AC	9.61	1	9.61	9.52	0.0177
BC	13.69	1	13.69	13.56	0.0078
A^2	40.46	1	40.46	40.09	0.0004
B^2	0.17	1	0.17	0.17	0.6951
C^2	1.27	1	1.27	1.26	0.2983
Lack of fit	7.07	7	1.01		
Pure Error	0.000	4	0.000		
Cor total	192.67	16			

df = degree of freedom

The R^2 and R^2adj values of 0.9855 and 0.9836 respectively are an indication that the regression model is a good one. The effect of the variables on the oil yield is shown in Figs. 1 to 3.

Fig. 1 showed the effects of extraction time and mass on okra oil yield. The mass of sample had the greater effect on the oil yield than the extraction time. This was also buttressed by the ANOVA result in Table 3 where the effect of sample mass was significant while that of the extraction time was not. Awolu et al. [4] also showed that the effect sample mass was

significant in the extraction of neem oil using solvent extraction process. Also, optimization of the soxhlet extraction of oil from Safou pulp (*Dacryodes Deulis*) showed that maximum oil yield was obtained after 2h [10], while it was at 3h that maximum oil yield was obtained when supercritical carbon dioxide extraction of oil from rapeseed (*Brassica napum L*) [11] as also recorded in this work that optimum oil yield were obtained in 4h. This showed that oil yield is time limit because after optimum yield, the yield reduces

The effect of solvent volume and extraction time on oil yield while sample mass was held constant was shown in Fig. 2. Solvent volume significantly affected the oil yield more than the extraction time as also buttressed by the ANOVA result.

The effect of solvent volume and sample mass on the oil yield was shown in Fig.3. The ANOVA result also showed the combination of both had significant effects on the oil yield. In summary, an optimized oil yield of 13.7 mL was produced at sample weight of 70 g, solvent volume 175 mL and extraction time of 4h.

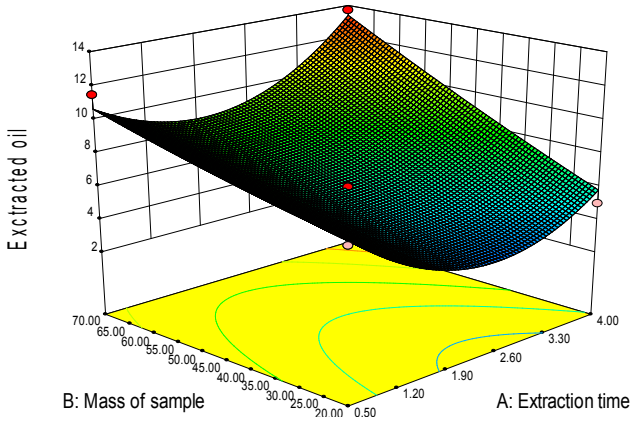

Fig. 1.3 D plot showing the effect of sample mass and extraction time on oil yield

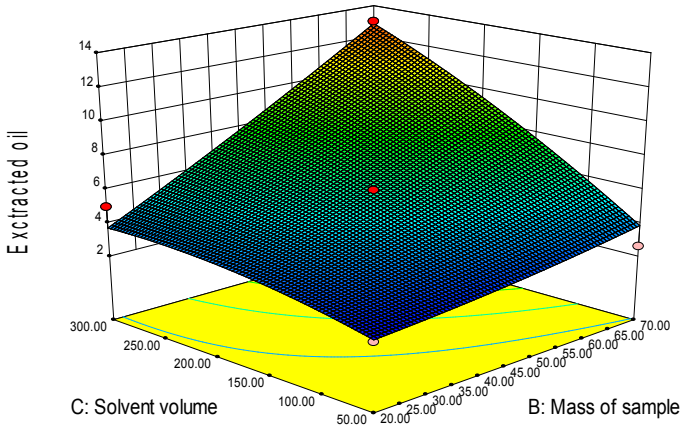

Fig. 2.3 D plot showing the effect of solvent volume and extraction time on oil yield

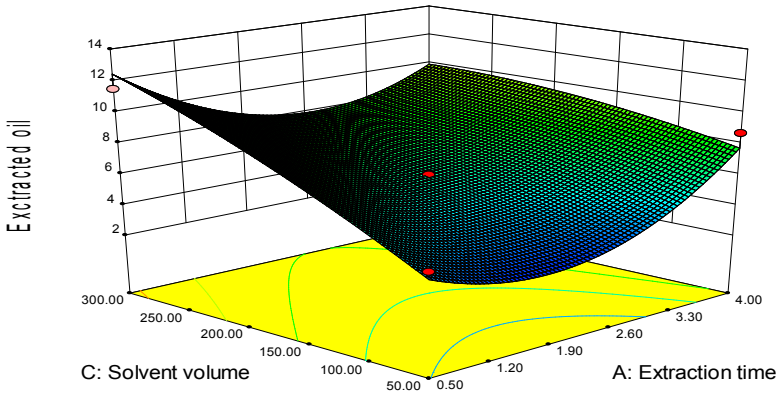

Fig. 3.3 D plot showing the effect of solvent volume and sample mass on oil yield

3.2 Fatty Acid Composition

The fatty acid profile of the enzyme- and solvent-extracted oils, respectively are shown in Table 4. There was no significance difference in the amounts of the major fatty acids in the enzyme- and solvent-extracted oil samples. The fatty acid composition of the oils obtained by enzyme and solvent extraction methods was not affected by the extraction process. The results of the present investigations of fatty acid profiles of extracted oils are in agreement with those of Abdulkarim et al. [6], who showed no significant variation in the major fatty acid contents of the enzyme- and solvent-extracted *Moringa oleifera*, soybean and rapeseed oils respectively.

Table 4. Relative acid composition of fatty acid in Okra seed oils

Fatty Acid composition	Enzyme (%)	Solvent (%)
Myristic Acid (C14:0)	0.20	0.23
Palmitic Acid (C16:0)	21.97	27.52
Palmitoleic Acid (C16:1)	0.98	0.41
Margaric Acid (C17:0)	0.17	0.22
Stearic Acid (C18:0)	3.39	3.80
Oleic Acid (C18:1)	23.74	20.98
Linoleic Acid (C18:2)	46.65	44.14
Linolenic Acid (C18:3)	2.64	2.16
Behenic Acid (C22:0)	0.25	0.52

The high percentage of linoleic acid in the oil makes it desirable in terms of nutrition and high stability cooking and frying oil. It has been demonstrated that a higher dietary intake of “bad” fats (saturated and trans fatty acids) is associated with an increased risk of coronary heart disease caused by high cholesterol levels in the blood [12,13] whereas a higher intake of ‘good’ fats is associated with decreased risk [14]. High linoleic-acid vegetable oils have been found to have enough oxidative stability to be used in demanding applications such as frying [15,16]. High-linoleic oils can be viewed as a healthy alternative to partially hydrogenated vegetable oils.

4. CONCLUSION

Okra seed oil has the potential to become a new source of high-linoleic acid oil. Enzymatic extraction of okra oil using cellulase will make it possible. Advantages of using solvent extraction include the production of higher yield oil as well as relative low operating cost. The use of enzymes in oil extraction is also encouraging as it had a higher yield of unsaturated fatty acids which have nutritional advantages than that of solvent extraction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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