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Optimization of Aqueous Extraction Conditions of Unrefined Shea Butter Using Response Surface Methodology

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Abstract The optimum condition for the extraction of unrefined Shea butter via (aqueous) traditional extraction method was determined using response surface methodology (RSM). A central composite design (CCD) was used to investigate the effect of three independent variables, namely- roasting time (min), roasting temperature (°C) and clarification time (min) on the responses, oil yield (%), peroxide value (meq/kg), free fatty acid (%) and unsaponifiable matter (%). The CCD consisted of 8 factors, 6 axial points and 5 central points to give total of 19 experimental points. Data were analyzed using design expert 7 software. Second order polynomial terms were used to predict the responses. The result indicated that oil yield, unsaponifiable and peroxide value were optimized with all having R^2 > 80% except for free fatty acid which was not a significant response ($p = 0.58$). The optimal extraction conditions for optimizing the responses were 106°C, 120 min and 90 min of roasting temperature, roasting time and clarification time respectively. Predicted response values were in agreement with experimental values indicating the success of RSM in optimizing the quality parameters of Shea butter. This result also reflects that it is more suitable to roast Shea kernel at low temperature for longer duration than at higher temperature for short time which is the current practice in Nigeria.

Keywords: *Shea butter, response surface methodology, extraction condition, quality, optimization*

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1. Introduction

Shea tree (*Vitellaria paradoxa*) commonly known as "tree of life" belongs to the Sapotaceae family. It is indigenous to the savanna belt of sub-Sahara Africa cutting across 19 countries including Nigeria. The shea tree (*Vitellaria paradoxa*) produces fruits with nuts from which enclosed kernels are used in the extraction of shea butter. In West Africa, shea butter has been utilized for almost 3,000 years as a cooking oil and oil for medicinal and cosmetic purposes [1]. The major edge of shea butter over other fats is its high percentage of unsaponifiables (4-16 %), mostly triterpene alcohol and phytosterols compared to other oils and fats (~2 %) [2]. The extraction and marketing of shea butter is mostly carried out by women and children, making the business a target for women empowerment and gender equity thereby playing a significant role in economic development and poverty alleviation in Africa.

In Nigeria, Shea butter price is very low compared to other exotic fats. The low price is as a result of poor quality leading to low international market value. The situation is no different from other African shea belt [3,4]. The low price of shea butter is a serious threat to the biodiversity of this indigenous crop because it encourages falling of shea trees for more lucrative coal business. The solution is to improve the quality of shea butter as well as value addition and usage diversification. Optimizing the potential of shea butter is proportional to upgrading the livelihood of women and children in the shea belt region [3,4,5]. At present, most obvious challenge is: awful odour which has been associated with poor postharvest practices leading to high peroxide value (PV) and subsequently poor consumer acceptability [6], forcing cosmetic industries especially in the US and Europe to refine the butter before usage. This act of refining strips the butter of its bioactive component that makes it unique.

The odour of Shea butter arises due to extreme heat utilized during the traditional extraction of Shea butter leading to formation of peroxides and subsequently secondary oxidation products during storage [7]. From the code of practice currently implemented in Nigeria Shea butter extraction, several unit operations are critical in achieving quality shea butter especially optimization of unsaponifiables and consumer acceptability (odour) [8]. Some of these critical control points have been partially studied such as nut drying mode and duration, roasting time [3] and sorting [9]. However, time and temperature used during clarification is yet to be optimised for effects on unsaponifiables and formation or acceleration of odour active compounds that may affects sensory. It is also important to note that most the works carried out on traditional method shea butter have failed to look at the effect of interaction among the critical control points of postharvest factors and impact on quality. There is need to use a holistic approach i.e. Design of experiment to determine interactions.

Response Surface Methodology (RSM) is a statistical tool, originally described by [10]. It is used to design experiments using quantitative data obtained to solve multivariate analysis [11]. RSM enables evaluation of the effects of several factors (independent variables) and their interactions on responses (dependent variables). A central composite design (CCD) is one of the statistical/mathematical techniques in RSM designed to determine optimum conditions of processes. CCD uses the response surfaces covered in the experimental design to produce efficient and effective optimum conditions [12]. RSM have successfully been used for developing, improving and optimizing processes in the food industry [12-20] including Fats and oils industry [21,22,23,24] among others. RSM have been used to optimize Shea kernel pretreatment and Shea butter extraction [6,25-30]. These optimizations studies are mostly centered on sohxlet and screw press extraction methods. There is need to optimize the traditional method of extracting Shea butter in Nigeria to ensure consistent quality [31]. This project therefore seeks to vary the critical extraction steps in the traditional (aqueous) method using RSM to create a model that optimizes the yield, unsaponifiables and odour of shea butter produced in Nigeria.

2. Materials and Methods

2.1. Materials

De-hulled Shea Kernel was purchased from Bida, Niger State. Kernel was sorted, washed and sundried for 1 hour. All reagents used were of analytical grade.

2.2. Extraction of Shea Butter

Shea butter was extracted using the traditional aqueous method employed in Nigeria [8]. Shea kernel was washed with warm water and sundried for 1 hour. Kernel was weighed (1 kg), crushed using a mortar and roasted at the appropriate temperature and time. Roasted kernel was allowed to cool and milled to a fine paste using a hand held manual grinder. Shea paste was kneaded using cold and hot water until the coluor changed from dark brown to creamy. Crude fat was collected from the top by adding cold water to paste. Crude Shea butter was clarified by boiling at appropriate time, cooled, filtered and kept in air tight container at 4°C.

2.3. Quality Parameters of Shea Butter

The yield was determined based on percentage of weight of oil produced to the weight of kernel used.

The equation is given as;

$$\%\,Yield = \frac{Weight\ of\ Oil}{Weight\ of\ kernel} x100.$$

Unsopiniable matter, peroxide value (PV) and free fatty acid (FFA) were determined according to MPOB p2.7 [32], IUPAC 2.501 [33] and AOACS Ca 5a-40 [34] respectively.

Table 1. Coded and actual values of independent variables used for optimization

Independent Variale	Unit	Symbol	Coded level				
			-1	0	+1	- α	+ α
Roasting time	min	X_1	10	65	120	0	137
Roasting temperature	°C	X_2	100	155	210	82	227
Clarification time	min	X_3	10	50	90	0	102

Table 2. Matrix of experimental central composite design (CCD) for Extraction of Shea butter

Treatment trial	Roasting time (min) X_1 (x_1)	Roasting temperature (°C) X_2(x_2)	Clarification time (min) X_3(x_3)
12	65(0)	227(+α)	50(0)
10	137(+α)	155(0)	50(0)
16	65(0)	155(0)	50(0)
18	65(0)	155(0)	50(0)
11	65(0)	82(-α)	50(0)
17	65(0)	155(0)	50(0)
2	120(+1)	100(-1)	10(-1)
14	65(0)	155(0)	102(+α)
19	65(0)	155(0)	50(0)
4	120(+1)	210(+1)	10(-1)
1	10(-1)	100(-1)	10(-1)
5	10(-1)	100(-1)	90(+1)
7	10(-1)	210(+1)	90(+1)
3	10(-1)	210(+1)	10(-1)
15	65(0)	155(0)	50(0)
8	120(+1)	210(+1)	90(+1)
6	120(+1)	100(-1)	90(+1)
13	65(0)	155(0)	0(-α)
9	0(-α)	155(0)	50(0)

2.3. Experimental Design

The Design of experiment and statistical analysis for the optimization of extraction conditions of Shea butter were determined using Design expert 7 (Stat-Ease, Inc. Minneapolis, MN, USA). The experiment was based on a central composite design (CCD) Cochran & Cox, (1957). Quadratic model was employed to study the interactive effect of three independent variables i.e. roasting time (X_1), roasting temperature (X_2) and clarification time (X_3). The effects of experimental error on the responses due to extraneous factors were minimized by randomizing the order of experiments.

Each independent variable had five levels which were -1, 0, +1, - α, and + α. A total of 19 combinations including five replicates of the center point and two extreme value; lower limit (-α) and upper limit (+α) were carried out (Table 1). Four dependent variables (y) were measured; oil yield (y1), unsaponifiable matter (y2), PV (y3), and FFA (y4). These dependent variables were expressed individually as a function of the independent variables known as response function. The variance for each factor assessed was partitioned into linear, quadratic and interactive components. Models were represented using the second order polynomial and regression coefficients. The second order polynomial used was as follows:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$

where b_0 is the regression coefficients intercept for the polynomial (constant term), b_1, b_2, b_3 represent linear coefficient, b_{11}, b_{22} and b_{33} (quadratic coefficient), and b_{12}, b_{13} and b_{23} (interactive coefficient) respectively. X_1, X_2 and X_3 are the independent variables.

The Design-expert 7 software was used to determine significance of all terms in the polynomial functions using F-value at a probability (p value) of 0.0.1. All significant model exhibited a lack of fit (p>0.05). Three-dimensional (3D) plots were generated by keeping one variable constant at the center point and varying the other variables within the experimental range.

2.4. Verification of Model

Optimal condition for the aqueous (traditional) extraction of Shea butter was based on extraction temperature, extraction time and clarification time. Model was generated using the predictive equations of RSM. Oil yield, PV and unsaponifiable matter were significant models and they were optimized using RSM. The predicted values were used to extract Shea butter (experimental values). Experimental values were compared to predictive values in terms of oil yield, PV and unsaponifiable matter using SPSS version 23 (IBM, Corp., NY) to determine statistical similarity of the independent sample T test in order to determine the validity of the model (Table 5).

3. Results and Discussions

The experimental value and analysis of variance for three response variables i.e. oil yield, unsaponifiable matter and PV under the different extraction condition are shown in Table 3 and Table 4 respectively. The p value obtained from the responses showed that RSM developed for all the models were adequate except for FFA. Oil yield, unsaponifiable matter and PV had R^2 values of 87%, 82% and 89% respectively.

3.1. FFA

The inability of RSM to optimize hydrolytic rancidity (FFA) indicates that hydrolytic rancidity might not be a function of extraction condition. Aculey, Lowor, Kumi, & Assuah [35] reported significant increase in FFA during heaping of Shea fruit beyond 3 days and improper drying of perboiled nuts. Heating of de-hulled Shea nut (nut perboiling) have been described as a post harvest CCP to inactivate lipase enzyme responsible for hydrolytic rancidity [36]. This result reinforce that in order to produce Shea butter with acceptable FFA, quality kernel must be used in the extraction process, it also indicate that roasting condition and clarification temperature does not significantly affect hydrolytic rancidity in fats and oils. This finding is in line with [9] study were germination of Shea fruit for 9 days led to 7-folds increase in FFA in Shea butter. The high value of FFA percentage observed (16.12-24.17) also indicates that the Shea kernel may not have undergone proper pretreatment. Although, this result is not in line with [27]. In the study, RSM optimization of Shea kernel roasting conditions showed a significant positive effect with roasting temperature and a significant negative effect with roasting time. There is need to further study effect of confounding factors such as initial FFA content of Shea kernel, effect of sorting e.t.c.

Table 3. Experimental data for the responses under different extraction conditions shown in Table 2

Treatment trial	Oil yield (%)	Unsaponifiable content (%)	PV (meq/kg)	FFA (%)
12	7.65	3.43	7.8	19.74
10	21.7	2.52	7	20.35
16	16.55	4.98	5.4	25.78
18	13	5.7	5.4	20.95
11	15.19	8.35	8.4	22.16
17	12.64	5.45	5.4	20.95
2	25.26	4.6	12.6	17.72
14	18.69	23.4	6.2	24.17
19	12.97	4.63	6.8	18.53
4	12.85	3.49	10	17.73
1	23.15	5.45	12.8	23.77
5	20.4	3.99	7.8	17.32
7	16.66	4.51	8	18.13
3	21.4	9.86	8.6	18.94
15	16.9	9.33	5.8	18.93
8	9.02	4.22	4.6	20.95
6	23.41	26.71	9.6	16.12
13	19.73	5.35	11.4	17.32
9	16.7	5.14	5.6	18.13

3.2. Oil Yield

Optimizing the yield of agricultural produce including fats and oil is one of the primary goals of any processor. Increased oil yield of Shea butter equates improved quality of life for women and children in Shea belt who are responsible for the production and marketing of Shea butter. The process of producing Shea butter is a tedious task which requires optimization. The yield of Shea butter ranged from 7.65% to 25.26%. The yield of 25.6% is in close range with earlier study on RSM (31.32%) [6]. The slight increase may be as a result of hexane maceration extraction techniques employed. Honfo, Akissoe, Linnemann, Soumanou, & Van Boekel [37] reported higher yield of Shea butter extracted with hexane compared to traditional extraction and other methods such as superficial CO_2. RIGHT SHEA, [38] reported that Shea butter yield was largely influenced by the kneading capacity of the processor. In this study, kneading was kept constant and one person was involved in the kneading of all the treatment trials to prevent cofounding effect. In Nigeria most persons involved in Shea butter production are experienced in kneading techniques, so kneading is not considered a CCP.

In this experiment, roasting temperature was the most significant factor that influenced oil yield with p <0.0001 for linear term and a negative effect. The interactive term and quadratic term were also significant with p value of <0.01 and <0.05 respectively, both also had a negative effect in the model. Clarification time and roasting time had no linear effect on yield. However, their quadratic term had significant negative effect (<0.05). The p value of 0.0004 and R^2 0.8726 showed that RSM adequately optimized the oil yield of Shea butter extracted using traditional method. From Figure 1, it is evident that at constant clarification time, oil yield increases with decrease in roasting temperature. This finding is in agreement with [7]. However, this findings is not in line with the current code of practice for the traditional extraction of Shea butter in Nigeria were roasting temperature is not controlled rather roasting time is controlled [39]. There is therefore need to address the roasting technology used in producing Shea butter in Nigeria.

Table 4. Regression coefficients, R^2, and p values for responses under different extraction condition

Regression coefficient	Oil Yield (%)	Unsaponifiable matter (%)	PV (meq/kg)
b_0	1.15	5.09	5.99
b_1	-0.021	1.03	0.38
b_2	-0.11***	-2.19	-1.29***
b_3	-0.033	3.47**	-2.03***
b_{12}	-0.073**	-3.57*	0.011
b_{13}	-2.884E-3	3.71**	-0.093
b_{23}	-0.022	-3.16*	-0.13
b_{11}	0.081**	-1.91	0.18
b_{22}	-0.061*	-0.64	1.26***
b_{33}	0.083**	3.88*	1.66***
R^2	0.8726	0.7881	0.8912
p value	0.0004***	0.0051**	0.0002***

Note: Subscripts: 1 = roasting time; 2 = roasting temperature; 3 = clarification time.
* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

3.3. Unsaponifiable Matter

The cosmetics and pharmaceutical usage of Shea butter is mostly as result of its high percent of unsaponifiable fraction [40]. The amount of unsaponifiable in fats and oils is directly proportional to antioxidant activity and its medicinal properties [37,41] Nahm [42] reported the use of fractionation and concentration of olein fraction to increase unsaponifiables in Shea butter. Using this technique oil loss may occur. Based on the CCPs identified and optimized using RSM, clarification was a significant factor in optimizing unsaponifiable matter of Shea butter. The linear, interactive and quadratic terms were all significant with p values <0.01, <0.01 and <0.05 respectively. These terms all had positive effects except for the interaction of roasting condition, roasting temperature and clarification time which showed a negative effect. The positive effect observed implies that longer clarification time increased percentage yield of unsaponifiable fraction. Figure 2 clearly shows the influence of clarification time on unsaponifiable matter at constant roasting time of 65 minutes. [43,44] implied that use of heating on coconut oil-water emulsion during processing increased antioxidant activity of coconut oil, as long as the temperature used was not high [43]. The negative interactive effect of b_{23} observed also suggests that lower temperature is required to optimize unsaponifiable content of Shea butter. The model showed a good fit with R^2 and p value of 0.8231 and 0.0051 respectively. The linear and quadratic terms of both roasting temperature and time were not significant contributors to amount of unsaponifiables in Shea butter. However, their interactive term was significant at p <0.005 with a negative effect.

3.4. Peroxide Value

Peroxide value is one of the most important quality parameter in fats and oils. It gives an indication of oxidation in fats and oil i.e. Formation of off flavour and subsequently, it is the most significant determinant of consumer acceptability of Shea butter. Linear terms of roasting temperature and clarification time both showed a negative significant effect (<0.0001) on PV. Their quadratic terms were also significant with p value < 0.0001 and positive effect (Table 4). The model was able to explain 89% of the variation in PV. The model showed a good fit with p value less than 0.0001.The negative effect observed for both X_2 and X_3 indicates that decrease in PV was observed at prolonged roasting and high temperature. This reason for this phenomenon was explained by [27] to be due to conversion of peroxides into secondary oxidation products such as hydrocarbons, ketones, aldehydes, furans which cannot be measured by PV. However, the higher order quadratic terms indicated an increase in peroxide value when roasting temperature was high and Shea butter clarified for longer period. This observation is in agreement with existing literature [7,41,42,45,46].

3.5. Overall Optimal Conditions

The optimal conditions covering all the significant responses (oilyield, unsaponifiable matter and PV) were determined using RSM. Oil yield and unsaponifiable matter were maximized, oil yield having 5+ importance and unsaponifiable matter 3+ importance. PV was minimized with 3+ importance. Optimal values of 24.42%, 25.08% and 7.76% were discovered for y_1, y_2 and y_3 respectively under the optimized extraction condition (Figure 4).

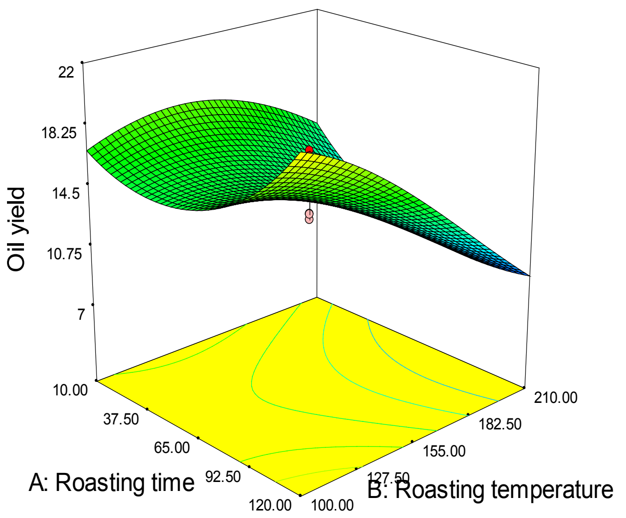

Figure 1. 3-D plot for oil yield of Shea butter as a function of roasting condition at 50min clarification time

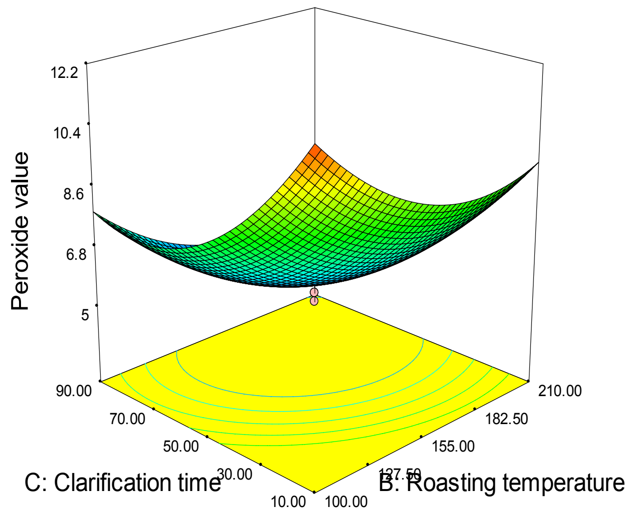

Figure 3. 3-D plot for PV of Shea butter as a function of roasting temperature and clarification time at 65min roasting time

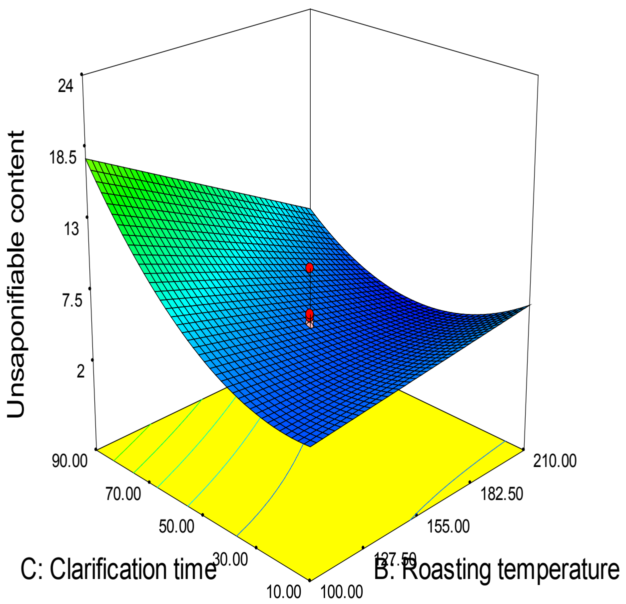

Figure 2. 3-D plot for unsaponifiable matter of Shea butter as a function of roasting temperature and clarification time at 65min roasting time

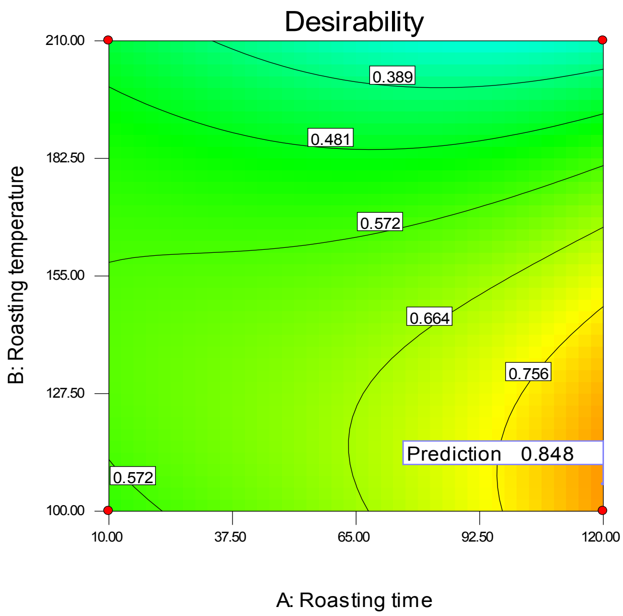

Figure 4. Contour plots for optimum combine condition as function of roasting temperature and roasting time at 90 minutes of clarification.

Table 5. Comparison of predicted and experimental values for the response variables

	X_1	X_2	X_3	Oil yield (%)	Unsaponifiable content (%)	PV (meq/kg)
Predicted	120	106	90	24.42	25.08	7.76
Experiment	120	106± 1°C	90	24.58	18.89	7.98

4. Conclusion

RSM was successfully used to establish the optimum extraction condition (roasting time, roasting temperature and clarification time) for traditional extraction of Shea butter. Base on the second order quadratic equation and 3-D plots, the optimum set of operating variables can be obtained. Roasting temperature influenced the responses to a great extent. Oil yield, unsaponifiable matter and PV were significantly affected by extraction conditions while FFA was not a significant response valuable.

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