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# **Optimization of Thermally Processed Bambara Groundnut Flour Nutrients using Response Surface Methodology**

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*Authors' contributions*

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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# **ABSTRACT**

White/cream colourbambara groundnut (BGN) seed (*Vigna subterranean L*.) Was grouped into 12 portions and subjected to different processing methods by soaking in water at room temperature  $(28^{\circ}$ C) for 12-48h and further boiling for different times  $(30 - 60)$ min). The soaked-boiled seeds were dried and milled into flours. Proximate composition and functional properties of these processed flours were determined and significant (*P = .05*) differences among the samples recorded. Combination of soaking and boiling of the seeds for different times resulted to irregular functional properties and nutrient losses of the BGN. Optimization of the responses showed that the seed soaked for 12h and further boiled for 46.26min would yield flour with optimum nutrient properties, while soaking for 12h before boiling for 9.96 min would produce flour with optimum functional properties.

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*Keywords: Proximate; functional properties; soaking; boiling; bambara groundnut flour.*

# **1. INTRODUCTION**

The cultivated Bambara groundnut (*Vigna subterranean L*.) is a pulse with subterranean fruit set, and is currently being cultivated by small farm holders [1,2]. The Bambara groundnut (*Vigna subterranean L*.) belongs to the plantea of the family of *fabaceae*and subfamily of *faboidea,* 

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[3]. Because of its composition, BGN produces almost a balanced diet with a reported good balance of essential amino acid; its lysine (6.8%) and methionine (1.3%), [4-7]. Due to its high protein score of 80% and predicted protein energy ratio (P-PER) of above 2.00. BGN is regarded as of high protein quality, [8]. Its other nutrients include 60-65% carbohydrate, [7] 6.34% to 6.85% crude fiber [3], [9] and is also credited with good quantities of micronutrient and vitamins. BGN has also historically been part of inexpensive meals throughout the world [10].

Bambara groundnut is regarded as the third most important legume crop after groundnuts *Arachis hypogeal* and cowpea, but it is still one of the lesser utilized and underexploited legume. Bambara groundnut has attracted very little scientific research and industrial utility, despite its nutritional and other reported potentials [11], therefore the objective of this study is to determine combination the better combination of two process variables: soaking time  $(X_1)$  and boiling time  $(X_2)$  that will give optimum quality of instant BGN flour, in terms of nutrition and functionality. The information generated from this study is expected prediction on its possible use in industrial processes. This will lead to diversification and utilization in addition to provision of enhanced food security.

Modeling and optimization are vital in product development. Also Response Surface Methodology (RSM) has been frequently used in optimization of food process variables [12]. Optimization of process conditions is one of the most critical stages in the development of any efficient and economic bioprocess, [13]. RSM is a mathematical and statistical technique for designing experiments, building models, evaluating the relative significance of several independent variables, in order to determine the optimum conditions for desirable responses, [14]. Therefore, the objective of this study is to determine through RSM the best combination of soaking time  $(X_1)$  and boiling time  $(X_2)$  that will give optimum quality of bambara groundnut (BGN) flour for nutrition and other processes.

# **2. MATERIALS AND METHODS**

#### **2.1 Source of Materials**

White/cream colour dry BGN seeds were procured from the "Akwata" section of Ogbete market, Enugu, Enugu State, Nigeria, through "999 Grains" retail shop, while the Giffion Laboratories, Umuahia supplied the reagents and used their standard equipment for the analysis.

#### **2.1.1 Experimental design**

A Response Surface Methodology (RSM) involving two process variables at three levels was applied to study the effect of soaking times  $(X_1)$  and boiling time  $(X_2)$  on the resultant quality of the BGN flours. The proximate and the functional properties of the flours were the response variables measured.

Table 1 shows 2-factor-3- levels Face Centered Central Composite design (FCCCD) experimental matrix of interaction effect of soaking time (h) and boiling time (min) employed to produce and evaluate the proximate and functional properties of Bambara ground nut flour. Table 2 shows the design key used to generate Table 1.

#### **2.2 Preparation of Raw Materials**

The BGN seeds were cleaned through dry cleaning process to remove extraneous materials such as pieces of stones, strings, pieces of metals and spoilt seeds. The BGN seeds were washed with potable water and then divided into twelve portions. Each portion was subjected to soaking and boiling as shown in Table 1. The categories of soaked and boiled samples were dried and then hammer milled to produce the BGN flours.

These 12 flour samples were later analyzed for proximate composition and functional properties. The results were presented in Tables 3 - 6 respectively.

#### **2.3 Analysis**

#### **2.3.1 Proximate composition**

The official method of [15] was used to determine the moisture, crude protein, lipid, ash and crude fibre contents of the Bambara groundnut flours. The carbohydrate was obtained by subtracting the summation of protein, fat, ash, crude fibre and moisture from 100.

#### **2.3.2 Functional properties**

Water absorption and oil absorption capacities; foam capacity and stability, gelation temperature, solubility and swelling power of the flours were determined using [16].

Run	Soaking time (h)	<b>Boiling time (min)</b>			
	A	В			
	$12(-1.00)$	$30(-1.00)$			
	$12(-1.00)$	45 (0.00)			
3	$12(-1.00)$	45 (0.00)			
4	$12(-1.00)$	60 (1.00)			
5	30(0.00)	$30(-1.00)$			
6	30(0.00)	45 (0.00)			
	30(0.00)	45 (0.00)			
8	30(0.00)	60 (1.00)			
9	48 (1.00)	$30(-1.00)$			
10	48 (1.00)	$30(-1.00)$			
11	48 (1.00)	45 (0.00)			
12	48 (1.00)	60 (1.00)			

**Table 1. Experimental Design with both coded and actual values**

*Values in the brackets are the codes while those outside are the actual*

**Table 2. Codes and actual levels of the independent variable (or conditions) and their corresponding levels and times**

<b>Independent Variables</b>	<b>Symbols</b>	<b>Coded levels</b>			
Soaking time		12h	30h	48h	
Boiling time	∧ว	30 <sub>min</sub>	45min	60 <sub>min</sub>	

#### **2.4 Statistical Analysis**

To determine the mean differences between treatments, the triplicate data were subjected to One Way Analysis of Variance (ANOVA). Duncan's multiple range tests was used to separate the means where differences existed using IBM SPSS, version 22. Design Expert version 12 was used to design the experiments and replications covering the full design of two factors were used for building quadratic models, which produced reasonable amount of information for testing the conditions for model adequacy and well suited for process optimization, [14]. Polynomial equations were determined and simplified based on the influence of the factors on the final response. The experimental data obtained from the model experiments can be represented in the form of the equation:

$$
Y = a + b_1 X_1 + b_2 X_2 + b_{12} X_1 Y_2 + X b_1 X_1^2 + b_2 X_2^2 + e \dots \dots \dots \dots \tag{1}
$$

A quadratic polynomial regression model was assumed for predicting various responses Montgomery, (2013). The model proposed for each response Y was represented as:

Y=  
\n
$$
\beta_0 \sum_{i=1}^3 + \beta_{i \times 1} + \sum_{i=1}^3 \beta_2 X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} \times
$$
  
\n $X_i^2$  (2)

Regression models and response surface plots were developed after examining the ANOVA of various response variables to select the terms whose p-value are < 0.05, significant model, high R<sup>2</sup>adj and non-significant lack of fit. The coefficient of determinations of responses was used to fit the models for only the responses that qualified. Table 3 shows the proximate composition of the samples

#### **3. RESULTS AND DISCUSSION**

The results obtained from the laboratory analysis of the BGN flours, ANOVA analysis and the numeric optimization solutions for the study were presented on Table 3 to 6. Tables 3 and 6 reveal that there were significant differences in the results obtained in both proximate and functional properties of the flours as a result of the varying treatments on the BGN seeds' flours. The regression models for moisture, ash, fat, water absorption capacity among other parameters yielded positive co-efficient signifying direct relationship between soaking and boiling times and their interactions. However these interactions were lower for carbohydrate and solubility properties of the flours. Optimization of the responses showed that 12h soaking and further boiling time of 46.26min resulted in BGN flour with optimum nutrients while 12h soaking and further boiling for 9.96min yielded BGN flours with optimum functional properties.





Values are mean ± standard deviation of replicated determinations. Mean values in the same column followed by different letters are significantly different (P=0.05)<br> $X_1$  = Soaking time;  $X_2$  = Boiling time, C. fibre = C

Table 4 shows the Summary of ANOVA and Coefficient Estimate of the proximate composition of the Bambara groundnut flour for the terms that showed significant model, nonsignificant lack of fit and P-value < 0.05. The ideal regression equation showing the response variables (proximate parameters) as a function of the independent (process variables) can be represented in Eq. 1.

$$
Y = \beta_{0+} \beta_{1} A + \beta_{2} B + \beta_{12} A B + \beta_{11} A^{2} + \beta_{22} B^{2} + \epsilon
$$
\n(1)

Where: *Y=* response variable, *β0=*intercept, *A*= soaking time,  $B =$  boiling time,  $\beta_1 \beta_2 \beta_{12} \beta_{11} \beta_{22} =$ coefficients of the linear, interaction and square of *A* and *B* respectively, *ε* = estimated error. ε is often neglected and not factored into the equation

#### **3.1 Moisture Content**

The regression model for the moisture content of the flour samples is presented in Eq.2 and Fig. 1 as the contour plot. It was observed from equation 1 and Fig. 1 that reducing the soaking time, while increasing the boiling time and their interaction, increased the moisture content of the flour. This is because soaking time exhibited positive coefficient, while boiling time and interaction of soaking time and boiling time had positive coefficients. The positive coefficients signify direct relationship between the soaking and boiling times and their interactions with the moisture content of the flour, while the negative coefficients indicate an inverse relationship between the process variables and the moisture content of the flour samples.  $R^2$ adj was 57.28% which is high, showing that the 57.28% change in the moisture content was caused by soaking and boiling times and their interactions.

Moisture = 6.60 -0.6137A +0.3082B +0.4197AB (2)

#### **3.2 Ash Content**

The regression model for the ash content of the flour samples is presented in Eq.3, while Fig. 2 shows the effect of soaking time and boiling time on the ash content of flour samples. Increasing the soaking time reduces the ash contentment of the flour since soaking time has negative coefficient. The square of soaking time however had a slight increase in the ash contentment of the flour shown in the 3D surface plot (Fig. 2) and as also shown in equation  $3. R^2$ adj was 66.48% which is high, and the coefficient of variation (% CV) wasn't high (12.31). This shows that the model explains 66.48% of the effect of the soaking and boiling times on the ash content of the flour samples, while the difference in the percentage (33.52%) could come from extraneous variables not factored into the design.

$$
Ash = 2.20-0.4688A+0.4063A2
$$
 (3)

#### **3.3 Fat Content**

The regression model of the fat content of the samples is presented in Eq. 4 and Fig. 3.

Fat = 
$$
5.84 + 2.42A
$$
 -0.0895B -0.9471AB +0.9213A<sup>2</sup> -0.0421B<sup>2</sup>

\n(4)

The model was significant with a satisfactory adjusted coefficient of determination,  $R^2_{adj}$  of 0.7857 (78.57%) as shown in Table 2. The high coefficient of determination showed excellent correlation between the independent variables (soaking time and boiling time) and the fat content. This is an indication that the response (fat) model is adequate, and can explain 78.57% of the total variability in the fat content of the Bambara ground nut flour samples.

**Table 4. Summary of ANOVA and Coefficient Estimate of the proximate composition of the Bambara groundnut flour for the terms that showed significant model, insignificant lack of fit and p-value < 0.05**

Term	<b>Coefficient</b>	<b>Moisture</b>	Ash	Fat	<b>Crude Fibre</b>	Carbohydrate
n Intercept	$\beta_0$	6.60	2.20	5.84	0.7266	81.12
(A)	$\beta_1$	$-0.6137$	$-0.4688$	2.42	0.1929	1.13
(B)	$\beta_{2}$	0.3082		$-0.0895$	$-0.3225$	
(AB) (A <sup>2</sup> )	$\beta_{12}$	0.4197		$-0.9471$	0.1329	
	$\beta_{11}$		0.4063	0.9213	0.0137	$-4.25$
$(\mathsf{B}^2)$	$\beta_{22}$	-		$-0.0421$	0.2055	
$R^2$ adj.		0.5728	0.6648	0.7857	0.6382	0.4710
$%$ CV		7.08	12.31	16.14	23.72	2.84

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**Fig. 1. Effect of soaking time and boiling time on the moisture content of the Bambara flour samples**



**Fig. 2. Effect of soaking time and boiling time on the ash content of soaked and boiled BGN flour**



**Fig. 3. Contour plot for the effect of soaking time and boiling time on the fat content of BGN flour**

## **3.4 Crude Fiber Content**

The regression model of the crude fibre content of the flour samples is shown Eq.5 and Fig.4

Crude fiber = 0.7266+0.1929A+0.3225B-0.1329AB-0.0137A² +0.2055B² (5)

Increasing both soaking time and boiling time increases the crude fibre content of the bambra ground nut flour samples. However, the interaction of the two independent variables and the square of soaking time reduced the crude fiber content as evidenced in equation 5 and Fig.4.

#### **3.5 Carbohydrate Content**

The regression model for the carbohydrate content is shown in Eq. 6 and Fig. 5 respectively.

Carbohydrate =  $81.12 + 1.13A - 4.25A^2$  (6)

The carbohydrate exhibited significant model. However, the adjusted coefficient of determination  $(R^2$ adj.) was low  $(0.4710)$ suggesting that only 47 % of the changes in carbohydrate were caused by the process variables and their interaction in the experiment. From Eq. 5, increasing the soaking time increased the carbohydrate content, while the square term reduced the carbohydrate also evidenced in Fig. 5 which shows the 3D surface plot of the carbohydrate content of soaked-boiled Bambara ground nut flour samples.  $R^2$ adj., was used in this work instead of  $R^2$ .  $R^2$ adj., is better for assessing model adequacy, than  $R^2$  since inclusion of more independent Variables in an experiment will surely increase  $R^2$ . [17]. However, if unnecessary independent variables are included in an experiment  $R^2$  increases while R<sup>2</sup>adj. values decrease.



**Fig. 4. Contour plot for the effect of soaking time (A) and boiling time (B) on the crude fibre content of soaked and boiled Bambara ground nut flour samples**



**Fig. 5. 3D surface plot of the carbohydrate content of soaked-boiled Bambara ground nut flour**

#### **3.6 Numeric Optimization for the Proximate Composition**

Table 5 shows the generated Numerical<br>Optimization solution of the proximate Optimization solution of the composition of soaked-boiled Bambara ground nut flour samples. The main criteria for constraints optimization of process parameters for the proximate composition were minimum possible soaking and minimum boiling time which generated the solution in Table 5 with desirability of 61.6%. The percentage desirability is high and acceptable. However, desirability of 100% is the most ideal if it could be obtained. It shows that if the selected critical values of 12 h of soaking and 46.26 min boiling are employed in the production of Bambara ground nut flour, that the flour would exhibit proximate composition of 7.15, 3.07, 4.79, 0.83 and 75.738 for moisture, ash, fat, crude fibre and carbohydrate respectively, with the desirability of 0.616 (62%).

# **3.7 Validation**

An experiment was further carried out using the optimized process parameters (12h of soaking and 46min of boiling) and the proximate composition of the flour produced was determined. The average result obtained showed the following properties; moisture, ash, fat, crude fibre and carbohydrate to have the respective mean values of 7.42%, 3.11%, 4.68%, 0.92% and 75.63%.

The mean of the triplicate result of the functional properties of the processed (soaked and boiled) BGN flours shown in Table 6 reveals that there was significant  $(P = .05)$  differences in the values obtained. The water absorption capacity (WAC) steadily increased (in a general term) from 0.665ml/g, the least value, to 2.70ml/g due to 30 h soaking and, 60 min boiling of sample H, and the 48 h soaking and, the 30min boiling of sample J. 12 h soaking yielded 38.46 % increase in WAC and 508.88 % relatively high increase due to 30 h soaking, while 48 h soaking yielded 130.77 % increase. A combination of soaking and boiling generally enhanced the WAC.

Table 7 shows the Summary of ANOVA and coefficient estimate of the functional properties of the Bambara groundnut flour for the terms that showed significant model, insignificant lack of fit and p-value < 0.05. The ideal regression equation showing the response variables (functional properties) as a function of the independent (process variables) can be represented in Eq.7

$$
Y = \beta_{0+} \beta_{1} A + \beta_{2} B + \beta_{12} A B + \beta_{11} A^{2} + \beta_{22} B^{2} + \epsilon
$$
\n(7)

Where: *Y=* response variable, *β0=*intercept, *A*= soaking time,  $B =$  boiling time,  $\beta_1$ ,  $\beta_2$ ,  $\beta_{12}$ ,  $\beta_{11}$ ,  $\beta_{22} =$ coefficients of the linear, interaction and square of *A* and *B* respectively, *ε* = estimated error. ε is often neglected and not factored into the equation

#### **3.7.1 Foam capacity**

The regression model for foam capacity is shown in Eq. 8 and the 3D surface and contour plots shown in Figs. 6a and 6b respectively.

Foam capacity

\n
$$
\text{(%)} = -2.74 - 2.13A - 2.35B + 7.15AB + 5.58A^2 + 6.76B^2
$$
\n
$$
\tag{8}
$$

#### **3.7.2 Gelatinization temperature**

The regression model for gelation temperature is shown in Eq. 9, while the Surface and Contour plots are shown in Figs. 7a and 7b respectively

Gelatinization temperature = +81.30+2.26A+3.12B+1.05AB-9.10A²- 10.34B² (9)

#### **3.7.3 Solubility**

The regression model for solubility of the samples is shown in Eq. 10, while the 3D surface and contour plots are shown in Figs. 8a and 8b respectively

Solubility (
$$
\%
$$
) = 17.56-2.71A-3.23B (10)

#### **3.8 Numeric Optimization for the Functional Properties**

The numeric optimization Solutions for the functional properties of the flour is shown in Table 8. The main criteria for constraints optimization of process parameters for the functional properties were minimum possible soaking and minimum boiling time which generated the solution in Table 8 with desirability of 60.30 %. This value is high and acceptable. However, desirability of 100 % is the most ideal if it could be obtained [18]. It shows that if the selected critical values of 12 h of soaking and 9.97 min boiling are employed in the production of Bambara ground nut flour, that the flour would exhibit functional properties of  $14.33, 63.95^{\circ}$ C and 22.42% for foam capacity, gelation temperature and solubility respectively.

# Table 5. Numeric optimization Solutions of the proximate composition of the BGN flour



#### Table 6. Functional Properties of Processed BGN (Soaked and Boiled) flour samples



Values are mean ± standard deviation of replicated determinations (n=21). Mean values in the same column followed by different letters are significantly (P = 05) different.  $X_1$  = Soaking time;  $X_2$  = Boiling time,

#### Table 7. Summary of ANOVA and coefficient estimate of the functional properties of the Bambara groundnut flour for the terms that showed significant model, insignificant lack of fit and p-value < 0.05



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**Fig. 6a. 3D Surface plot for the foam capacity of soaked and boiled BGN flour,**



**Fig. 6b. Contour plot for the effect of soaking time (h) and boiling time (min) on the foam capacity of soaked and boiled BGN flour**



**Fig. 7a. 3D Surface plot for the gelatinization temperature of soaked and boiled BGN flour**

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**Fig. 7b. Contour plot for the effect of soaking time and boiling time on the gelatinization temperature of soaked and boiled BGN flour**



**Fig 8a. 3D Surface plot for the solubility of soaked and boiled BGN flour**



**Fig. 8b. Contour plot for the effect of soaking time (A) and boiling time (B) on the solubility of soaked and boiled BGN flour**

<b>Soaking</b> time (h)	<b>Boiling time</b> (min)	Foam Capacity	Gelatinization Temp.	<b>Solubility</b>	<b>Desirability</b>	
12.000	9.966	14.327	63.945	22.423	0.603	Selected
	Design-Expert <sup>®</sup> Software <b>Factor Coding: Actual</b> <b>Overlay Plot</b> Foam Cap <b>Gel Temp</b> Solubility Design Points $X1 = A$ : SOAKING TIME X2 = B: BOILING TIME	B: BOILING TIME (Min)	60 $50 -$ Foam Cap: 0.42 $40 -$ $30 -$ 20 - Foam Cap: 14.329 Gel Temp: 63.9435 Solubility: 22.4238 X1 12 X2 9.96646 $10 -$ $\mathbf{0}$ 21 12	Overlay Plot $\bullet$ $\bullet$ Gel Temp: 81 30 39 A: SOAKING TIME (h)	48	

**Table 8. Numeric optimization Solutions for the functional properties of the flour**

#### **Fig. 9. Optimization plot of the functional properties of Bambara ground nut flour**

#### **3.8.1 Graphical optimization for the functional properties**

The solution displayed in the numerical optimization could be graphically shown as Fig. 9.

# **4. CONCLUSION**

From this study, it may be concluded that the combination of soaking and boiling/water blanching had significant effect on nutrient losses however; longer soaking times yielded the most fat. Minimum soaking time yielded better functional properties. Models for the prediction of optimal nutrient responses and optimal functional properties were developed.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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