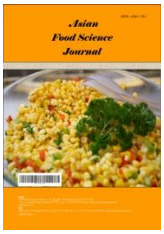

*Asian Food Science Journal*

***21(1): 42-52, 2022; Article no.AFSJ.77687***
***ISSN: 2581-7752***

# Effect of Soaking and Germination on the Functional Properties of Kpaakpa (*Hildegardia barteri*) Seed Flour Using Response Surface Methodology

**J. I. Anyadioha [a*], R. N. Attaugwu [a] and E. C. Okoli [b]**

*[a] Department of Food Science and Technology, Madonna University Nigeria, Akpugo Campus, Enugu, Nigeria.*
*[b] Department of Food Science and Technology, Ebonyi State University, Abakaliki Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/AFSJ/2022/v21i130399

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77687

***Original Research Article***

***Received 08 November 2021***
***Accepted 16 January 2022***
***Published 17 January 2022***

## ABSTRACT

**Aim:** The effects of soaking and germination on the functional properties of the kpaakpa (H.barteri) seed flour was evaluated.
**Introduction:** Kpaakpa (Hildegardia barteri) plant is a tropical leguminous plant in the family of steruliacea which is grown mostly in semi arid forest with other plants especially in Ivory Coast and Nigeria. The seeds are consumed in West Africa as raw or roasted nuts and have a flavour resembling peanut.
**Method:** The seeds were soaked for 12, 24 and 36 hours and allowed to germinate for 2, 4 and 6 days respectively. The germinated seeds were dried milled into flour and analyzed for functional properties. Results were statistically analyzed and fitted into second order polynomial equation. A face centered response surface method was employed to optimize the process parameters that gave the targeted optimum responses.
**Results:** The functional properties of the flours; bulk density, water absorption capacity and oil absorption capacity, foam capacity and swelling index were in the range of 0.50-0.60, 1.6-2.30 g/ml, 1.68-2.82 g/ml, 4.0-12 %, 1.2-11.respectively.The Water Absorption Capacity and Oil Absorption Capacity of the flour were enhanced by soaking and germination while foam capacity and bulk density decreased. Response optimization showed that

**Corresponding author: Email: anyadioha@madonnauniversity.edu.ng;*

soaking time of 12.6646 h with 3.7530 days of germination were derived as the closest process parameters that could give the targeted value of 0.58 for bulk density.
**Conclusion:** Soaking and germination imparted positively to the functionality of the flours with respect to Water absorption capacity and Oil absorption capacity of the flour.

*Keywords: Hildegardia barteri; seed flour; soaking; germination; functional properties.*

## 1. INTRODUCTION

*Kpaakpa* (*Hildegardia barteri)* plant is a tropical leguminous plant in the family of steruliacea which is grown mostly in semi arid forest with other plants. The plant grows from Ivory Coast to Nigeria. The seeds are consumed in West Africa as raw or roasted nuts and have a flavour resembling peanut.

The kernel is eaten raw or roasted or used as condiments in traditional food preparation [1]. In Nigeria, the seeds of *H. barteri* are consumed in few rural communities in Ebonyi and Enugu states of Nigeria, respectively. The seeds are not widely known and consumed due to lack of literature on its functionality in food systems. This research, therefore, is designed to evaluate the effect of processing treatments on functional properties of the seed using a response surface methodology. *H.barteri,* like other leguminous seeds, is an excellent source of proteins for both human and animal consumption. Functionality of food is defined as those physical and chemical properties, which affect the behavior of proteins in food systems during processing, storage, preparation and consumption [2]. The functional behavior of proteins in food is influenced by some physicochemical properties of the proteins. The functional behavior of proteins in food is influenced by some physicochemical properties of the proteins such as their size, shape, amino acid composition and sequence, net charge, charge distribution, hydrophobicity, hydrophilicity, type of structure, molecular flexibility/rigidity in response to external environment such as pH, temperature, salt concentration or interaction with other food constituents [3]. The functional properties are the intrinsic physicochemical characteristics which affect the behavior of a food ingredient food system during processing, manufacturing, storage and preparation. Such functional properties include water holding, oil binding, emulsification, foam capacity, gelatin, whipping capacity, viscosity and others. Functional properties are important in determining the quality of the final product as well as facilitating processing such as improved machinability of cookie dough or slicing of processed meats [4]. However, these properties vary with the type of food products; for example, proteins with high oil and water binding are desirable for use in meats, sausages, bread and cakes, while proteins with high emulsifying and foaming capacity are good for salad dressing, Sausages, bologna, soups, confectionery, frozen desserts and cakes [5].

Modification in protein structure of cereals during germination process have been reported to largely responsible for functional changes such as foaming, emulsification, nitrogen solubility and water absorption capacity [6].

Response surface Methodology (RSM) is a statistically-based optimization technique that uses experimental design and regression analysis to relate a response variable to the changing levels of a set of input variables. RSM can reveal curvilinear relationships and describe interactions between variables and their responses. Response surface analysis is deemed to be an important tool in food product development if several ingredients interact with one another to give specific physical characteristics [7].

This study is justified from the stand point of value addition as it has generated scientific information regarding its functionality in food systems.

## 2. MATERIALS AND METHODS

### 2.1 Sample Sourcing

The *Kpaakpa* (*H. barteri*) seed was hand picked around the trees at Independence Layout Area of Enugu metropolis Enugu State, Nigeria

### 2.2 Sample Preparation

#### 2.2.1 Raw *kpaakpa (H. barteri)* seed flour

The outer cover of the seed was removed, winnowed and oven dried at a temperature of 50$^{O}$C for 24 hours. The dried seeds were manually dehulled, milled into flour using Binatone blending machine and sieved through a 500 micron mesh sieve. The milled and sieved flour was analysed for functional properties.

#### 2.2.2 Soaked *kpaakpa (H. barteri)* seed flour

Two hundred grams each of the oven dried seeds were weighed and put in germination bags and soaked in clean water for 12, 24 and 36 hours respectively. After soaking, the seeds were drained off, oven dried at a set temperature of 50 $^{O}$C for 48 hours, dehulled, milled, sieved into flour and analyzed for functional properties.

#### 2.2.3 Germinated *kpaakpa* seed flour

Two hundred grams of *Kpaakpa* (*H. barteri)* seeds were weighed and soaked as above. After the soaking process, the seeds were spread inside the germination bags and placed in a jute bag which has previously been soaked with water and covered also with the jute bag. These samples were allowed to germinate for 2, 4 and 6 days respectively. After the germination, the seeds were oven dried at a temperature of 50 $^{0}$C for 48 hours. After the oven drying, the seeds were dehulled, milled, sieved into flour and analyzed for functional properties.

### 2.3 Experimental Design

This experiment was designed using Minitab software version 14.0. It is a face centred central composite design that has two major factors where each factor has three levels ($3^2$) given a total of nine runs as shown in Table 1.

### 2.4 Determination of Functional Properties of the Flour Samples

The functional properties of each of the flour samples were analyzed in each to ascertain the functionality of the protein of each flour samples. The bulk density was determined by method of Onwuka; Musa [8,9]. The method developed by Coffmann CW and Garciaj [10] was used for determining foaming capacity. The swelling Index was calculated using the method described by Akinyele [11]. The method of Beuchat [12] was used to determine the water absorption capacity and oil absorption capacity of the flour sample.

## 3. STATISTICAL ANALYSIS

All the responses were determined in triplicates. Data were analyzed statistically using a statistical software package for social science (SPSS version 17.0 for windows, SPSS Inc. Illinois, USA). Mean separation were carried out using Least Significant difference (LSD) at $p > 0.05$.

Experimental data generated from the functional properties were further analyzed using Minitab software (version 14.0). The above analysis involved fitting data into the simple second order polynomial model equation for the theoretical prediction of the response variables. The model equation is represented as below:

$$y = a + b_1x_1 + b_2x_2 + b_{11}x^2_1 + b_{22}x^2_2 + b_{12}x_1x_2 + e \quad [1]$$

Where,

**Table 1. Central Composite Design** (**Coded Experimental Design)**

| Run | A | B |
|---|---|---|
| 1 | -1 | 1 |
| 2 | 0 | -1 |
| 3 | -1 | -1 |
| 4 | 0 | 0 |
| 5 | 1 | 0 |
| 6 | 0 | 1 |
| 7 | 1 | 1 |
| 8 | 1 | -1 |
| 9 | -1 | 0 |

***A= Soaking time:*** *-1=12 h, 0=24 h, +1=36 h,* ***B= Germination time:*** *-1=2 days, 0=4 days, +1=6 days,* ***Factors:*** *2, Replicates: 1,*
***Base run:*** *9, B 9,* ***Base, blocks:*** *1,* ***Total blocks:*** *1.* ***Two-Cube factorial:*** *Full factorial,* ***Cube points:*** *4,* ***Center points in cube: 1,***
***Axial points:*** *4,* ***Center points in axial****: 0,* ***Alpha: 1***

Y = the responses, a = constant regression coefficient, $b_1$, $b_2$ = linear coefficients of the independent variables, $b_{12}$= coefficient of the interaction, $x_1$, $x_2$ = independent variables, $x_{12}$ = the interaction, $b_{11}$ & $b_{22}$ are quadratic regression coefficient terms, e = error associated with the observation of y. The test for the significance of the model (p = 0.05) and the adjusted coefficient of determination ($R^2$ adjusted) which showed the quality of the model were determined from the regression analysis.

The model search was started with linear through quadratic and the equation that gave the highest coefficient of determination was selected as being significantly adequate for the prediction of the functional properties [13,14].The term found to be statistically insignificant were excluded from the model (p>0.05). The term found to be statistically insignificant were excluded from the model (p = 0.05)

## 4. RESULTS AND DISCUSSIONS

### Bulk Density

The Raw *Hildegardia barteri* Flour had the highest bulk densty of 0.6 g/ml and this value decreased significantly (p=0.05) as the germination time increased. This was confirmed by the response surface regression analysis performed on the bulk density data which showed that linear effect of soaking and germination were significant (p=0.05).The test for fit conducted revealed very high $R^2$ and $R^2$ adj of 0.906 and 81.1 % respectively. This implied very high model adequacy and that the model predicted 90.6 % variation. This model adequacy was further confirmed by the low p –value of 0.025 and high F –value of 9.59 obtained from the analysis of variance table. Neverthless, the main effect of soaking time, germination time and the quadratic effect of germination time had antagonistic effect on bulk density whereas the quadratic effect of soaking time influenced bulk density positively.

Bulk Density = $0.537778 - 0.02500X_1 - 0.016667x_2$ ………………… [2]

Bulk density is important in determining the packaging requirement and material handling. Although the low bulk density may be undesirable as it will impair ease of dispersability of food powder, it is also a very relevant factor in weaning foods [15].

## 5. EFFECT OF PROCESSING ON THE FUNCTIONAL PROPERTIES OF *H. BARTERI* FLOUR

Values are mean ±std deviations of Triplicate samples. Means with different superscript within the same row are significantly different from each other (P<0.05).

**Table 2a. Analysis of variance for bulk density**

| Source | DF | SeqSS | AdjSS | AjMS | F | P |
|---|---|---|---|---|---|---|
| **Regr** | 4 | 0.0061 | 0.0061 | 0.0015 | 9.59 | 0.25 |
| **Resid** | 4 | 0.006 | 0.006 | 0.0001 | | |
| **Total** | 8 | 0.0068 | 0.0068 | | | |

**Table 2b. Estimated regression coefficient for bulk density**

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| **Const** | 0.5377 | 0.0094 | 6.843 | 0.000* |
| **A** | -0.0250 | 0.0051 | -4.825 | 0.008 |
| **B** | -0.0166 | 0.0051 | -3.216 | 0.032* |
| **A*A** | 0.0183 | 0.0089 | 2.043 | 0.111 |
| **B*B** | -0.0066 | 0.0089 | -0.743 | 0.499 |
| **$R^2$** | 0.906 | $R^2$ (adj) | 0.811 | |

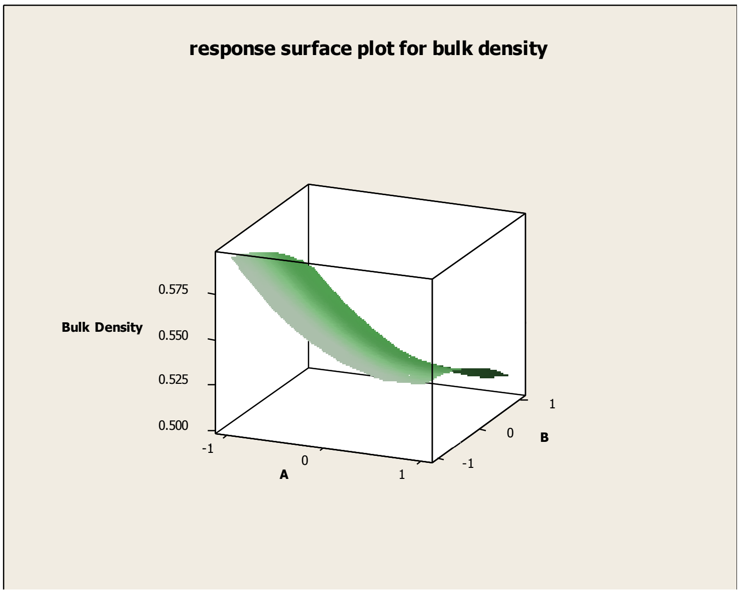


**Fig. 1. Surface plot for bulk density**

**Table 4a. Analysis of variance for foam capacity**

| Source | DF | SeqSS | AdjSS | AdjMS | F | P |
|---|---|---|---|---|---|---|
| **Regr** | 2 | 33.9000 | 33.9000 | 16.9500 | 95.15 | 0.000 |
| **ResidErro** | 6 | 1.0689 | 1.0689 | 0.1781 | | |
| **Total** | 8 | 34.9689 | | | | |

**Table 4b. Estimated regression coefficient for foam capacity**

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| **Const** | 13.511 | 0.5072 | 26.635 | 0.000* |
| **A** | -0.1833 | 0.0143 | -12.768 | 0.000* |
| **B** | -0.4500 | 0.08616 | -5.223 | 0.002* |
| **$R^2$** | 0.969 | $R^2$(adj) | 0.611 | |

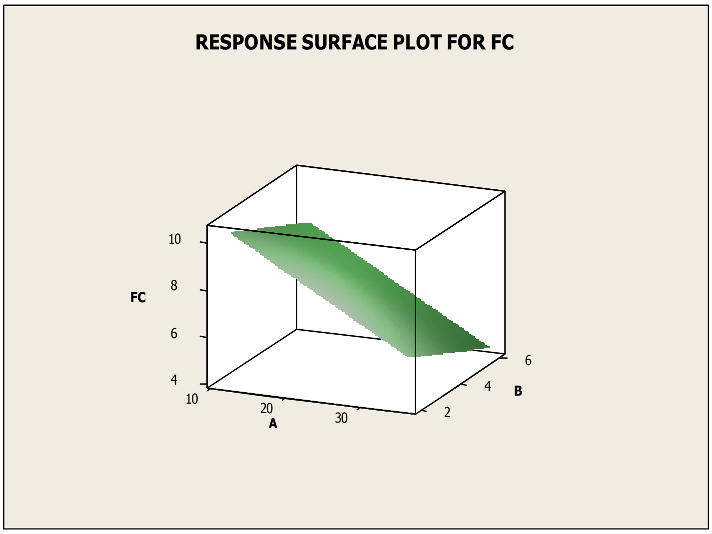


**Fig. 2. Surface plot for foam capacity**

## 5.1 Key

RHBF – Raw *Hildegardia barteri* Flour. S12 – Soaked *H. barteri* for 12 h. S12G2 – Soaked 12 h Germinated for 2days. S12G4 – Soaked 12 h Germinated for 4days. S12G6 – soaked 12 h Germinated for 6days S24 – Soaked for 24 h. S24 G2 – Soaked 24 h Germinated for 2days S24 G4 – Soaked for 24 h germinated for 4days. S36 – Soaked for 36 hS36 G2 – Soaked for 36 h germinated 2days. S36 G4 – Soaked for 36 h germinated for 4daysS36 G6 – Soaked for 36 h germinated for 6days, BD – Bulk Density, WAC – Water Absorption Capacity, OAC - Oil Absorption Capacity, FC - Foam Capacity, SI – Swelling Index.

## 5.2 Foam Capacity

The regression analysis carried out showed that the main effect of soaking and germination (linear effect) had negative impact on the foam capacity. The coefficient of determination ($R^2$) of 0.969 and $R^2$ (adj.) of 95.9 % showed high model adequacy and this means that 96.9 % of the total variation is explained by the regression equation. Results of the analysis of variance showed high level of linear model significance going from the p –values and F –values.

**Foam capacity= 13.5111 – 0.1833$x_1$ - 0.4500$x_2$……………. [3]**

As germination duration increased, the foamability decreased. The poor foaming properties exhibited by these samples was similar to the report of the study by [16]. Foamability is related to the rate decrease of surface tension of air – water interface caused by absorption of protein molecules. The report of Graham [17] showed that flexible protein molecules which can rapidly reduce surface tension give good foamability.

## 5.3 Swelling Index

The quadratic effect of soaking time and the interaction term affected swelling index positively and all the terms were significant p= 0.05. The $R^2$ of 0.998 and $R^2$ (adj.) of 99.5 % indicated significant goodness of fit of the model equation.

The results of the Analysis of variance confirmed it with p – value of 0.000 and F – value of 290.74. The model for swelling index is as below:

**Swelling Index = 13.511 – 0.1833$X_1$ – 0.4500$X_2$ …….. [4]**

The variation in swelling index could be related to associative binding within the starch granules while the strength and character of the miscellar network may be related to the amylose content which confers high swelling power [18]. Flours with good swelling capacities are primarily used for thickening of soups, sauces gravies etc.

## 5.4 Water Absorption Capacity

The water binding capacity ranges from 1.6ml/g in control (RHBF) to 2.3ml/g in $S_{36}G_6$. Samples $S_{12}G_4$ through sample $S_{36}G_4$ followed the same trend as the Water absorption capacity continued to increase with increase in soaking and germination time, respectively. The main effect of soaking time was statistically significant (P=0.05). However the quadratic effect of germination influenced WAC negatively as is evidenced from the Analysis of Variance (ANOVA) performed. The $R^2$ of 0.998 and $R^2$ (adj.) of 99.5 % indicated that the water absorption capacity data could be fitted to the model equation as could be seen from the very low probability value and high F-value.

**WAC = 1.42444 + 0.01917 $x_1$ ……………. [5]**

This suggest an increase in cellular water uptake with increased germination time and due to changes in the quantity and quality of proteins in flour upon germination [19]. The Water absorption capacity is a functional property used in determining the suitability of using a material in a baked food such as bread where high water absorption is needed [20]. The water absorption capacity could be used to determine the rate of water intake when used in food formulations. The high water absorbed in some samples shows that when used in foods the rate of water uptake will be higher in those samples that had higher values than the control samples. This, therefore, suggest that this flour can be used in composite flour for bread and confectioneries.

## 5.5 Oil Absorption Capacity

All the terms were significant on the oil absorption capacity. Although the quadratic effect of both soaking and germination time had antagonistic effect on this parameter. The high $R^2$ and $R^2$ (adj.) indicated a good fit of the model equation.

**OAC= $0.13750X_1+0.28666X_2-0.001782X_1^2-0.01666X_2^2-0.0039X_1X_2$……………[6]**

Since oils contribute to flavour retention in foods and increase mouth feel, it is an important property in food formulations. As a result, germinated *Kpaakpa* flour could be used in various food formulations where flavor enhancement is a priority especially in baby foods. This result was consistent with the reports of [15] who reported increase in OAC of germinated cowpea and moth bean flour respectively.

Response Optimization of the parameters were performed by using numerical optimization. The Minitab software used, searched for a combination of factor levels that simultaneously satisfied the expected requirements placed on each of the responses and the factors. Optimization requires that goals (i.e. minimum, target and maximum) are set for the independent variables and responses where all the goals then get combined into one desirability function. To find a good set of conditions that will meet all the goals, the two variables [1] Soaking time (12 – 36 h) and [2] Germination time (2 – 6 days) were set within range while the responses were set at target. Desirability ranges from zero to one for a given response. To maximize a response, the closer the desirability to 1, the better the response values and to minimize, (''smaller is better'') i.e. the closer the desirability to zero the better the response values. After setting the goals for each response, the Minitab software generated the optimum levels of soaking and germination times respectively with the predicted responses. Below were the optimization plots for the targeted responses. Bulk density has the global solution of 12.6646 h for soaking and 3.7530 days for germination with desirability of 1.000. Water absorption capacity has a targeted value of 2.0 and an achieved value of 1.9992 with desirability of 0.99745 which was very close to 1 (good desirability).

**Table 5a. AVOVA for swelling index**

| Source | DF | SeqSS | AsjSS | MS | F | P |
|---|---|---|---|---|---|---|
| **Regr** | 3 | 1.2097 | 1.2097 | 0.40326 | 15.18 | 0.054 |
| **Residual Error** | 5 | 0.3891 | 0.38917 | 0.077834 | | |
| **Total** | 8 | 1.5989 | | | | |

**Table 5b. Estimated Regression Coefficient for swell index**

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| **Const** | 1.2578 | 0.0930 | 13.525 | 0.000* |
| **A** | 0.3183 | 0.11390 | 2.795 | 0.038* |
| **B** | 0.2233 | 0.11390 | 1.961 | 0.107 |
| **A*B** | -0.2750 | 0.13949 | -1.971 | 0.106 |
| **$R^2$** | 0.757 | $R^2$ (adj) | 0.611 | |

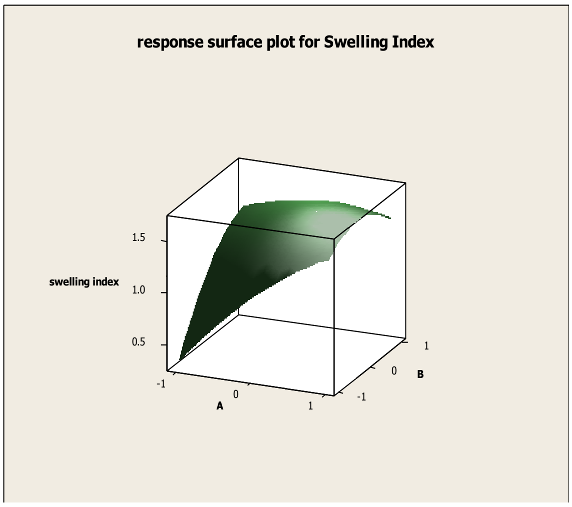


**Fig. 3. Surface plot swelling index**

**Table 6a. ANOVA for water absorption capacity**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **Regre** | 5 | 0.4232 | 0.4232 | 0.0846 | 300.73 | 0.000 |
| **Resid Error** | 3 | 0.0008 | 0.0008 | 0.0002 | | |
| **Total** | 8 | 0.4240 | | | | |

**Table 6b. Estimated regression coefficient for WAC**

| Term | SE Coef | T | P | |
|---|---|---|---|---|
| **Constant** | 1.4244 | 0.0682 | 20.866 | 0.000* |
| **A** | 0.01917 | 0.00423 | 4.528 | 0.020* |
| **B** | 0.02000 | 0.02539 | 0.787 | 0.488 |
| **A*A** | 0.00002 | 0.00008 | 0.281 | 0.797 |
| **B*B** | -0.0016 | 0.0029 | -0.562 | 0.613 |
| **A*B** | 0.0004 | 0.0003 | 1.192 | 0.319 |
| **$R^2$** | 0.998 | $R^2$ (adj) | 0.995 | |

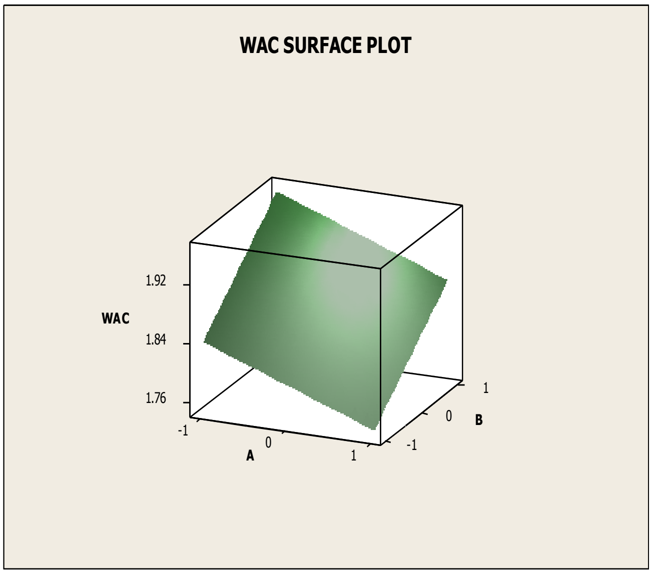


**Fig. 4. Surface Plot Water absorption capacity**

**Table 7a. Analysis of variance for OAC**

| Source | DF | SeqSS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **Regre** | 5 | 1.38508 | 1.385078 | 0.277016 | 375.85 | 0.000 |
| **Resid Error** | 3 | 0.00221 | 0.002211 | 0.000737 | | |
| **Total** | 8 | 1.38729 | | | | |

**Table 7b. Estimated regression coefficient for OA C**

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| **Const** | -0.0988 | 0.1104 | -0.895 | 0.437 |
| **A** | 0.1375 | 0.0068 | 20.074 | 0.000* |
| **B** | 0.2866 | 0.0410 | 6.975 | 0.006* |
| **A*A** | -0.0017 | 0.0001 | -13.370 | 0.001* |
| **B*B** | -0.0166 | 0.0047 | -3.473 | 0.040* |
| **A*B** | -0.0039 | 0.0005 | -6.999 | 0.006* |
| **$R^2$** | 0.998 | $R^2$ | 0.996 | |

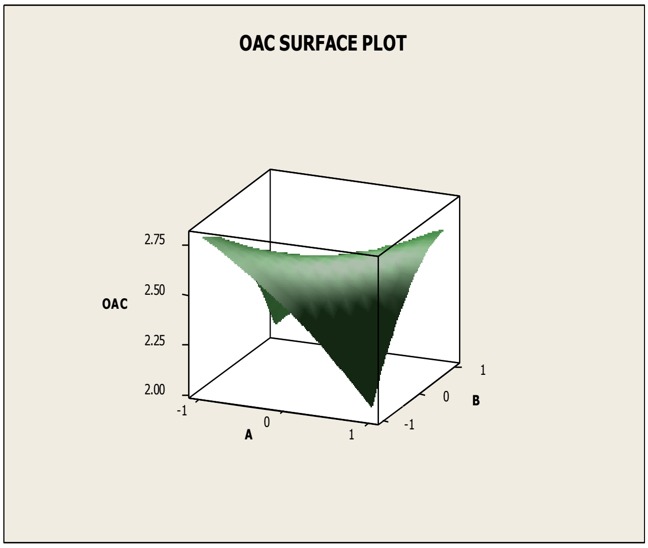


**Fig. 5. Surface plot for OAC**

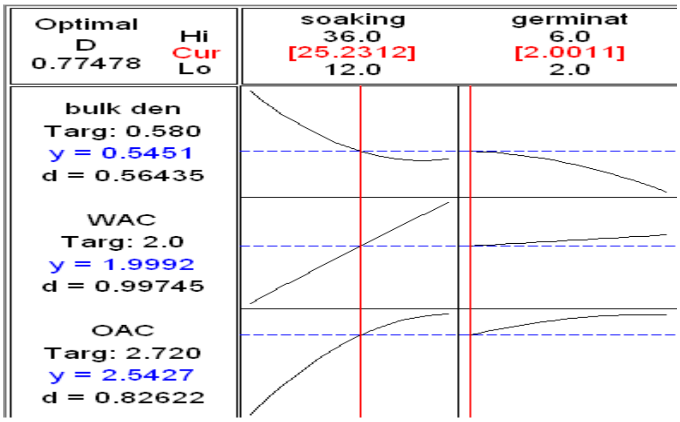


**Fig. 6. Optimization plot for selected functional properties**

## 6. CONCLUSION

The study showed that, soaking and germination, as processing treatments, could be used to improve the functional properties of *Kpaakpa* seed. However, applying these independent processing variables on *Kpaakpa* for functionality purposes generated a flour that has enhanced water absorption capacity, Oil absorption capacity and swelling index and so could be beneficial in food systems especially in confectionary industries where high oil and water absorption properties respectively are required. The enhanced swelling Index makes the flour suitable for soup condiments. The reduction in bulk density after soaking and processing was an advantage when issues relating to packaging is being considered. Response optimization showed that soaking time of 12.6646 h with 3.7530 days of germination were derived as the closest process parameters that could give the targeted value of 0.58 for bulk density. Response optimizer applied to Water absorption capacity and Oil absorption capacity of the flour gave the desirability of 0.997 and 0.826 respectively and achieved target of 1.999 and 2.542 respectively as against 2.0 and 2.7 targeted values for WAC and OAC.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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