

The proximate and mineral composition of soaked and germinated kpaakpa (*Hildegardia barteri*) seed flour: A response surface methodology approach

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Abstract

This paper evaluated the effects of soaking and germination on the proximate and mineral composition of the *kpaakpa* (*H. barteri*) seed flour. 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 proximate and mineral composition. Results were statistically analyzed and fitted into second order polynomial equation. A face centered response surface method using three level-two factor full factorial central composite design was employed to optimize the process parameters that will give the targeted optimum responses. Germination was found to increase protein, dietary fibre, reduce carbohydrate and fat. Germination enhanced potassium in virtually all the processed samples but reduced in Ca, Fe, Zn and phosphorus. The multiple regression model developed showed that the optimized processing conditions for the responses were 12 h of soaking and 4.75 days for germination for protein; 12 h of soaking and 5.97 days of germination for Iron and 36h of soaking and 5.95 days germination for Zinc.

Keywords: kpaakpa, flour, germination, proximate, mineral composition

1. Introduction

At a time like this, when the gap between global populations continues to widen, ways and means of bridging the gap has become a matter of urgent importance. The current surge in the search for nutritious plant foods is, thus, not surprising. Legumes refer to edible seeds of leguminous plants belonging to the leguminosae family. Generally, legumes are not only cheap and popular food for many people but are the main plant source of proteins in human diet containing 17-25 % except soybeans which contain 40 % protein. They are also rich in dietary fibre, carbohydrates, lipids and minerals [1]. Minor compounds of legumes are polyphenols and bioactive peptides [2]. Due to animal protein sources often containing large amounts of saturated fat and cholesterol, most health organizations recommend the frequent consumption of vegetable protein, since it is known that it may reduce serum cholesterol levels, the risk of coronary heart diseases and diabetes [3]. Also protein malnutrition is one of the major nutritional problems in the developing world, so much research has focused on various sources of plant proteins [4]. The ultimate has not been achieved as several plants exist with very high nutritive value, that may help in increasing the nutritional value of food products at low cost and yet remain unexploited for human and animal benefits [5]. This is evidenced by the lack of literature available on the subject.

Kpaakpa (*Hildegardia. Barteri*) seeds belong to this group of unexploited food materials. The 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 [6]. The seeds of *H. Barteri* are consumed in few rural communities in Ebonyi state and Enugu state of Nigeria. It is a lesser known and utilized legume that contains 17.5 % crude protein, 37.5 % crude fat, 2.8 % ash and 6.5 % crude fibre [7]. Studies on the fatty acid characterization and profile have been carried out. The study showed that the *H. barteri* oil contain an almost equal amount of myristic, palmitic, stearic and linolenic acids which is quite uncommon among all oil seeds. Palmitic acid was the major fatty acid, up to 29.4 % followed by stearic acid. This is similar to that of palm kernel oil which has 82 % saturated fatty acids and 18 % unsaturated acids [7].

It is in view of these huge nutritional potentials of this seed that this research was designed to evaluate the effect of soaking and germination on the proximate and mineral composition of Kpaakpa seed flour using a response surface methodology.

2. Materials and Method

2.1 Preparation of Raw kpaakpa (*H. barteri*) seed flour

2.2 Sample sourcing

The *Kpaakpa* (*Hildegardia barteri*) seed was handpicked around the trees at Independent Layout Area of Enugu metropolis and Ezzaa Local Government Area of Ebonyi State.

2.3 Preparation of Raw kpaakpa (*H. Barteri*) seed flour

The outer cover of the seed was removed, winnowed and oven

dried at a temperature of 50 °C for 24 hours. The dried seeds were manually dehulled, milled into flour using Panasonic blending machine and sieved through a 500 micron mesh sieve. The milled and sieved flour was analyzed for functional properties.

2.4 Preparation of 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 °C for 48 hours, dehulled, milled, sieved into flour and analyzed for functional properties.

2.5 Preparation of Germinated kpaakpa (H. Barteri) 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 °C for 48 hours. After the oven drying, the seeds were dehulled, milled, sieved into flour and analyzed for proximate and mineral composition.

2.6 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 (3²) given a total of nine runs as shown in Table 1

Table 1: CCD (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, Total runs: 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

2.7 Determination of proximate composition of the flour samples

The proximate analysis of each of the flour samples (raw and processed) was carried out using standard methods of [8]. The parameters determined included moisture, ash, crude fibre, protein and fat. The total carbohydrates was calculated by difference [9]. The mineral composition of the various samples was extracted from each sample according to the dry ash method of [9]. The zinc and iron content of each of the samples was determined using Atomic Absorption Spectrophotometer 2380, Perkin Elmer (Shelton, Conn.) using zinc and iron hollow cathode lamps at a wave length of 213.9 nm and 248 nm respectively. The potassium content of each sample was estimated according to the flame photometric method of [10]. The calcium content of each sample was determined according to the Ethylene di amine tetra acetic acid versanate complex method of [10]. All determinations was performed in triplicates.

2.8 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 responses were further analyzed using Minitab software (version 14.0).The 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:

$$y = a + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 + e \dots\dots\dots (1)$$

Where, y = the response variables, a = the intercept b₁, b₂ = linear coefficients of the independent variables, b₁₂ = coefficient of the interaction, b₁₁& b₂₂ = quadratic regression coefficient terms, x₁, x₂ = independent variables, x₁₂ = the interaction, e = error associated with the observation of y

3. Result and discussions

Table 2: Effect of Processing on the Proximate Composition of *H. barteri* Seed Flour (mg/100 g)

Responses	RHBT	S12	S12G2	S12G4	S12G6	S24	S24G2	S24G4	S24G6	S36	S36G2	S36G4	S36G6
Moisture	7.17 ^b ± 0.05	10.06 ^k ± 0.05	10.78 ^j ± 0.01	10.85 ⁱ ± 0.02	10.96 ^h ± 0.02	11.55 ^s ± 0.02	11.05 ^e ± 0.05	10.14 ^f ± 0.05	11.23 ^e ± 0.011	11.27 ^d ± 0.04	11.34 ^e ± 0.05	11.39 ^b ± 0.05	11.43 ^a ± 0.05
Crude fibre	3.7 ^a ± 0.15	3.13 ^b ± 0.01	2.94 ^c ± 0.02	3.96 ^d ± 0.05	2.87 ^e ± 0.05	2.99 ^{ef} ± 0.12	2.73 ^{fg} ± 0.04	2.76 ^g ± 0.05	2.70 ^g ± 0.06	2.68 ^g ± 0.01	2.80 ^{ef} ± 0.07	2.68 ^g ± 0.01	2.76 ^h ± 0.02
Ash	3.46 ^a ± 0.58	3.05 ^b ± 0.01	2.91 ^{bc} ± 0.58	2.88 ^b ± 0.06	2.82 ^b ± 0.02	2.76 ^{bc} ± 0.02	2.78 ^{bc} ± 0.01	2.65 ^{bc} ± 0.02	2.78 ^{bc} ± 0.03	2.70 ^c ± 0.01	2.71 ^c ± 0.07	2.74 ^{bc} ± 0.04	2.75 ^{bc} ± 0.03
Protein	16.15 ^b ± 0.01	15.02 ^a ± 0.05	15.23 ^a ± 0.05	17.50 ^{ab} ± 0.58	18.26 ^c ± 0.05	17.43 ^{ab} ± 0.01	16.14 ^b ± 0.05	22.01 ^d ± 0.01	18.29 ^c ± 0.05	16.95 ^b ± 0.05	18.47 ^c ± 0.54	19.31 ^d ± 0.02	19.33 ^d ± 0.02
Fat	33.50 ^a ± 0.05	33.13 ^{bc} ± 0.11	33.00 ^{bc} ± 0.05	32.83 ^{bc} ± 0.15	31.80 ^c ± 0.15	31.46 ^d ± 0.15	29.13 ^d ± 0.15	30.00 ^e ± 0.00	31.33 ^d ± 0.57	30.0 ^e ± 0.00	30.0 ^e ± 0.00	30.0 ^e ± 0.00	30.07 ^e ± 0.04
CHO	36.02 ^b ± 0.5	35.56 ^{bc} ± 0.05	35.61 ^{bc} ± 0.20	32.00 ^b ± 0.5	33.30 ^c ± 0.15	33.82 ^a ± 0.10	36.31 ^a ± 0.20	35.31 ^{bc} ± 0.15	35.07 ^{bc} ± 0.52	35.10 ^{ba} ± 0.13	34.7 ^a ± 0.05	33.98 ^{bc} ± 0.05	33.66 ^c ± 0.05

Values are means ±std deviations of Triplicate samples. Means with different superscript within the Same raw are significantly different from each other (p<0.05)

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 Germinate for 2days. S24 G4 – Soaked for 24 h germinate for 4days. S36 – Soaked for 36 h S36 G2 – Soaked for 36 h germinate 2days. S36 G4 – Soaked for 36 h germinate for 4days S36 G6 – Soaked for 36 h germinate for 6days

3.1 Moisture content

The regression analysis performed on the moisture data showed that, none of the effects (main, quadratic and interaction) was significant (P>0.05). This was confirmed by the results of analysis of variance which showed high p – values of 0.125, 0.149 and 0.116 and low F –values of 3.53, 3.17 and 3.88. The test for fit was checked by determining the R2 (0.779) and R2 adjusted (0.558) and this further validated the statistical insignificance of the model. Therefore, the moisture data could not be fitted in the regression model.

3.2 Carbohydrates

The interactive effect of soaking and germination led to increased carbohydrate losses in all the processed samples studied which was in line with the report of [11]. The significant (p>0.05) decrease in the total carbohydrate content was in consonance with the report of [12] who observed decrease in carbohydrate after germination. The decreased Carbohydrate level of the germinated seeds might be due to increase in the amylase activity. The alpha amylase breaks down complex carbohydrate to simpler and more absorbable sugar which are utilized by the growing seedlings during early stages of germination. Analysis of variance and estimated regression coefficients, showed that none of the terms was significant in predicting carbohydrates going by the high p values of 0.494, 0.065, 0.077 and 0.052 on one hand, and low F- values on the other hand. The low F- values indicated that most of the variation cannot be explained by a regression equation whereas the high P –values showed that the model is considered statistically insignificant. However, the negative coefficients indicated that linear (main) effect of germination time and quadratic effect of soaking time had antagonistic effect on carbohydrates, but the test of fit was significant with the R2 of 0.833 [13].

3.3 Protein

Values obtained for protein range from 16.15 % -22.01 % which fell within the range of protein in legumes (17-25 %) as

reported by [14]. The increase in protein content after germination was in line with earlier reports of increase in protein content during germination of various cereals, legumes and other seeds [12] and values are above the minimum range of 14 % (Nx6.25) for management of moderate malnutrition. Also according to [15], germination improves protein owing to decrease in carbohydrates and fat sine these macro nutrients were used as major sources of carbon needed for seed growth. Hence, germination would advantageously be utilized to improve the nutritional quality of food products. The data generated from analysis of variance and estimated regression coefficient for protein indicated that the quadratic effect of soaking and germination time exerted a statistically insignificant negative effect on protein. This was confirmed by the high p values of 0.152 and low F values of 3.05.

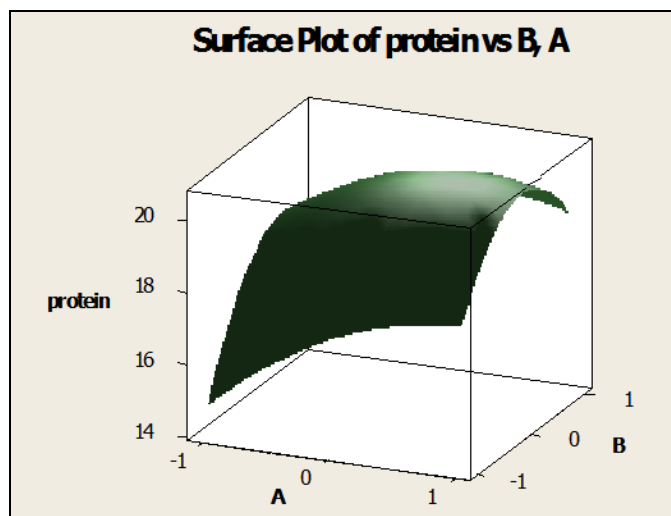


Fig 1: Response Surface Plot for Protein

3.4 Crude Fibre

The response surface regression performed on the experimental data revealed that neither the linear, quadratic

nor the interaction terms was significant with p values of 0.293 and 0.339 respectively. Although the main effect of soaking time and quadratic effect of germination time brought about decrease in level of crude fibre, the effect was statistically insignificant as confirmed by the result of the analysis of variance. The R² of 0.61 and R² adj of 22 % as obtained from the table below indicated high model inadequacy as only 61 % of the total variation is explained by the model leaving about 39 % of the total variation attributed to extraneous factors not accommodated in the experimental design. For a good fit model, R² should be at least 0.75 [16, 17] hence obtained R² of 0.61 showed significant lack of fit and so could not be used.

3.5 Fat Content

There was significant decrease in germinated samples. The observed decrease was in consonance with the result of study by [18] where fat content decreased with increase in germination time. This was as a result of the fact that fat was also used as a source of carbon for seed growth [20, 19] suggested that fatty acids were oxidized to carbon (iv) oxide and water to generate energy needed for germination. Also [19] reported that during germination, there was increase in the activity of lypolytic enzymes which hydrolyse fat into simpler products needed by developing embryo as a source of energy. This fact was further confirmed by the analysis of Variance and estimated regression coefficient results which showed that both the main and quadratic effects of soaking time were significant in predicting fat with p values of 0.036 and 0.045 respectively. The R²=0.897 and R² adj. =72.6 % showed model adequacy and so the empirical relationship between fat and the studied variables in terms of coded units as in equation 2.

$$\text{Fat} = 29.441 - 1.1283X_1 + 1.7883X_1^2 \dots\dots\dots (2)$$

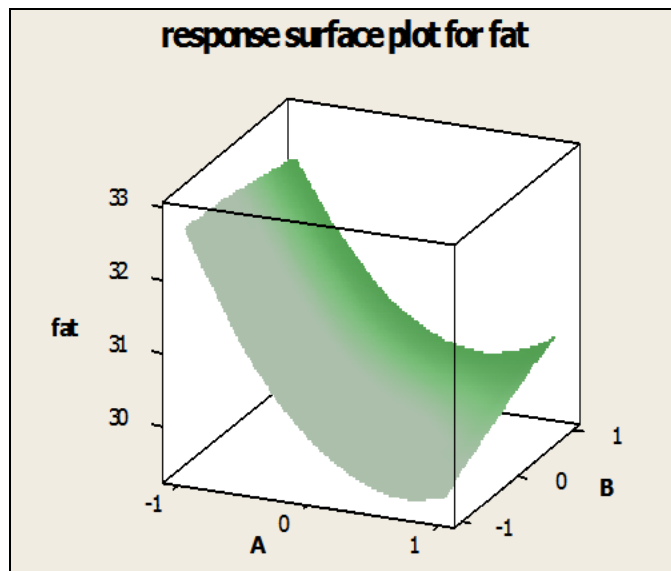


Fig 2: Response Surface Plot for Fat

3.6 Ash Content

From the results of the analysis of variance and response surface regression performed on Ash data, R² and R² adj. of 0.598 and 0.357 respectively indicated significant model inadequacy as it could only explain 59.8 % variability of the response. Furthermore, the p- values of 0.176, 0.208 and 0.140 respectively and the F –values of 2.48, 2.19 and 3.07 confirmed the model insignificance, hence the ash data could not be fitted into a regression model.

Table 3: Effect of soaking and germination on the Mineral Composition of *H. barteri* flour (mg/100 g).

Mineral	RHBF	S12	S12G2	S12G4	S12G6	S24	S24G2	S24G4	S24G6	S36	S36G2	S36G4	S36G6
Calcium	2653e ± 54	2608e ± 20.4	1777f ± 81.66	2426e ± 57.29	2426f ± 40.79	2019f ± 31.69	2308f ± 63.75	2581 ± 36.22	2464 ± 44.30	1004 ± 32.9	2502 ± 35.55	2553 ± 58.38	2466 ± 52.6
Zinc	3.04bc ± 0.31	2.65 ± 0.06	3.11b ± 0.58	3.12b ± 0.14	3.68a ± 0.10	2.28e ± 0.32	2.37e ± 0.037	2.59de ± 0.05	2.70abcd ± 0.33	2.09f ± 0.23	2.25e ± 0.22	2.69abcd ± 0.14	2.83abc ± 0.14
Phosphorus	180.67ab ± 1.15	181.95ab ± 1.78	182.a40ab ± 2.77	180.0ab ± 1.5	177.40ab ± 2.7	176.77ab ± 5.1	182.07ab ± 2.5	184.87a ± 3.77	163.6c ± 15.38	171.90bc ± 13.95	178.0ab ± 2.0	177.33ab ± 1.15	184.63a ± 5.18
Iron	3.3a ± 0.24	2.7b ± 0.11	2.39cde ± 0.13	2.28def ± 0.23	2.50bed ± 0.23	2.38cde ± 0.08	2.18ef ± 0.16	2.24def ± 0.18	2.04f ± 0.06	2.13ef ± 0.01	2.55bc ± 0.10	2.54bc ± 0.18	2.44bed ± 0.33
Potassium	5.7ed ± 0.12	5.8bc ± 0.15	6.72abc ± 0.16	6.6abc ± 0.13	7.06ab ± 0.92	5.68cd ± 0.20	6.31bc ± 0.69	6.14bc ± 1.7	5.84d ± 0.66	6.2bc ± 0.39	6.88ab ± 0.67	7.04ab ± 0.12	7.54a ± 0.92

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). 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 Germinate for 2days.S24 G4 – Soaked for 24 h germinate for 4days. S36 – Soaked for 36 h S36 G2 – Soaked for 36 h germinate 2days. S36 G4 – Soaked for 36 h germinate for 4days S36 G6 – Soaked for 36 h germinate for 6 days

3.7 Phosphorus

The analyses of variance and regression analysis was performed on phosphorus data. From the analysis, neither the linear, quadratic nor interaction terms were significant as evidenced by the high probability values. However, the main effect of soaking has negative effect on the phosphorus level as increase in soaking time resulted in significant reduction of phosphorus level. The coefficient of determination of 0.812

and the R² adj. Of 49.8 % showed model inadequacy in predicting phosphorus and thus the phosphorus data could not be fitted into the model.

3.8 Iron

The combined effect of soaking and germination also brought about reduction in iron levels as in Table 3.0. The reduction of iron in soaked samples were due to leaching out of iron into

the soak water as it is water. From the regression coefficient and Analysis of variance of Iron, the quadratic effect of soaking time was significant whereas the linear effect of soaking and germination was not significant. Furthermore, the interaction effect of soaking and germination contributed immensely towards explaining the relationship between the variables. The correlation coefficient (R^2) of 0.909 and R^2 (adj.) of 75.7 % was strong enough to predict the iron content better.

Hence the model $Fe = 2.4288 + 0.21333X_1^2 + 0.155X_1X_2$ (3)

A surface plot of interactions of soaking and germination on Fe confirm the significant quadratic effect of these independent variables on Fe as in figure

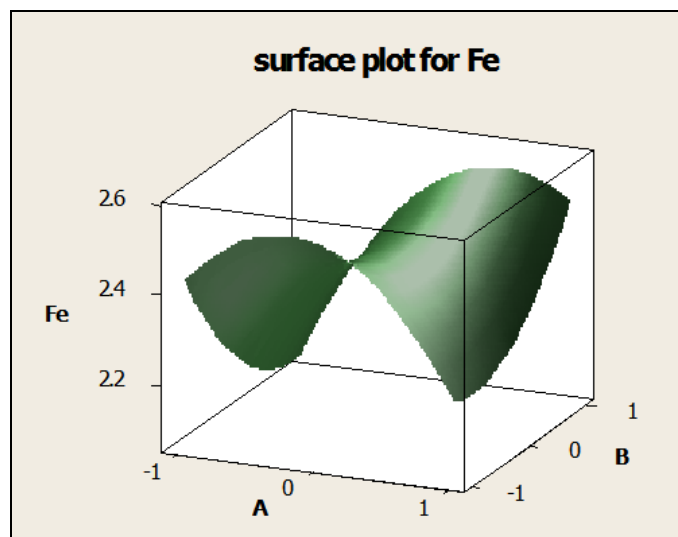


Fig 3: Surface Plot for Iron

3.9 Zinc

Data on the application of face centred CCD to zinc content of *kpaakpa* flour showed that the linear effect was significant ($p < 0.05$) on both soaking and germination time respectively; quadratic effect was also significant on soaking time but not germination time. The main effect of soaking has negative effect on zinc. The R^2 of 0.943 and R^2 adj. 0.887 obtained from table 12b showed a good fit of the model with the experimental data. The regression model developed for zinc is given as

$Zn = 2.5377 - 0.3566x_1 + 0.2466x_2 + 0.3933x_1^2$ (4)

The above model was highly significant and adequate for prediction of zinc in similar food systems with the same treatment.

3.10 Potassium

The Analysis of variance and response surface estimated regression coefficient for potassium. There was a significant contribution of quadratic terms to soaking time as could be

seen from the p value ($p = 0.042$). The correlate on coefficient of 0.85 provided a good measure of 85% variability in the observed potassium values. Therefore, the equation $K = 7.1955 - 0.798x_1^2$ (5) Could be adequately applied in the theoretical prediction of potassium.

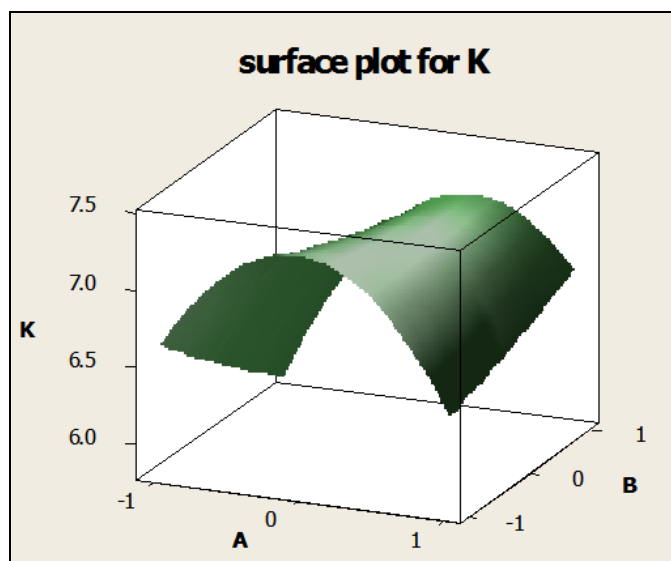


Fig 4: Surface plot for Potassium

3.11 Calcium

The Analysis of variance and response surface regression coefficient determination performed on Calcium showed that none of the terms was significant on processing variables (soaking and germination) time as was evident from the probability values. Similarly, the R^2 and R^2 (adj.) of 0.72 and 0.459 indicated model inadequacy and so calcium data could not be fitted into the model

3.12 Optimization

Response Optimization of the parameters were performed by using numerical optimization. The Minitab software used, searches for a combination of factor levels that simultaneously satisfy 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 (16). 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. Desirabilities 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 as shown in table 4. Below are the optimization plots for the targeted responses.

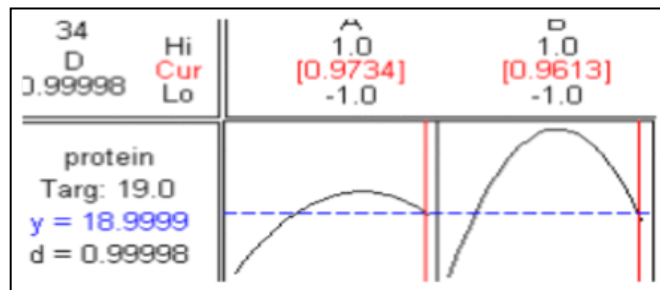


Fig 5: Optimization plot for protein

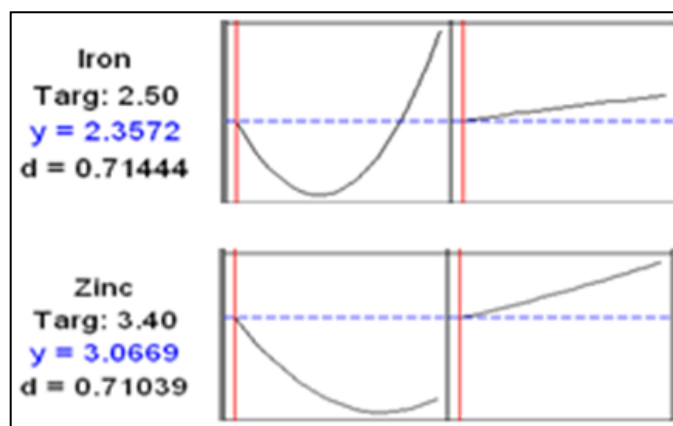


Fig 6: Optimization plot for Iron and Zinc

The figures 5 and 6 above are graphical optimization of some selected responses. They include protein, Iron and Zinc, From the protein optimization, the current process conditions, the one written in red ink, soaking time (A)= 12 h and Germination time (B) 4.7530 days was derived as the closest process parameters that could give the targeted value for protein. It has an optimum desirability of 0.90674. For Iron, the response optimizer generated 12 h soaking and 5.5972 days as the process condition that could give the predicted value of 2.4202 with desirability of 0.8404 and optimal desirability of 0.78289. For Zinc, the following optimization results were obtained: target=3.4, y= 2.833, d=0.50697 and optimal desirability y=0.76095. The current process condition that yield the above results was 36 h soaking and 5.955 days.

Table 4: Target Response values and predicted values of selected responses

S/N	Response	Target	Predicted	Desirability
1.	Protein	19.0	18.999	0.9999
2.	Zinc	3.4	3.066	0.7104
3.	Iron	2.5	2.36	0.7144

Conclusion

This research work has been able to generate information on the effect of soaking and germination on the compositional properties of *kpaakpa* seed flour. The proximate analysis result showed that protein was slightly enhanced, fat and carbohydrates were reduced. Thus, soaking and germination, as processing treatments, could be used to improve the nutritional value of *Kpaakpa* seed. The flour generated from this seed could be beneficial in food systems especially in confectionary industries where high oil and water absorption

properties respectively are required. The optimization showed that in the current process conditions, soaking time of 12h with 4.7530 days of germination were derived as the closest process parameters that could give the targeted value of 19 % for protein. Response optimizer was also applied to iron and 12 h soaking and 5.972 days were generated as the process condition that could give the 2.42 % level of iron targeted with desirability of 0.08404.

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