Modelling and optimization of proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour using response surface methodology

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***Abstract*: Three components augmented simplex centroid design of Response Surface Methodology (RSM) was applied to model and optimized the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour. Blends were variously pre-treated, millet by soaking, mungbean by malting and tigernut by defatting. The experimental values were obtained and subjected to regression analysis to generate regression equations. The linear, binary and ternary effects of the blends were generated and graphically expressed on 3-D surface plots. The developed models were validated at $p<0.05$. Furthermore, optimization of outcomes was selected at desirability of 0.464. The result showed that experimental values for protein; 11.83%, 21.59%, 8.56% respectively for millet, mungbean and tigernut. The regressed values showed good correlation with the predicted values. The optimum blends selected at a desirability of 0.464 were 0.00g millet, 70.36g mungbean, and 29.63g tigernut will give the best breakfast meal. Confirmatory runs were done to ascertain 95% confidence of the optimum values. The three component argumented simplex centroid experimental design of response surface methodology was adequate in modelling and optimization of the parameters: moisture, ash, crude fiber, fat protein, carbohydrate, oxalate, phytate and tannin.**

***Keywords*: Proximate composition, Anti-nutritional composition, Breakfast cereals, Simplex centroid design**

I. INTRODUCTION

Nigeria is a country with an abundance of food that can be used for proper nutrition, as well as for the formulation of breakfast cereals. Despite the abundance of foods, there is malnutrition. Malnutrition does not only persist but remains widespread in many developing countries. Protein Energy Malnutrition (PEM) and micronutrient deficiencies among infants and children have been shown to be directly and individual associated with more than 50% of all childhood morbidity and mortality in the developing world (Standing Committee on Nutrition, 2004). The WHO and UNICEF are very much concerned about this trend. This is due mainly to lack of adequate and good quality formulated foods, poor processing methods and the presence of anti-nutritional factors such as tannin, oxalate, and phytate (Gee & Harold, 2004). The amount of cereal grown in Nigeria is high compared to its utilization. This is due to post harvest losses incurred from cereals, thus there is need to diversify the use of cereals into producing some products which can be made available all year. Cereals are most important part of human diet because they provide energy and nutrient intake of humans (Jones, 2003).

Breakfast cereals are foods made by swelling, grinding, rolling or flaking of any cereal (Sharma & Caralli, 2004). Breakfast cereals are rapidly gaining acceptance in most developing countries and gradually replacing most traditional diets that are used as breakfast meals due to convenience, nutritional values, improved income and status symbol and job demands especially among urban dwellers, (Abbey & Ibeh, 1988).According to Jones (2003) breakfast cereals facilitate independence because of their ease of preparation which means that children and adolescents can be responsible for their own breakfast or snacks. Such foods may need to be reconstituted, preheated in a vessel or allowed to thaw if frozen before consumption, or they may be eaten directly without further treatment (Okaka, 2005). Their consumption has also been extended to non-breakfast hours and often serve as in-between meals. A study has clearly shown that 42% of 10-year-olds and 35% of young adults consumed cereal at non-breakfast period (Haines *et al.,* 1996); it could be taken dry as snack food, with or without cold or hot milk, based on their location, availability of resources and habits.

The growing third world population requires more protein and nutritious food to combat malnutrition. The cheapest source of protein and other nutrients could be derived from underutilized plant materials that are in abundance in the developing countries. It is evident that plant nutrients are the best alternative to proteins derived from animal source (Sharma & Caralli, 2004). The utilization of cereals and legumes-based foods by the human race offers them an essential place in global nutrition which plays a vital part in the conventional food practice of many provinces all over the world. Underutilized crops make a significant position in human nutrition particularly in the dietary pattern of low economic population from budding countries are said to be the best combination for delivering good nutrients

(Anderson *et al*., 1999 & Messina, 1999). Underutilized cereals and legumes provide positive health responses when they are properly examples positioned in the daily diet (Kushi *et al.,* 1999). Kaur *et al.* (2014) revealed associations between the utilization of legumes and declining prevalence of numerous diseases for example: aging, cancer, diabetes and cardiovascular diseases. Jacobs (1998) reported that cereals and its products show beneficial effects in reducing the risk of cancer. The relationship between cereal intake and different type of cancer has been evidenced by several researchers (Anderson *et al*., 1999)

Millet are rich in resistant starch, soluble and insoluble dietary fibers, minerals and antioxidants (Ragee *et al.,* 2006). It contains about 92.5% dry matter, 2.1% ash, 2.8% crude fiber. 7.8% crude fat, 13.6% crude protein, 63.2% starch, (Ali & EI-Tinay,2003). Thus, the availability of these nutrients in millet makes it suitable for large scale utilization in the production of food products such as: breakfast foods. Mung bean (*Vigna radiata*) is still underutilized as food due to its tough texture, long cooking time and lack of knowledge on its nutritional composition. Its consumption is occasional. Mung bean can provide significant amounts of protein (240 g/kg), carbohydrate (630 g/kg) and a range of micronutrients in diets (Anwar *et al.,* 2007). Mungbean protein and carbohydrates are easily digested and create less flatulence than those derived from other legumes. Tigernut (*Cyperus escullentum*) tuber is an underutilized crop, which contain 38% Kcal (1635kg), 7.15% protein, 35% fat (oil), 46% starch, 6% fibre. It is also rich in mineral especially phosphorus and potassium and vitamin E and C (Belewu & Abodunrin, 2008, Oladele & Aina 2007).

Apart from supplementation and processes that reduces or removes antinutrient substances in breakfast cereals, there is need to investigate the nutrient composition as well. Knowledge of food composition (Greenfield & Southgate, 2003) and anti-nutritional composition of food is important for nutrition planners and also understanding their behavior during preparation. Response surface methodology (RSM) is a statistical technique for the design, empirical modelling and optimization of processes, where the responses of interest are influenced by several process variables (Freeny, Box, & Draper,1988; Gunst *et al.,* 1996).Response surface methodology (RSM) was applied to investigate the relationships between different independent and response variables while minimizing the number of experiments and usage of resources. Hence, the objective of this study was to model and optimize the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour using response surface methodology.

II. MATERIALS AND METHODS

2.1 Materials

Millet (Green pearl millet) and tigernut were purchased from Relief market, Imo state, Nigeria while mungbean (Green) was supplied from Kingsway Market Apapa Lagos state, Nigeria. The millet, tigernut and mungbean were identified in Crop Science Technology Department, FUTO. The chemicals used were of analytical grade; and both the equipment were obtained from the Department of Food Science and Technology, Federal University of Technology Owerri, Imo State; Polytechnic, Umuagwo-Ohji; University of Ilorin, University of Jos Plateou State, Nigeria and National Root Research Institute Umudike, Umuahia Abia State Nigeria. Proximate composition analysis was carried out at Nigerian Store Product Research Institute Port Harcourt. Anti-nutritional composition analysis was carried out at Nigeria and National Root Research Institute Umudike, Umuahia.

2.2 Methods

2.2.1 Production of Millet flour

The method of Jideani (2005) was adopted for the production of millet flour. Two (2kg) kilograms of millet was cleaned, sorted to remove dirt and any form of impurity. The cleaned and washed millet were soaked for 12 h at ambient temperature and rinsed thoroughly with distilled water. The millet was dried in an oven (DHG-9023A, Zenith Laboratory China) at 65^{o}C for 6 h. It was further milled into flour with attrition mill and sieved (300 mm sieve) to obtain fine flour. The flour sample obtained was packaged in an air tight container and kept for further analysis. The flow chart for millet four production is shown in Figure 1.

2.2.2 Production of Mungbean flour

Sprouted mungbean flour was processed following the method described by Mubarak (2005) with slight modification. Two kilograms (2kg) of mungbean seed was cleaned, sorted and washed with distilled water, soaked by submerging the sample in distilled water at ambient temperature in a transparent container for 12 h at ambient temperature. Thereafter, the seeds were spread out on a sterile jute bag and covered with a muslin cloth and kept at an ambient temperature. The sample was allowed to germinate (sprout for 24 h), during this period, distilled water was sprinkled on the white muslin cloth every 6 h. After 24 h, the sprouted seeds were cleaned and dried in an air oven (DHG-9023A, Zenith Laboratory China) at 60^{o}C, for 9 h, and milled to obtain malted mungbean flour, packaged and stored in an air tight container for further analysis.

2.2.3 Production of Tiger nut flour

Two kilograms (2kg) of fresh tigernut was cleaned, sorted, and washed thoroughly to remove any form of impurity. Cleaned tigernuts were milled into paste, homogenized in boiled water (100^{o}C) and poured into a muslin cloth and squeezed to express the milk. The tigernut spent mash was dried (60^{o}C for 8 h) in an oven (DHG-9023A, Zenith Laboratory China), packaged in air tight container for further analysis (Adejuyitan, 2011).

2.2.4 Production of breakfast cereals

The method of Okafor & Usman (2014) was adopted for the production of breakfast cereals. One hundred grams (100 g) each of millet flour, sprouted mungbean flour and tigernut flour were blended. Two grams (2 g) of sugar, 0.5 g of salt, 5 ml of vanilla flavor and 20 ml of distil water were added. They were mixed together and toasted at 150^0C for 10 min in an oven. Thereafter, it was cooled, milled and packaged (Figure 1)

2.3 Determination of Proximate Composition

The proximate (moisture, ash, fiber, fat, and protein) content of the blends were determined following the standard methods of AOAC (2015). The carbohydrate content of the samples was calculated by simple difference method as reported by Onwuka (2018).

2.3.1 Moisture Content

Two grams (2 g) of each of the sample was weighed into dried weighed crucible. The samples were put into a moisture extraction oven at 105^0C and heated for 3 h. The dried samples were put into desiccator, allowed to cool and reweighed. The process was repeated until a constant weight was obtained. The difference in weight was calculated as a percentage of the original sample.

$$\text{Percentage moisture} = \frac{W_2 - W_2}{W_2 - W_3} \times \frac{100}{1} \quad (1)$$

Where

W_1 = Initial weight of empty dish, W_2 = Weight of dish + un-dried sample

W_3 = Weight of dish + dried sample

2.3.2 Ash Content

Two grams (2 g) of each of the samples were weighted into crucibles and heated in a moisture extraction oven for 3 h at 100^oC before being transferred into a muffle furnace at 550^oC until it turned ash/ white and free of carbon. The sample was then removed from the furnace, cooled in a desiccator to a room temperature and reweighed immediately. The weight of the residual ash was then calculated as

$$Percentage\ ash\ content = \frac{Weight\ of\ Ash}{Weight\ of\ original\ \ sample} \times \frac{100}{1} \quad (2)$$

2.3.3 Crude Protein

Two grams (2 g) of each of the samples was mixed with 10 ml of concentrated H_2SO_4 in a test tube. One tablet of selenium catalyst was added to the test tube and mixture heated inside a fume cupboard. The digest was transferred into distilled water. A 10 ml portion of the digest, mixed with equal volume of 45% NaOH solution was poured into a kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into a flask containing 4% boric acid solution and 3 drops of methyl red indicator. A total of 50 ml distillate was collected and titrated against sodium hydroxide. Triplicate values were measured and the mean obtained. The Nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein content. This is given as percentage

$$\text{Nitrogen} = \frac{(100 \times N \times VF)T}{100 \times V_a} \quad (3)$$

Where

N= Normality of the titrate (0.1N), VF= Total volume of the digest= 100 ml, T= Titre Value

Va= Aliquot Volume distilled

2.3.4 Fat Content

Two grams (2 g) of the sample was loosely wrapped with a filter paper and put into the thimble which was fitted to a clean round bottom flask, which has been cleaned, dried and weighed. The flask contained 120 ml of petroleum ether. Thesample was heated with a heating mantle and allowed to reflux for 5 h. The heating was then stopped and the thimbles with the spent samples kept and later weighed. The difference in weight was calculated as mass of fat and is expressed in percentage of the sample.

$$\text{The percentage oil content is percentage fat} = \frac{W_2 - W_1}{W_3} \times \frac{100}{1} \quad (4)$$

Where

W_1 = weight of the empty extraction flask, W_2 = weight of the flask and oil extracted

W_3 = weight of the sample

2.3.5 Crude Fiber

Two grams (2 g) of the sample and 1g asbestos were put into 200 ml of 1.25% of H_2S0_4 and boiled for 30 min. The solution was poured into Buchner funnel equipped with muslin cloth and secured with elastic band. This was allowed to filter and the residue was then put into 200 ml boiled NaOH and boiling continued for 30 min, then transferred to the Buchner funnel and filtered. It was then washed twice with alcohol, the material obtained washed thrice with petroleum ether. The residue obtained was put in a clean dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. The difference in weight (i.e. loss in ignition) was recorded as crude fibre and expressed as

$$\text{Percentage Crude Fibre} = \frac{W_1 - W_2}{W_t} \times \frac{100}{1} \quad (5)$$

Where

W_1 = weight of sample before incineration, W_2 = weight of sample after incineration

W_t = weight of original sample

2.3.6 Carbohydrate

The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameters as Nitrogen free Extract (NFE) percentage carbohydrate (NFE) $= 100 - (M + P + F1 + A + F2)$ (6)

Where:

M = Moisture, P = Protein, F_1 = Fat, A = ash, F_2 = Crude fiber

2.4 Determination of Anti-nutritional Factors

2.4.1 Oxalate

The titration method of (AOAC, 2015) was followed in the determination of oxalate. Two grams of sample was suspended in a mixture of 190 ml of distilled water in a 250 ml volumetric flask. Ten (10 ml) of 6M HC1 and the suspension was heated for 1 h at 100°C in a water bath. The mixture was cooled and made up to 250 ml mark with distilled water before filtration. Duplicate portion of 125 ml of the filtrate was measured into 250 ml beakers. Each extract was made alkaline with concentrated sodium hydroxide then made acid by drop wise addition (4 drops) of acetic acid until the test solution is changed from salmon pink to faint yellow (pH 4-4.5) (methyl red indicator used). Each portion was heated at 90°C to remove precipitate containing ferrous ions. The filtrate was heated again to 90°C on a hot water bath and 10 ml of 5% calcium chloride solution added while being stirred constantly. After heating, it was centrifuged at full speed (2500 rpm) for 5 min. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution and the total filtrate resulting from 2 g of the sample was made up to 300 ml.

*Permaganate titration***:** Aliquot of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05M $KMNO_4$ solution to a faint pink color which persisted for 30 sec. Oxalic acid content was calculated using the formula,

$$\%\text{Oxalic acid} = \frac{T\ x\ (V_{me})(D_f)x\ 10^5}{ME\ x\ M_f} \quad (7)$$

Where: T = Titre of $KMN0_4$ (ml), V_{me} = volume - mass equivalent (1 ml of 0.05M MNO_4 solution is equivalent to 0.0022 g anhydrous oxalic acid), Df = the dilution factor (i.e. 300 ml) 125 ml, ME = the molar equivalent of $KMNO_4$ in oxalic acid ($KMNO_4$ redox reaction is 5), Mf = the mass of the sample used.

2.4.2 Phytate or Phytic Acid

The phytate determination was carried out as described by A.O.A.C (2015). Two grams of each of the samples was placed in a flask into which 100 ml of 1.2 HC1 and 10% Na_2S0_4 were added. The flask was stoppered and shaken for 2 h on a mechanical shaker. The extract was vacuum filtered through No. 4 Whatman paper. 10.0 ml of the filtrate was pipetted into a 50 ml centrifuge tube. Ten (10ml) deionized water was added, followed by 12 ml of FeCl solution (2.0g $FeCl_3.6H_20$) + 16.3 ml cone. HC1 per litre). The mixture was stirred, heated for 75 min in boiling water and cooled, covered for 1.0 h at room temperature. The tube was centrifuged at l000Xg for 15 min. The supernatant was decanted and discarded and the pellet was thoroughly washed thrice with a solution of 0.6% HC1 and 2.5% $NaSO_4$. After each wash, the mixture was centrifuged at l000Xg for 10 min and the supernatant discarded. 10 ml concentrated HNO_3 was added to the resulting pellet and transferred quantitatively to a 400 ml beaker with several small portions of deionized water. Four drops of concentrated H_2SO_4 was added and content heated approximately 30 min in a hot plate until only the H_2S0_4 is left. Approximately 5ml of 30% H_2O_2 was added and the mixture returned to the hot plate at a low heat until bubbling ceases. The residue was dissolved in 15 ml 3N HC1 and heated for 15 min. The resulting solution was made up of 100.0 ml volume diluted 15 and then analyzed for iron using Franson *et al.* (1975) procedure.

2.4.3 Tannin

The Folin-Denis colorimetric method as described by Kirk & Sawyer (1991) was used for the determination of tannin content in the samples as follows: 5g of the sample was dispersed in 50 ml of distilled water and agitated. The mixture was allowed to stand for 30 min at room temperature and shaken every 5min. After 30min it was centrifuged at 1000xg for 30min and the extract obtained. The extract (2ml) was taken into a 50ml volumetric flask. Similarly, 2ml standard tannin solution (tannic acid) and 2ml of distilled water was put in separate 50ml volumetric flask to serve as standard. The reagent (1.0ml of Folin-Denis) was added to each of the flasks, followed by addition of 2.5ml of saturated sodium carbonate solution. The content of each flask was made up to 50ml with distilled water and allowed to incubate for 90min at room temperature. Their respective absorbance was measured in a spectrophotometer (SP-1901, Shnaghai Spectrum Instrument Co., China) at 250nm using reagent blank to calibrate the instrument at zero. The tannin content was calculated using the formula,

$$\%\ \text{Tannin} = \text{An/W x C/V}_a \text{ x Vf x } 100/1 \quad (8)$$

Where:

An = Absorbance of test sample, AS = Absorbance of standard solution, C = Concentration of standard solution, W = Weight of sample used, Vf = Total volume of extract, V_a =Volume of extract analyzed.

2.5 Experimental Design and Statistical Analysis

The three simplex centroid experimental design of response surface methodology (RSM) as described by Scheffe (1963) was used to develop predictive models and to investigate the linear, binary and ternary blends of process parameters (millet flour, mungbean flour, tigernut flour) on the proximate and anti-nutritional composition of breakfast cereals in which 14 runs/design points were conducted. Four runs were replicated to estimate the internal error within the design as shown in

Table 1. Analysis of variance (ANOVA) was carried out on data from proximate and anti-nutritional composition of flours (millet, mungbean and tiger nut). A p-value ($p<0.05$) was considered significant as shown in Table 2. The generation of response surface plots and statistical analysis were performed using Design-Expert (Version 12.0.6.2, State-Ease, Inc. Minneapolis, 2015) software. ANOVA was also performed using this software and model significance ($p<0.05$), lack of fit and adjusted regression coefficients (R^2_{adj}) which indicate the model fitness were determined from the analysis. Special cubic model was adopted as stated below:

$$y = \sum_{i=1}^{q} \beta_i x_i + \sum_{i \neq j}^{q} \beta_{ij} x_i x_j + \sum_{i \neq j \neq k}^{q} \beta_{ijk} x_i x_j x_k + \varepsilon_{ijk} \quad (9)$$

Where, β_i are the main effects, β_{ij} are the binary joint effects between the i^{th} and j^{th} components, β_{ijk} are the ternary joint effects between the i^{th}, j^{th} and k^{th} components.Y is the predicted response, q is the number of process parameters (q = 3 in this study), ε_{ijk} is error involved in estimating the components from the experimental data. The special cubic model equation proposed for each response of Y can also be written as

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \quad (10)$$

where Y is the predicted response, β's are the parameter estimates for each linear and cross product term for the prediction model, x_1, x_2, x_3, x_1x_2, x_1x_3,x_2x_3 and $x_1x_2x_3$are the linear terms of *millet*, *mungbean* and *tiger nut* and the cross product terms of *millet* x *mungbean*, *millet* x *tiger nut*, *mungbean* x *tiger nut*, and *millet* x *mungbean* x *tiger nut* flours respectively. The model chosen was based on a significant model ($p<0.05$), insignificant lack of fit and highest R^2 as recommended by Cornell (1986).The criteria for the responses were stated and numerically optimized (Myers, Montgomery,& Anderson-Cook, 2009) as shown in Table 3& 4.The model was validated by plotting the actual values or experimental values against the predicted values (Vining, Cornell, &Myers, 1993) as shown in Figure 2

III. RESULTS AND DISCUSSION

3.1 Proximate composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour

3.1.1 Moisture content

The moisture content of the breakfast cereals is shown in Table1. The values ranged from 5.57% to 7.38%. The variation in moisture content was due to the processing methods of individual flour. This was subjected to statistical analysis as indicated in Table 2. Statistically, significant ($p<0.05$) linear effects of millet (6.37), mungbean (7.43) and tigernut (5.85) were observed in moisture content as shown in Table 2. This is in line with the findings of Ghavidel & Davoodi (2014). Moisture content of foods is influenced by type, variety and storage condition (Eshun, 2012). Therefore, the storage of this product would require careful reduction of the moisture content by drying. A very high degree of precision and a good deal of the reliability of the experiment was indicated by a low value of the coefficient of variation (5.78%) as shown in Table 2. The R^2 (0. 6783) and non-significant ($p>0.05$) lack of fit showed the adequacy of the model. The model was significant ($p = 0.0020$) and it explained 61.98% of all variance in the data. The remaining 38.02% are attributed to factors not included in the model. The 3-D surface plot for the moisture content is shown in Figure 2. The model was validated by plotting the graph of the predicted values against the actual values as shown in Figure 11. Good correlation existed between the predicted values and actual values. Hence, the significant ($p<0.05$) model equation for the moisture content was

$$Moisture\ content = 6.37x_1 + 7.43x_2 + 5.85x_3 \quad (11)$$

3.1.2 Ash content

Table 1 showed the ash content of the breakfast cereals. The values ranged from 1.13% to 3.33%. The difference in ash content between the samples was probably due to processing technique, heat treatment, and difference in cultivar (Mbaeyi-Nwaoha & Uchendu, 2015; Mateos-Aparicio *et al.*,2010). Ash content is an indication of mineral content of a food. However, statistically as shown in Table 2, there was highly significant ($p<0.0001$) linear effects of millet ($1.12x_1$), mungbean ($3.33x_2$) and tigernut ($1.77x_3$) which resulted in high ash content of the breakfast cereal. Also, significant ($p<0.05$) binary effects of millet and mungbean flours ($-0.71x_1x_2$), millet and tigernut flours ($-0.74x_1x_3$) produced low ash content. The highest increase in the ash content was observed in ternary effects of millet, mungbean and tigernut flours ($5.12x_1x_2x_3$).The increase in ash is indicative of high mineral content of the breakfast cereals from blends of millet, mungbean and tigernut flours. The low ash content observed may be as a result of the sprouting of mungbean.Mbaeyi & Onweluzo, (2010) reported that sprouting decreased ash content. The treatments (sprouting) aided the removal of the vegetative part of the seeds during milling leading to losses in dry matters and leaching of the nutrients.Furthermore, low value of the coefficient of variation (1.82%) indicated higher precision and reliability of the experiment. The R^2 (0.9986) and non-significant ($p>0.05$) lack of fit showed the adequacy of the model. The model was highly significant ($p<0.0001$). It explained 99.74% of all variance in the data while the remaining 0.26% was caused by factors not included in the model. The plot of predicted values and actual values showed the validity of the model as indicated in Figure 12. This showed that the predicted and actual values were closely related. The 3-D surface plot for the ash content is shown in Figure 3. The significant ($p<0.05$) model equation for ash content was:

$$Ash\ content = 1.12x_1 + 3.33x_2 + 1.77x_3 - 0.71x_1x_2 - 0.74x_1x_3 + 5.12x_1x_2x_3 \quad (12)$$

3.1.3 Crude fiber

Crude fiber ranged from 4.72% to 24.63% as shown in Table 1. Crude fiber is a measure of the quantity of indigestible cellulose, pentosans, lignin and other components of this type in present foods (Arawande & Borokini, 2010). Table 2 showed that linear effects of millet ($5.40x_1$), mungbean ($6.38x_2$) and tigernut flour ($24.46x_3$) were highly significant ($p<0.0001$). This produced high crude fiber. Oladunmoye *et al*., (2010) reported that food rich in crude fiber helps in the treatment of heart diseases, colon cancer, diabetes etc. Furthermore, Table 2 indicated that a low value of the coefficient of variation (17.08%) showed a very high degree of precision and a good deal of the reliability of the experiment. The R^2 (0.9549) and non-significant ($p>0.05$) lack of fit showed the adequacy of the model. The model was highly significant ($p<0.0001$). The model explained 92.67% of all variance in the data. The predicted values were plotted against actual values as shown in Figure 13. A close relationship between predicted and actual values was observed which showed the validity of the model. The 3-D surface plot for the ash content is shown in Figure 4.Therefore, the significant ($p<0.05$) model equation for the crude fiber was:

$$Crude\ fiber = 5.40x_1 + 6.38x_2 + 24.46x_3 \quad (13)$$

3.1.4 Fat content

Table 1 indicated that the fat content ranged from 1.77% to 16.21%. The differences in fat content may be due to location and varietal differences (Moss, Gore, & Murray, 1987). Statistically, high fat content of the breakfast cereals was observed in the highly significant ($p<0.0001$) linear effects of millet ($5.96x_1$), mungbean ($1.56x_2$) and tigernut flour ($15.92x_3$) while the significant ($p<0.05$) binary effects of millet and tigernut flours ($-20.93x_1x_3$) produced low fat content as shown in Table 2. Aiyesanmi & Oguntokun (1996) revealed that diets with high fat content contribute significantly to the energy requirement for humans. High fat flours are also good for flavour enhancers and useful in improving palatability of foods in which it is incorporated. This implies that this product would be energy dense foods suitable for people such as sportsmen that require lot of energy to work. Also, a very high degree of precision and a good deal of the reliability of the experiment was indicated by a low value of the coefficient of variation (22.77%) as shown in Table 2. The R^2 (0. 9254) and non-significant ($p>0.05$) lack of fit showed the adequacy of the model. The model was significant ($p = 0.0003$) and it explained 87.88% of all variance in the data. The remaining 12.12% are attributed to factors not included in the model. The 3-D surface plot for the fat content is shown in Figure 5. The model was validated by plotting the graph of the predicted values against the actual values as shown in Figure 14. This showed that the predicted values and actual values were closely related. Hence, the significant ($p<0.05$) model equation for the fat content was:

$$Fat\ content = 5.96x_1 + 1.56x_2 + 15.92x_3 - 20.93x_1x_3 \quad (14)$$

3.1.5 Protein content

The protein content of breakfast cereals ranged from 8.56% to 21.59% as observed in Table 1. The protein content differences can be attributed to the geographical location. Since soils with high nitrogen levels can influence protein levels (Brown, 1991). Table 2 showed that highly significant ($p<0.0001$) linear effects of millet ($11.30x_1$), mungbean ($22.06x_2$) and tigernut ($9.11x_3$) produced high protein content of the breakfast food product. The high protein content of the products may be attributed to the presence of mungbean flour component used in the product. Mungbean has been reported to contain 25% protein (Dongyan *et al.*, 2014). Temple & Bassa (1991) reported that addition of legume to cereals improves the level of protein. The protein content of the flours in this product suggests that they may be useful in food formulation systems. Protein is needed for tissue replacement, deposition of lean body mass and growth. Moreover, a very high degree of precision and a good deal of the reliability of the experiment was indicated by a low value of the coefficient of variation (8.74%) as shown in Table 2. The R^2 (0.9344) and non-significant ($p>0.05$) lack of fit showed the adequacy of the model. The model was highly significant ($p<0.0001$) and it explained 92.25% of all variance in the data. The remaining 7.75% are attributed to factors not included in the model. The 3-D surface plot for the moisture content is shown in Figure 6. The model was validated by plotting the graph of the predicted values against the actual values as shown in Figure 15. Good correlation existed between the predicted values and actual values as observed from the plot. Thus, the significant ($p<0.05$) model equation for the protein content was:

$$Protein\ content = 11.30x_1 + 22.06x_2 + 9.11x_3 \quad (15)$$

3.1.6 Carbohydrate

Table 1 showed that the carbohydrate content ranged from 43.26% to 70.3%. Carbohydrates are good sources of energy and that a high concentration of it isdesirable in breakfast meals and weaning formulas. Highly significant ($p<0.0001$) linear effects of millet ($70.06x_1$), mungbean ($59.14x_2$) and tigernut ($43.43x_3$) produced high carbohydrate while the significant ($p<0.05$) binary effects of mungbean and tigernut flours ($20.68x_2x_3$) produced the highest carbohydrate as indicated in Table 2. The high carbohydrate content of this product suggested that it could be used in managing protein-energy malnutrition since there is enough quantity of carbohydrate to derive energy from in order to spare protein so that protein can be used for its primary function of building the body and repairing worm-out tissues rather an as a source of energy (Butt & Batool, 2010). Awolu *et al.,* (2015)

reported that carbohydrates are good sources of energy and that a high concentration of it is desirable in breakfast meals and weaning formulas. A low value of the coefficient of variation (2.08%) showed a very high degree of precision and a good deal of the reliability of the experiment. The R^2 (0.9864) and non-significant (p>0.05) lack of fit showed the adequacy of the model. The model was highly significant (p<0.0001). The model explained 97.78% of all variance in the data. The predicted values were plotted against actual values as shown in Figure 7. The plot indicated that the predicted and actual values were closely related. This showed the validity of the model. The 3-D surface plot for the ash content is shown in Figure 16.

Hence, the significant (p<0.05) model equation for the carbohydrate was:

$$Carbohdrate = 70.06x_1 + 59.14x_2 + 43.43x_3 + 20.68x_2x_3 \quad (16)$$

3.2Anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour

3.2.1 Oxalate

Oxalate content ranged from 0.27 to 2.05 mg/100g as shown in Table 1. Oxalate is an abundant metabolic end product in plants. The variation in the oxalate content of foods is mainly determined by the plant family (Siener *et al*., 2006). Statistically, significant (p<0.05) linear effects of millet ($1.83x_1$), mungbean ($3.12x_2$) and tigernut ($0.89x_3$) produced high oxalate as indicated in Table 2. Franziska (2017) reported that many foods that contained oxalates are delicious and provide many health benefits. Avoiding them is not necessary for most people and may even be detrimental. Most healthy people can consume oxalate rich foods without problems but those with altered gut function may need to limit their intake (Habauzet & Morand, 2012).Morrison & Savage (2003) revealed that the consumption of high-oxalate foods is more likely to pose health problems in those who have an unbalanced diet or those with intestinal malfunction. In healthy individuals, the occasional consumption of high-oxalate foods as part of a balanced diet does not pose any particular problem. Moreover, low value of the coefficient of variation (33.07%) indicated higher precision and reliability of the experiment. The non-significant (p>0.05) lack of fit showed the adequacy of the model. The plot of predicted values and actual values showed the validity of the model (Figure 17). Good correlation was observed between the predicted and actual values. The 3-D surface plot for the oxalate is shown in Figure 8. The significant (p<0.05) model equation for oxalate was:

$$Oxalate = 1.83x_1 + 3.12x_2 + 0.89x_3 \quad (17)$$

3.2.2Phytate

Phytate ranged from 0.57 to 2.82mg/100g as shown in Table 1. The variation may be caused by many factors, such as genetics, environmental fluctuations, location, irrigation conditions, type of soils, year and fertilizer application that can affect the phytic acid content and phosphorus availability in cereal grains. Phytate are the principal storage form of phosphorus and are particularly abundant in cereals and legumes (Reddy *et al.*,1982). Table 2 showed that the linear effect of millet ($0.27x_1$), mungbean ($1.15x_2$) and tigernut ($1.43x_3$) produced high phytate. Graf *et al*. (1987) reported that the most important beneficial functions of phytates were their anti-cancer activity which inhibits the growth of cancer cells. Furthermore, the non-significant (p>0.05) lack of fit showed the adequacy of the model (Table 2). The 3-D surface plot for phytate is indicated in Figure 9. The model was validated by plotting the graph of the predicted values against the actual values as shown in Figure 18. Good correlation existed between the predicted values and actual values. Thus, the significant (p<0.05) model equation for phytate was:

$$Phytate = 027x_1 + 1.15x_2 + 1.43x_3 \quad (18)$$

3.2.3Tannin

Table 1 showed that tannin ranged from 0.15 to 1.62mg/100g. The linear effect of millet ($-0.34x_1$), mungbean ($-0.55x_2$) and tigernut ($-1.78x_3$) significantly (p<0.05) decreased the tannin while the binary effects of millet and tigernut ($4.77x_1x_3$)), mungbean and tigernut ($4.58x_2x_3$)significantly (p<0.05) increased the tannin as observed in Table 2.Consumption of tannin rich foods helped to treat and prevent cancer (Huang *et al*., 2010). Tannins are water-soluble polyphenols that are present in many plant foods (Chung *et al*., 1998). Moreover, the R^2 (0.8453) and non-significant (p>0.05) lack of fit showed the adequacy of the model. The model was significant ($p = 0.0042$). The model explained 74.86% of all variance in the data. The model was validated by plotting the predicted values against actual values as shown in Figure 19. Good correlation was observed between the predicted and actual values. This showed the validity of the model. The 3-D surface plot for tannin is shown in Figure 10. Hence, the significant (p<0.05) model equation for the tannin was:

$$Tannin = -0.34x_1 - 0.55x_2 - 1.78x_3 + 4.77x_1x_3 + 4.58x_2x_3 \quad (19)$$

3.3 Optimization

Numerical optimization of Design Expert" software (Version 12.0.5, Stat-Ease, Inc., Minneapolis, USA) statistical package was adopted to optimize the individual responses to search for a combination of independent variables levels that simultaneously satisfy the target requirement placed on each response and factors. The goal, lower limit, upper limit, lower weight, upper weight and importance were set for each independent variable and response variable as shown in Table 3. Setting the importance at 3 for both the independent and response variables means that no goals are favored over others. The optimum values (Millet flour: 0.000g, Mungbean flour: 70.37g, Tigernut flour: 29.62g, moisture content:

6.96%, ash content: 2.92%, crude fiber: 10.02%, fat content: 6.82%, protein content: 18.22%, carbohydrate: 58.80%, oxalate: 0.40mg/100g, phytate: 0.81mg/100g and tannin: 1.04mg/100g) with desirability of 0.464 were selected as shown in Table 4. Numerical optimization ramps view for the variables were indicated in Figure 20. Ramps are graphical representation of optimal solution. Flat ramps indicate uniform desirability (millet flour, mungbean flour and tigernut flour), whereas inclined ramps represent minimum/maximum desired value. Red and blue dots represent factors and responses, respectively. The height of dot corresponds to the level of desirability achieve upon optimization. Desirability bar graph was shown in Figure 21. The bar graph shows how well each variable satisfies the criteria: values near one are good. 3D desirability plot was shown in Figure 22.

3.4 Confirmation of optimum values (Two-sided Confidence = 95%)

Table 5 showed the confirmation of optimum values (Two-sided Confidence = 95%) for modelling and optimization of the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour. Six confirmatory runs were done to ascertain 95% confidence. It was observed that predicted mean and data mean for each response was close and also, within the range of actual or experimental values. The standard deviation (SD) of the responses (moisture content, protein content, ash content, fat content, crude fibre, carbohydrate, oxalate, phytate and tannin) were low. This showed how close the individual data are from the mean value. Also, the standard error predicted for the responses was small. A small standard error (SE) is an indication that the sample mean is a more accurate reflection of the actual population mean. The standard error is an indication of the reliability of the mean. Furthermore, 95% prediction interval (PI high) based on the sample showed that there is a 95% probability that a future observation will be contain within the prediction interval. Conversely, there is also a 5% probability that the next observation will not be contained within the interval. A prediction interval is a range of values that is likely to contain the value of a single new observation given specified settings of the predictors. Prediction interval account for the variability around the mean response inherent in any prediction (Scheffé, 1963). Thus, 0.00 g of millet, 70.36 g of mungbean and 29.63 g of tigernut yielded the best breakfast product.

IV. CONCLUSION

The three component argumented simplex centroid experimental designed of the response surface methodology (RSM) was adequate in modelling and optimization of the protein, carbohydrate, moisture content of the breakfast meal. Also, the RSM also predicted the oxalate, phytate and the tannin adequately. The predictive equations were generated for all the parameters investigated. The response surface plots (3D) graphically represented the linear, binary and ternary interaction of the millet, mungbean and tigernut flours. A good correlation was obtained between the predicted and actual (experimental) values, which validated the developed models. The optimum quantity of the blends in the breakfast food selected at a desirability of 0.464 were 0.00 g millet, 70.36 g mungbean and 29.63 g tigernut; suggesting that blends of the above will give the best breakfast product. The developed model would be of great importance to the food manufacturers to maximize the nutritive needs of consumers who are protein, mineral and energy deficient. This product would be of health importance to cancer patients.

COMPLIANCE WITH ETHICAL STANDARDS STATEMENT

Declaration of Interest: None

Ethical Statement

Hereby, I consciously assure that for the manuscript "Modelling and optimization of proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour using response surface methodology", the following is fulfilled:

1. This material is the authors' own original work, which has not been previously published elsewhere.
2. The paper is not currently being considered for publication elsewhere.
3. The paper reflects the authors' own research and analysis in a truthful and complete manner.
4. The paper properly credits the meaningful contributions of co-authors and co-researchers.
5. The results are appropriately placed in the context of prior and existing research.
6. All sources used are properly cited

Consent Statement

I hereby declare that we participated in the study and in the development of the manuscript titled "Modelling and optimization of proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour using response surface methodology". Authors and co-authors have read the final version and give our consent for the article to be published.

Data Availability Statement

Authors can confirm that all relevant data are within the article.

ACKNOWLEDGEMENTS

I sincerely express my gratitude to the Almighty God for His infinite mercy, direction, protection, guidance, provision and love throughout the duration of this study.

Author Statement

Eweama, A.U.: Conceptualization, Writing-Original draft, Writing-Reviewing and Editing, Investigation, Formal Analysis.

Nwosu, J.N.: Supervision, Resources.

Owuamanam, C.I.: Supervision, Resources. **Obeleagu, S.O.:** Methodology, Validation.

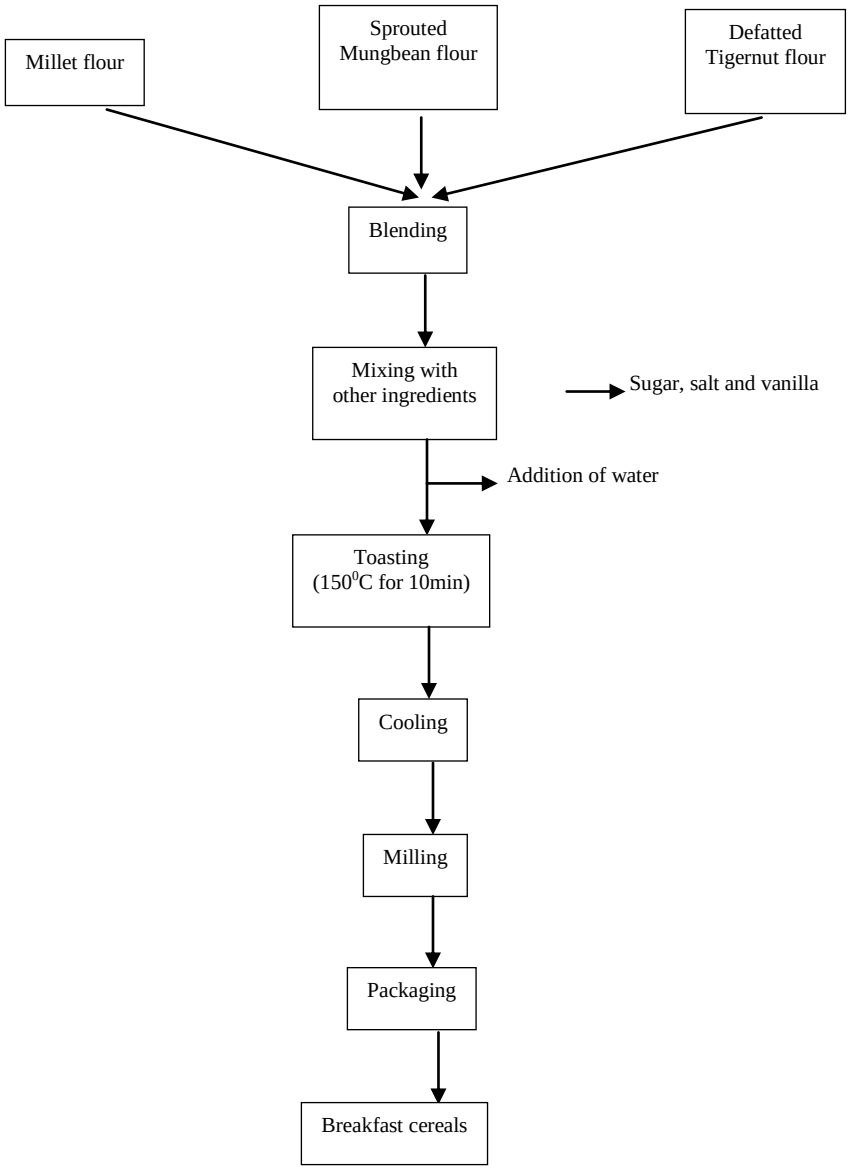

Figure 1: Modified flow diagram for the production of breakfast cereals from blends of Millet, mungbean and tigernut flours
Source: Okafor & Usman (2013)

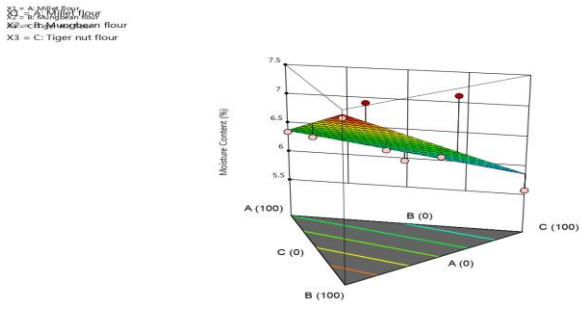

Figure 2: 3-D surface plot for the moisture content of breakfast cereals

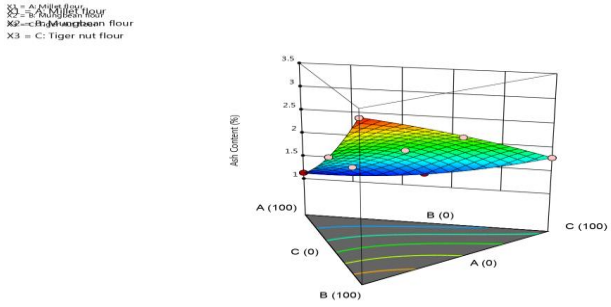

Figure 3: 3-D surface plot for the ash content of breakfast cereals

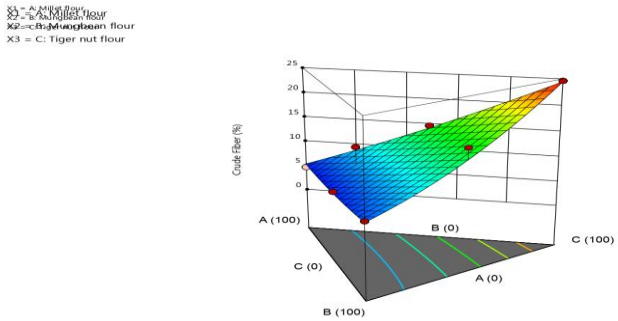

Figure 4: 3-D surface plot for the crude fiber of breakfast cereals

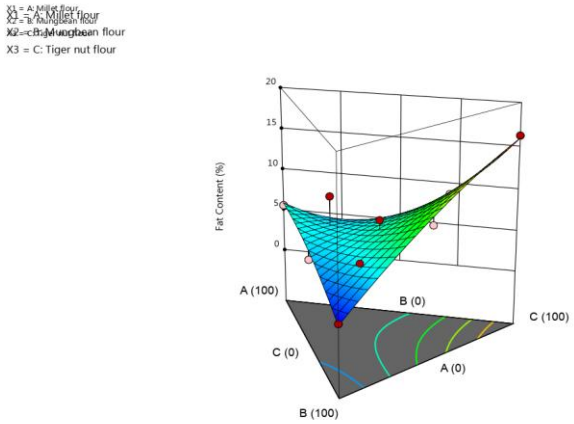

Figure 5: 3-D surface plot for the fat content of breakfast cereals

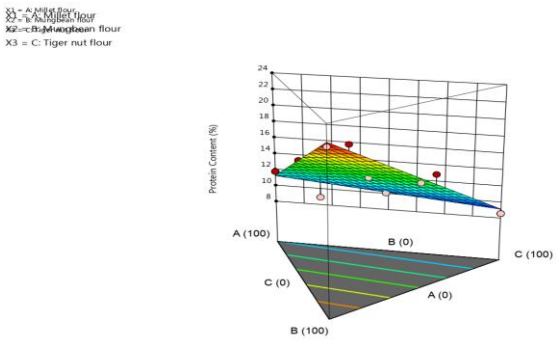

Figure 6: 3-D surface plot for the protein content of breakfast cereals

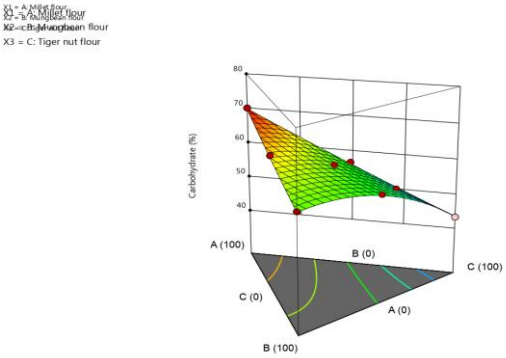

Figure 7: 3-D surface plot for the carbohydrate content of breakfast cereals

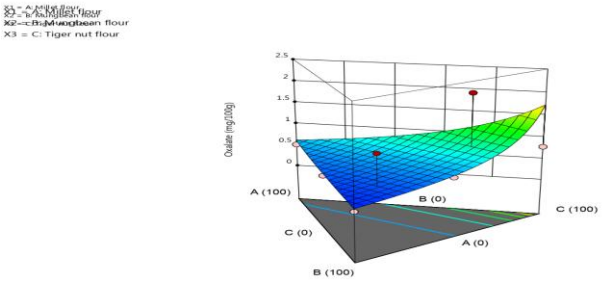

Figure 8: 3-D surface plot for the oxalate of breakfast cereals

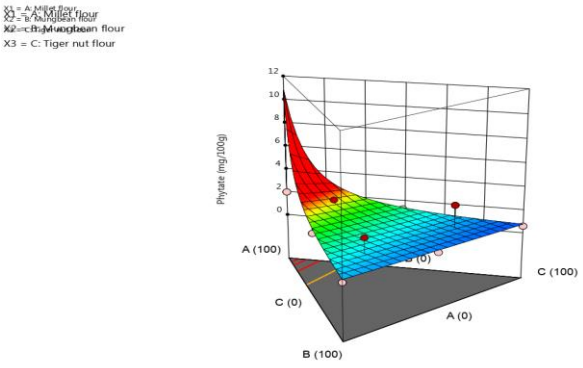

Figure 9: 3-D surface plot for the phytate of breakfast cereals

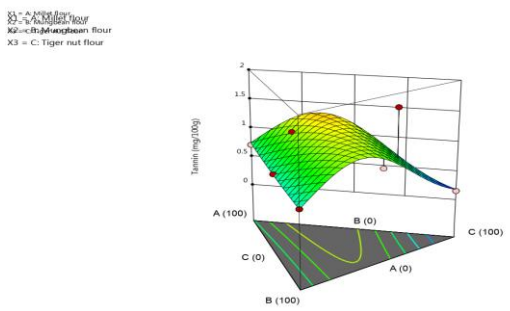

Figure 10: 3-D surface plot for the tannin of breakfast cereals

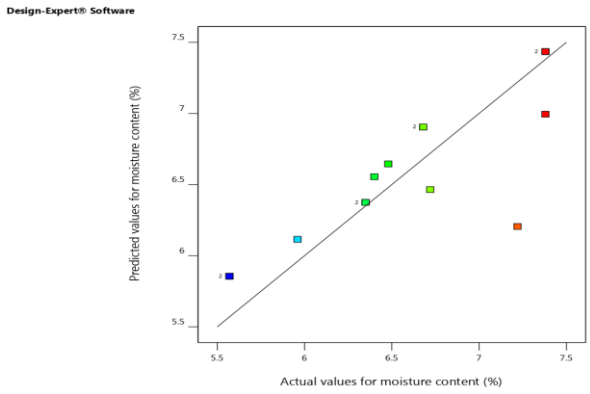

Figure 11: Graph of predicted against actual values for the moisture content of breakfast cereals

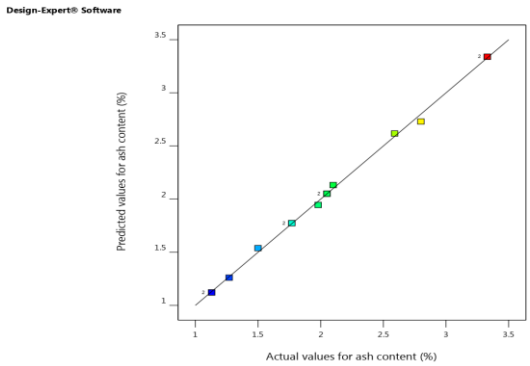

Figure 12: Graph of predicted against actual values for the ash content of breakfast cereals

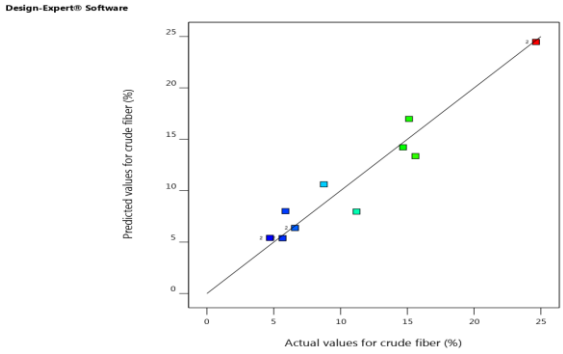

Figure 13: Graph of predicted against actual values for the crude fiber of breakfast cereals

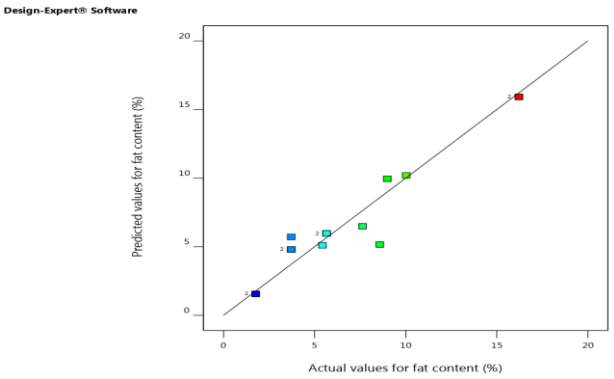

Figure 14: Graph of predicted against actual values for the fat content of breakfast cereals

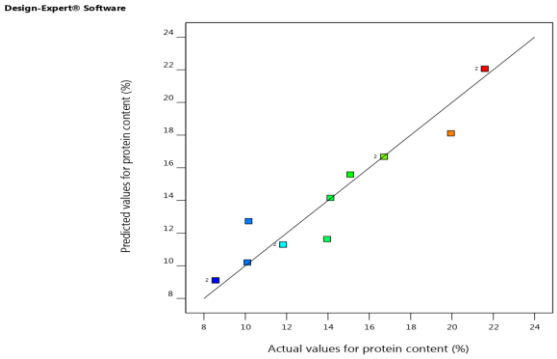

Figure 15: Graph of predicted against actual values for the protein content of breakfast cereals

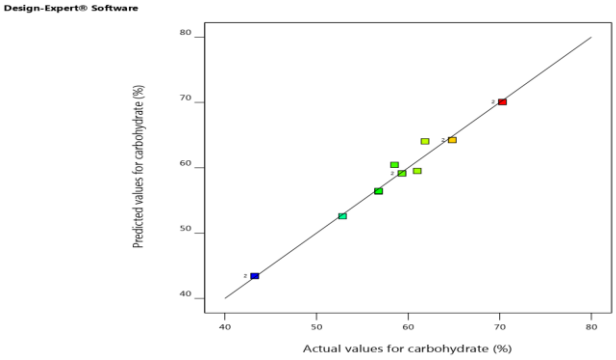

Figure 16: Graph of predicted against actual values for the carbohydrate content of breakfast cereals

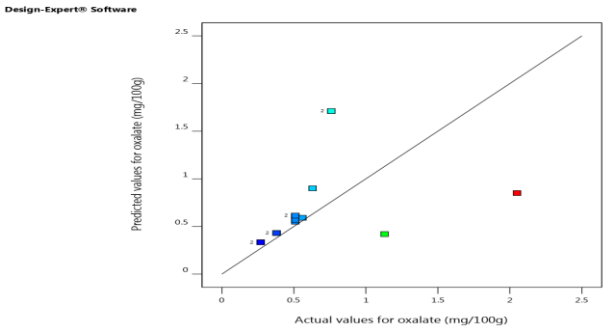

Figure 17: Graph of predicted against actual values for oxalate of breakfast cereals

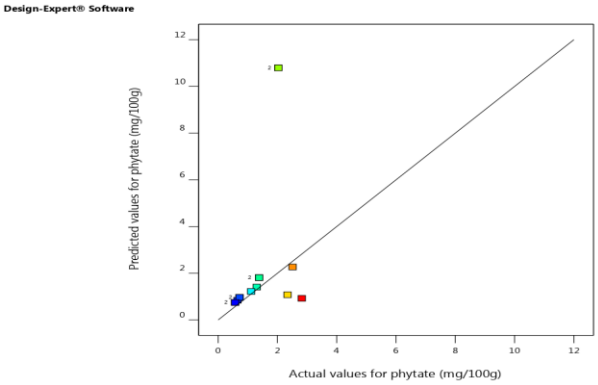

Figure 18: Graph of predicted against actual values for phytate of breakfast cereals

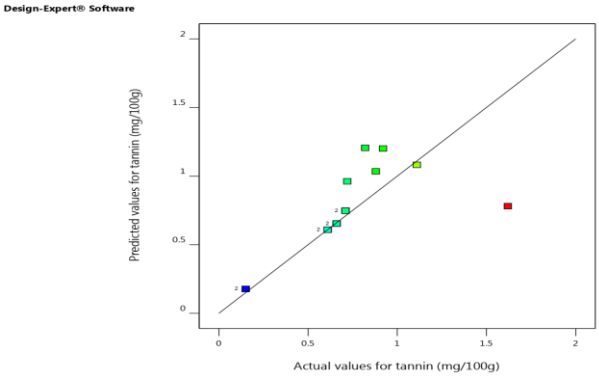

Figure 19: Graph of predicted against actual values for tannin of breakfast cereals

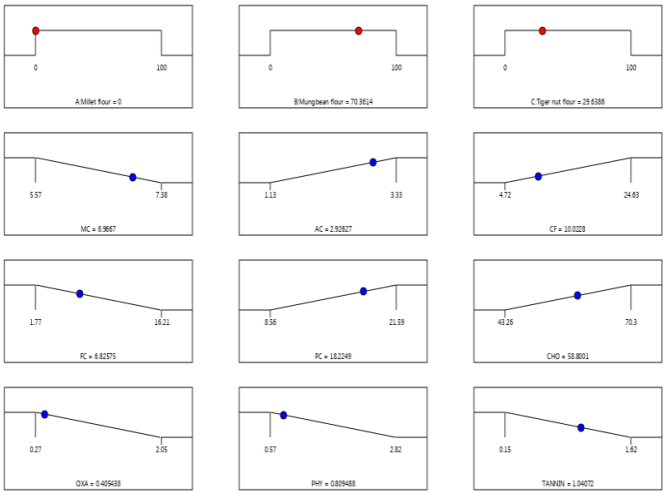

Figure 20: Numerical optimization ramps view for the variables

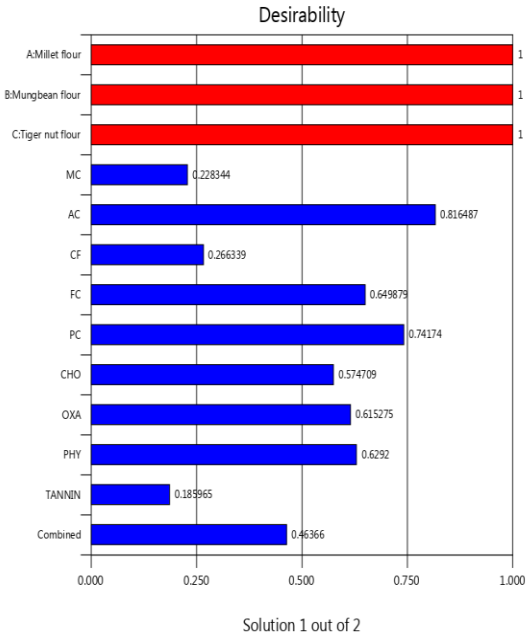

Figure 21: Desirability bar graph

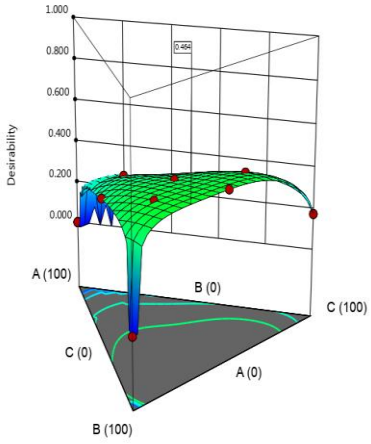

Figure 22: 3-D desirability plot

Table1: Three component augmented simplex centroid design matrix for modelling and optimization of the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour

Design point	Independent variables			Dependent variables								
	x_1 (g)	x_2 (g)	x_3 (g)	MC (%)	AC (%)	CF (%)	FC (%)	PC (%)	CHO (%)	Oxalate (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)
1	100	0	0	6.35	1.13	4.72	5.66	11.83	70.30	0.51	2.03	0.71
2	0	100	0	7.38	3.33	6.59	1.77	21.59	59.33	0.27	0.72	0.61
3	0	0	100	5.57	1.77	24.63	16.21	8.56	43.26	0.76	0.57	0.15
4	50	50	0	6.68	2.05	5.66	3.72	16.71	64.82	0.38	1.38	0.66
5	50	0	50	5.96	1.27	14.68	3.72	10.10	56.78	0.63	1.30	0.92
6	0	50	50	6.48	2.59	15.61	8.99	15.08	56.78	0.51	0.65	0.88
7	33.3	33.3	33.3	6.40	2.10	8.75	7.63	14.12	61.00	0.51	1.11	0.82
8	66.6	16.6	16.6	6.72	1.50	11.20	8.58	10.16	61.85	0.56	2.51	1.11
9	16.6	66.6	16.6	7.38	2.80	5.88	5.43	19.95	58.52	1.13	2.34	0.72
10	16.6	16.6	66.6	7.22	1.98	15.13	10.03	13.96	52.85	2.05	2.82	1.62
11	100	0	0	6.35	1.13	4.72	5.66	11.83	70.30	0.51	2.03	0.71
12	0	100	0	7.38	3.33	6.59	1.77	21.59	59.33	0.27	0.72	0.61
13	0	0	100	5.57	1.77	24.63	16.21	8.56	43.26	0.76	0.57	0.15
14	50	50	0	6.87	2.05	5.66	3.72	16.71	64.82	0.38	1.38	0.66

Key: MC- Moisture Content; AC- Ash Content; CF- Crude Fiber; FC- Fat Content; PC- Protein Content; CHO- Carbohydrate content.X_1- *millet*flour; X_2- *mungbean*flour; X_3- *tigernut* flour.

Table 2: Regression equation coefficients for modelling and optimization of the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour

Coefficient	Dependent variables								
	MC (%)	AC (%)	CF (%)	FC (%)	PC (%)	CHO (%)	Oxalate (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)
Linear									
x_1 (p-value)	6.37* (0.002)	1.12* (<0.0001)	5.40* (<0.0001)	5.96* (<0.0001)	11.30* (<0.0001)	70.06* (<0.0001)	1.83* (0.008)	0.27* (0.01)	-0.34* (0.01)
x_2 (p-value)	7.43* (0.002)	3.33* (<0.0001)	6.38* (<0.0001)	1.56* (<0.0001)	22.06* (<0.0001)	59.14* (<0.0001)	3.12* (0.008)	1.15* (0.01)	-0.55* (0.01)
x_3 (p-value)	5.85* (0.002)	1.77* (<0.0001)	24.46* (<0.0001)	15.92* (<0.0001)	9.11* (<0.0001)	43.43* (<0.0001)	0.89* (0.008)	1.43* (0.01)	-1.78* (0.01)
Binary									
x_1x_2 (p-value)	-	-0.71* (0.0009)	-2.03 (0.750)	4.13 (0.457)	-	-1.42 (0.732)	-	-	-0.12 (0.91)
x_1x_3 (p-value)	-	-0.74* (0.002)	-2.86 (0.709)	-20.93* (0.010)	-	-1.536 (0.758)	-	-	4.77* (0.006)
x_2x_3 (p-value)	-	0.24 (0.183)	-8.24 (0.298)	4.83 (0.467)	-	20.68* (0.002)	-	-	4.58* (0.008)
Ternary									
$x_1x_2x_3$ (p-value)	-	5.12* (0.002)	-	-	-	-	-	-	-
R^2	0.6783	0.9986	0.9549	0.9254	0.9344	0.9864	0.5772	0.5371	0.8453
Adj R^2	0.6198	0.9974	0.9267	0.8788	0.9225	0.9778	0.5003	0.4529	0.7486
LOF	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	1.17	1.82	17.08	22.77	8.74	2.08	33.07	41.04	72.12
Model	0.0020*	<0.0001*	<0.0001*	0.0003*	<0.0001*	<0.0001*	0.0088*	0.0145*	0.0042*

Key: LOT-Lack of Fit; * Significant at the 5% level ($p < 0.05$). NS - Not Significant; CV- Coefficient of Variation;x_1- *millet*flour; x_2- *mungbean*flour; x_3- *tigernut* flour.

Table 3: Numerical optimization criteria for modelling and optimization of the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour

Variables	Goal	Lower limit	Upper limit	Lower weight	Upperweight	Importance
Independent Variables						
Millet flour (g)	is in range	0	100	1	1	3
Mungbean flour (g)	is in range	0	100	1	1	3
Tigernut flour (g)	is in range	0	100	1	1	3
Dependent Variables						
Moisture content(%)	minimize	5.57	7.38	1	1	3
Ash content(%)	maximize	1.13	3.33	1	1	3
Crude fiber(%)	maximize	4.72	24.63	1	1	3
Fat content (%)	minimize	1.77	16.21	1	1	3
Protein content (%)	maximize	8.56	21.59	1	1	3
Carbohydrate (%)	maximize	43.26	70.3	1	1	3
Oxalate (mg/100g)	minimize	2.05	0.27	1	1	3
Phytate (mg/100g)	minimize	2.82	0.57	1	1	3
Tannin (mg/100g)	minimize	0.15	1.62	1	1	3

Table 4: Optimization value, prediction and desirability for modelling and optimization of the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour

S/N	Millet (g)	Mung (g)	TF (g)	MC (%)	AC (%)	CF (%)	FC (%)	PC (%)	CHO (%)	Oxalate (mg/100 g)	Phytate (mg/100 g)	Tannin (mg/100 g)	DES	Selection
1	0.00	70.36	29.63	6.96	2.92	10.02	6.82	18.22	58.80	0.40	0.80	1.04	0.464	Selected
2	20.40	79.60	0.00	7.21	2.77	5.85	3.13	19.86	61.13	0.34	1.02	0.58	0.402	

Key: Mung- Mungbean flour; TF- Tigernut flour; MC- Moisture Content; AC- Ash Content; CF- Crude Fiber; FC- Fat Content; PC- Protein Content; CHO- Carbohydrate; DES- Desirability

Table 5: Confirmation of optimum values (Two-sided Confidence = 95%) for modelling and optimization of the proximate and anti-nutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour

Response	Predicted Mean	Predicted Median	Observed	Std Dev	n	SE Pred	95% PI low	Data Mean	95% PI high
MC	6.55502	6.55502	6.4	0.380161	6	0.393563	5.6888	6.58	7.42125
AC	2.13257	2.13257	2.1	0.0374603	6	0.0473228	2.02067	2.06	2.24447
CF	10.6245	10.6245	8.75	1.88447	6	2.08397	5.81886	11.03	15.4302
FC	6.48707	6.48707	7.63	1.61205	6	1.78271	2.37612	7.08	10.598
PC	14.1591	14.1591	14.12	1.25333	6	1.29751	11.3033	14.34	17.0149
CHO	59.5172	59.5172	61	1.22412	6	1.35372	56.3956	58.80	62.6389
OXA	0.569763	0.511639	0.51	0.191039	6	0.18675	0.289388	1.99	2.20535
PHY	1.21895	1.04862	1.11	0.486454	6	0.48231	0.546629	0.93	12.8407
TANNIN	1.20597	1.14119	0.82	0.412044	6	0.39207	0.489046	0.92	2.66298

Std Dev – Standard Deviation, SE Pred – Standard Error Predicted, PI – Prediction Interval, n- number of confirmations run

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