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EFFECT OF VARIED FLOUR COMPOSITION ON SOME NUTRIENT CHARACTERISTICS OF COOKIES FROM *AKIDI*, WHEAT AND YELLOW MAIZE COMPOSITE FLOUR

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ABSTRACT

*The effect of varied flour composition on the proximate and anti-nutritional properties of high fibre cookies from flour blends of wheat, akidi (black bean) and whole yellow corn was studied using D-optimal mixture design of response surface methodology (RSM) of which ten (10) experimental runs were generated while 100% wheat flour served as control. The cookies were subjected to proximate and anti-nutrient analysis using standard methods. Statistical analysis and optimization of mixture components were carried out using Design-Expert software version 6.0.8 while means were separated using ANOVA. Significant differences were accepted at $p<0.01$ for regression analysis and $p<0.05$ for mean separations. The results of proximate composition of the cookies showed significant ($P<0.05$) difference among the cookies with the ranged of 5.12-6.49% for moisture, 3.53-4.93% for ash, 3.82-5.44% for fibre, 9.84-11.69% for fat, 10.32-18.47% for protein, and 54.28-65.04% for carbohydrate. The anti-nutrients factors of the cookies ranged from 0.07-0.35% for saponin, 0.21-0.56 for phytate, 0.05-0.41% for Trypsin inhibitor and 0.17-0.50 for tannin content. The study revealed that quality and nutrient-dense cookies can be processed from blends of wheat, cowpea and whole yellow corn flour of the proportion (10:50:40) which exhibited high level of fibre, fat, protein and lower level of anti-nutrients as evaluated. Optimization of mixture variables showed that optimal response is generated for flour mixture comprising 10% wheat, 10.89% akidi and 79.11% whole yellow corn with desirability of 55.80%***.**

Keywords: *Yellow maize, akidi, D-optimal design, high fibre, postharvest loss*

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I. INTRODUCTION

Composite flour has been described as blend of two or more flour proportions which may usually contain wheat flour or not, and are used for making cookies and other confectioneries with the intention of increasing the essential nutrients in human diet and increase the economic relevance of indigenous crops (Okoye and Obi, 2017). The use of composite flour in food system has the potential to provide better amino acid balance, dietary fibre, antioxidants and mineral content. Aparana (2018) stated that the utilization of composite flours in regularly will not only enhance the nutritional status of the general population but also help individuals suffering from degenerative diseases. Cookies are well-known snacks with an exceptional texture and taste, long shelf-life and a comparatively inexpensive price (Petrović *et al.,* 2016). They are mainly processed using various raw materials like wheat flour that is a major source of calorie, protein, B-vitamins, dietary fibre and phytonutrients which contribute to a healthy diet (Shewry and Hey, 2015). Besides being nutrient dense, wheat is versatile due to the ability of its proteins to interact and form the gluten network, which is the basic framework of many baked products (Zhou and Hui, 2014). However, the fact that wheat commonly used in cookies production cannot be economically grown in tropical countries like Nigeria due to climatic conditions (Adegunwa*et al.,* 2017), the need to improve the nutrient value of cookies and in turn enhance utilization of indigenous crops brought about supplementation of cookies with *akidi* and yellow corn.

Black beans (*Phaseolus vulgaris*) popularly known as *akidi* amongst the Igbo speaking tribes of Southeastern States of Nigeria, West Africa plays a crucial role in the livelihood of many people of African origin and other developing world, where it contributes majorly as important source of dietary protein that nutritionally complements low-protein staple cereal and other tuber crops (Hussein *et al.,* 2020). *Akidi* contain appreciable quantity of amino acids like valine, leucine, phenylalanine and lysine (Elhardallou*et al.*, 2015). The seed comprises iron, calcium, phosphorus, potassium, magnesium, sodium, zinc, manganese, copper, selenium, vitamin A, vitamin C, vitamin B_6, thiamin, riboflavin, niacin, pantothenic and folate (Agugo*et al*., 2013). Despite its loaded nutrients, *akidi* has received little attention from farmers and food players, and it is fast growing into extinction. Corn is one of the staple foods in many parts of the world, and recognised as the third leading crop of the world after rice and wheat (Sandhu *et al*., 2017). Its colour ranges from white to yellow, red and black (Ranum *et al*., 2014), and provides various nutrients like minerals, vitamins and antioxidant compounds (Revilla *et al*., 2022). Kidist (2018) reported that yellow corn variety contains high amount of total carotenoids than the white variety. Therefore, the objective of this research is to improve utilization of *akidi* and yellow corn in composite technology to produce cookies.

II. MATERIALS AND METHODS

A. PROCUREMENT OF SAMPLES

Wheat, *akidi* seeds and yellow corn grains (**Figure 1**) were purchased from Ubani Main Market, Umuahia North LGA Abia State while reagents and conduct of the research work was carried out at the Department of Food Science and Technology, Michael Okpara University of Agriculture, UmudikeAbia State.

B. SAMPLE PREPARATION

Wheat flour production

The method described by Bello *et al.* (2020) was used for processing of wheat flour. The wheat grains were sorted, washed in tap water, drained and dried in the oven at 60ºC for 20 h prior to milling with hammer mill and sieved through 2 mm mesh size to obtain wheat flour and were properly packaged and stored.

Akidi flour production

The method described by Linus-Chibuezeh*et al.* (2023) was adopted for production of *akidi* flour. *Akidi* seeds was sorted, washed in tap water and steeped in tap water for 12 h before manually dehulled and dried in oven at 60°C for 5 hours before milling and sieved with 1 mm mesh size to obtain fine *akidi* flour. The flour was properly packaged and stored properly.

Processing of yellow corn flour

Yellow corn grains were sorted, washed in water and drained prior to drying in the oven at 50ºC for 24 h and milling with hammer mill to obtain fine yellow corn flour that was properly packaged in Ziploc bag.

C. EXPERIMENTAL DESIGN

The design of the experiment was according to D-optimal design of mixture experimental design for optimal formulation. The component for each flour samples were recorded as a fraction of the mixture such that each treatment combination had the sum of the component proportion equal to 100% where:

$\sum X_i = X_1 + X_2 + X_3 = 100$ (1)

$1 = \sum_{i=1}^{n} xj$

Where X_1 = wheat flour criteria 10% to 50%

X_2 = *akidi* flour criteria 10% to 50%

X_3 = corn flour criteria 40% to 80

$Yi=\beta_1 Xi_1+\beta_2 Xi_2+\ldots+\beta k X_{ik}+\varepsilon i$ (2)

Ten experimental runs were generated based on D-optimal mixture design with additional point that served as control, statistical analysis and optimization of mixture variables using Expert Design Software version 8 as shown in **Table1**. To study the relationship between each factor's component and its experimental levels, adequate polynomial equations were obtained as response surface plots. Insignificant terms for each factor were excluded from the equations and response surface plots. The adequacy of the model was determined by assessing the coefficient of determination (R^2), adequate precision ratio, and ANOVA F-value of the responses.

D. PRODUCTION OF COOKIES

The recipe (**Table 2**) and rubbing-in-method described by Ndife *et al.* (2020a) were used in production of cookies as shown in **Figure 2**. The wheat, *akidi* and corn flours were mixed together with ingredients (unsalted fat, sugar, whole egg, water, salt, baking powder and milk flavor) before manually kneading and rolling out. Afterwards, the dough was rolled out and cut into circular shapes before baking at 212 ºC for 15 min to obtain cookies. The cookies were cooled and packaged for analyses.

E. METHODS OF ANALYSES

Determination of proximate composition of the cookies

The proximate composition of the cookies; moisture, ash, fat, crude fibre protein and carbohydrates were determined according to standard methods described in Onwuka (2018).

Determination of anti-nutrients of the cookie samples

Determination of tannin

The method described by Onwuka (2018) was used to determine the tannin content of the cookies. One gram of each sample was weighed into different centrifuge tube with 2 ml of distilled water. It was centrifuged at 1500 rpm for 10 min. The centrifuge samples were then poured out into a beaker and the supernatant (extract) dispersed. One ml of $NaCO_3$ and Folin Denis reagent was added in the beaker and allowed to settle. Therefore, the readings were taken using a spectrophotometer. Tannin was calculated as follows:

$$\text{Tannin (mg/100g)} = \frac{A_n}{A_s} \times C \times \frac{100}{W} \times \frac{V_f}{V_a} \qquad (3)$$

Where: An= absorbance of test sample
As = absorbance of standard sample
C = concentration of standard solution
Vf = Total volume of extract
Va = volume of extract analyzed
W = Weight of sample

Determination of saponin

Saponin was determined using the colorimetric method AOAC (2012). The sample (0.5 g) was weighed and put into a test tube followed by the addition of 10 mL of distilled water. The mixture was shaken and allowed to stand for 1 h. The formation of stable foaming froth was observed. About 1 mL of the mixture was pipetted into another test tube with about 5 mL of distilled water added to the extract. This was followed by addition of a drop of olive oil. The test tube with its content was shaken and it became cloudy. The absorbance was measured at 620 nm using spectrophotometer. The quantity of saponin contained in each sample was estimated from the standard saponin curve obtained from plotting the concentration of the standard concentration against the absorbance. Hence, amount of saponin calculated as:

PS = Ab x S x DF x 100 (mg/100g saponin) (4)

Where PS = Percentage of saponin
Ab = Absorbance
S = Slope
DF = Dilution factor

Determination of phytate

The spectrophotometric method was used for the determination of phytate. One gram of the sample was extracted in duplicate for 4 h with 20 ml of 0.1M nitric acid with constant agitation. The tubes were stoppered and placed in a boiling water bath for 20 min and allowed to cool. Five milliliters of amyl alcohol were added to each tube followed by 1.0 ml of ammonium thiocyanate (100 g/l). The tubes were shaken thoroughly and centrifuged at 2000 rpm and then absorbance of the amyl alcohol layer was determined at 465 nm against amyl alcohol exactly 15 min after the addition of the ammonium thiocyanate using spectrophotometer. One milliliter of the extract was pipetted into a test tube fitted with a ground glass stopper together with 1ml of ferric solution. Ferric solution was prepared by dissolving 0.2 g hydrated ammonium iron (III) sulphate IN 100 ml 2N HCL and made up to 1000 ml with diluted water and the absorbance was measured at 519 nm against distilled water using spectrophotometer. A standard solution was also prepared for the analysis. Phytic acid was then calculated using the absorbance of the test sample and that of the standard solution (AOAC, 2012).

Determination of trypsin inhibitor

Trypsin inhibitor of the cookie samples was determined using the spectrophotometric method described by Nwosu (2011). A measured weight (10 g) of the sample was dispersed in 50 ml of 0.5 M NaCl solution and stirred for 30 min at room temperature. It was centrifuged and the supernatant filtered through Whatman filter paper. The filtrate was used for the assay. Standard trypsin was prepared and used to treat the substrate solution (N-benzoyl –DI-arginine-P-anilide; BAPA). The extent of inhibition was used as a standard for measuring the trypsin inhibitory activity of the test sample extract into a test tube containing 2 ml of extract and 10 ml of the substrate (BAPA) 2 ml of the standard trypsin solution was added. Also, 2 ml of the standard trypsin solution was added in another test tube containing only 10 ml of substrate. The latter served as the blank. The content of the tubes was allowed to stand for 30 min and then absorbance of the solution measured at 430 nm wavelength with a colorimeter. One trypsin activity unit inhibited is given by an increase of 0.01 absorbance unit at 430 nm. Trypsin unit inhibited was calculated as follows:

Trypsin unit inhibited (mg/100g) = $\frac{A_u}{A_s} \times 0.01xF$ (5)

Where: Au =Absorbance of test sample
As = Absorbance of standard (uninhibited) sample

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F = Experiment factor given as $\frac{V_f}{V_a} x \frac{1}{W}$

Vf = Total volume of extract

Va = Volume of extract analyzed

W = Weight of sample analyzed

III. RESULT AND DISCUSSION

A.Percentage proximate Composition of Cookies from Wheat, Cowpea and Corn Flour blends

Result of proximate composition of cookies from blends of wheat, akidi and whole yellow corn flour is presented in **Table 3**.

Moisture content of cookies

Result of moisture content of the cookies showed a range of 5.12% to 6.49%. There was significant difference (P<0.05) in the mean moisture content among the various treatments. Highest moisture content (6.49%) was recorded for the control cookies (ABA), while sample FDE had the lowest moisture content of 5.12%. Moisture content is recognized as a critical parameter in food products as it plays a vital role in determining their shelf life and overall quality. Higher moisture content has the potential to promote microbial growth and spoilage, while lower moisture content tends to extend shelf life. Johnson *et al.* (2018) and Smith *et al.* (2021) reported similar trends in moisture content for cookies. Variation in moisture content of the cookies indicates that the effect of moisture content on shelf life might be context-specific and dependent on the particular food product or formulation under investigation. Interaction of the studied variables showed significant (p≤0.01) predicted model and linear mixture term. The quadratic interactions of the mixture variables showed significant (p≤0.05) for factors AB (wheat and akidi flour) and BC (*akidi* and corn flour), whereas interaction of AC (wheat and corn flour) showed no significant (p≥0.05) impact on moisture content. However, these interactions resulted to high correlation factor (R^2) of 99.40% and adequate precision ratio of 35.76 which is an indication that the model could be used to navigate the design space. R^2 measures how well the empirical model fits the actual data (Koocheki*et al.*, 2009) while aadequate precision ratio greater than 4 is desirable in RSM studies (Boluk*et al.*, 2023). The response surface curve (Figure 3) showed a falling ridge on the axis of *akidi*flour (factor B) revealing the effect of changing *akidi* flour on the response (moisture content) while the regression equation of significant terms with respect to moisture content is presented in equation 6.

Regression equation for moisture = +5.95393+4.25052+5.27022+3.07891+2.43119+0.19823 (6)

Ash content

The range of 3.53 to 5.08% was recorded for ash content of the cookies. The cookies showed significant ($p\leq0.05$) difference in the ash content. These variations can likely be attributed to the distinct mineral compositions of each flour sample, as ash content of food materials connotes total mineral content of the food which corresponds to the reports of Dabels*et al.* (2016) and Okwunodulu*et al.* (2023) that ash content of a food material is a measure of mineral content or inorganic residue remaining after water and organic matter have been removed by open air incineration. Ndife *et al.* (2020a) reported lower ash content of 1.50 - 2.20% for cookies from enriched biscuits made from selected cereals and groundnut composite flours. The interactions of the independent variables revealed significant ($p\leq0.01$) linear mixture and predicted model. Their quadratic interactions showed insignificant ($p\geq0.01$) factors with respect to ash content of the cookies. These interactions accounted to R^2 of 97.54% which denotes model fit and high adequate precision ratio of 16.995 and is adequate to navigate the design space.

Fibre content

The result of fiber content showed a range of 3.82 to 5.44 %. The cookies showed significant ($p\leq0.05$) difference in fibre content. Highest fibre content was recorded for cookies FDE (cookies from 10% wheat, 50% *akidi* and 40% whole yellow corn flour) while the control cookies (from 100% wheat flour) recorded least fiber content of 3.82%. These disparities in fiber content are likely attributed to both the flour source of the samples and the methods employed during processing. It's worth noting that prior research studies conducted by Green *et al.* (2015) and Black *et al.* (2019) have also documented variations in fiber content within similar food products. High fibre content recorded for the cookies compared to 0.84-1.15%% (Hama-Ba *et al.,* 2018) and 1.25 - 2.25% (Ndife *et al.,* 2020a) could be attributed to whole corn flour used in the processing of the cookies and impact of *akidi* flour. Green *et al.* (2015) reported similar fibre of 4.6% for cookies from blends of wheat and soybean flour. In the context of health-conscious consumers and the growing demand for high-fiber foods, these findings underscore the potential for tailoring cookie recipes to meet specific dietary preferences and nutritional goals. High fibre as recorded in this work could help facilitate peristalsis which has been implicated to reduce many gastrointestinal diseases, serum cholesterol, risk of coronary heart diseases, colon and breast cancer, and hypertension (Adeyeye *et al.,* 2005). Interaction of the mixture variables showed insignificant ($p\leq0.01$) quadratic interaction of the variables and could suggest that variation in fibre content could be brought about by singular effect of whole yellow corn flour. These interactions accounted to high correlation of 89.11% and adequate precision ratio of 7.88 which indicates that the model could be used to navigate the design space.

Fat content

Fat content of the cookies ranged from 9.84% (control) to 15.37 % [(ABE) cookies from 30% wheat, 10% *akidi* and 60% corn flour)]. There was significant ($p\leq0.05$) difference in the fat content of the cookies. Higher content of fat recorded for the cookies could directly be attributed to the quantity of fat used for baking of the cookies and fractions of fat in the individual flour components. Regression analysis on fat content showed that the only significant terms are factors AC (p=0.0186) and linear interaction ABC (p=0.073) this accounted to R^2 of 89.11% which conferred goodness of fit to the model. The response surface curve showed a quadratic curve indicting the changing effect of various flour mixtures on fat content of the cookies.

Protein content

Protein content of the cookies ranged from 10.32% (cookies CAB) to 18.47% (cookies FDE). There were significant ($p \leq 0.05$) differences among the cookies. High protein values were recorded for cookies containing higher *akidi* (a legume known to have appreciable protein content) and wheat flours. Ndife *et al.* (2020a) reported protein values of 8.21 - 28.05% for enriched biscuits produced from selected cereals and groundnut which is slightly higher than protein content of this research and could be attributed to difference in origin of protein supplementation for the biscuits. However, other authors have reported lower protein values for biscuits/cookies. Hama-Ba*et al.* (2018) reported protein range of 7.85 – 12.85% for biscuit from blends of millet and cowpea (not *akidi*) flours; Bolarinwa*et al.* (2016) reported protein range of 7.28 – 11.78% for biscuit from blends of sorghum and soybean while Usman*et al.* (2015) reported protein range of 8.81 – 12.70% for biscuit from blends of wheat and maize bran flour. Protein is an important macronutrient in human being and protein rich food is source of amino-acids needed for the synthesis of the body protein and repair of worn-out tissues in the body. Interaction of the studied variables had significant ($p \leq 0.01$) linear mixture and predicted model. Interactions of the mixture variables could not be predicted reliably using quadratic models of the chosen model (Table 3) but these interactions accounted to good R^2 of 62.56% which conferred goodness of fit to the model (Adindu-Linus *et al.*, 2024). Low adequate precision ratio of 6.93 recorded for protein content is also adequate to navigate the design space as a ratio greater than 4 has been reported to be adequate to navigate the design space.

Carbohydrate

The result on carbohydrate content ranged from 54.28 to 65.04%. Significant ($p<0.05$) difference was observed for carbohydrate content of the cookies. These findings shed light on the variations in carbohydrate content among the samples. Sample ABA (control sample) displayed the highest carbohydrate content (65.04%), while Sample FDE exhibited the lowest carbohydrate content, with a value of 54.28%. Low carbohydrate content recorded for the composite cookies compared to the control (from 100% wheat flour) could be attributed to the high fibre content of the cookies due to addition of whole corn flour and higher protein content of the cookies, as carbohydrate was calculated as difference between other proximate parameters (moisture, ash, fibre, fat and protein) and 100%. Robinson *et al.* (2014) reported similar carbohydrates of 58.66 to 61.85% for cookies from wheat, cowpea, and corn flour blends. The differences in carbohydrate content among samples could impact the nutritional profile and sensory characteristics of the end products, such as cookies made from these flour blends. Interaction of the mixture variables accounted to R^2 of 97.81% which conferred goodness of fit to the model.

B. Antinutrient composition of cookies

The result of the anti-nutrient properties of the cookies samples is presented in **Table 4**. Anti-nutrients are substances presence in plant foods that have negative impact on bioavailability, digestibility, and utilization of nutrients available in foods.

Saponin content

The range of 0.07 to 0.35mg/100g was recorded for saponin content of the cookies. Notably, Sample FDE exhibited the highest saponin content at 0.35 mg/100g, while Sample ABA (100% wheat cookies) had the lowest 0.07 mg/100g. Saponins are naturally occurring plant compounds recognized for their potential anti-nutrient effects. Ojinnaka *et al.* (2019) reported similar saponin value of 0.09 to 0.33 mg/100g for cookies made from wheat-aerial yam flour blends. Low saponin content of the cookies is desirable and poses no anti-nutrient effect on humans as saponin is known to lower plasma cholesterol concentrations (Okpala and Chinyelu, 2011). Interactions of the variables showed that all the studied factors had significant impact on saponin content of the cookies at different probability levels. This resulted to high level of correlation coefficient of 99.08% which conferred goodness of fit to the model and adequate precision ratio of 25.55 which indicates a desirable ratio and can be used to navigate the design space, as adequate precision ratio greater than 4 is adequate enough to use to navigate any design space (Adindu-Linus *et al*., 2024). The RSM curve (Figure 5) showed the impact of changing different flour blends on the cookies, while equation 13 shows the final regression equation in term of real components for significant terms.

Regression for saponin = +0.31386+2.49158E-003+0.078249+0.83586-0.80051+1.38131 (7)

Phytate content

The result of phytate content of the cookies ranged from 0.21 to 0.56 mg/100g. There was significant ($p \leq 0.05$) difference in phytate content of the cookies with highest score recorded for FDE (0.56 mg/100g) while Sample ABA displayed the lowest phytate content of 0.21 mg/100g. Phytate has been reported have adverse impact on mineral absorption, particularly for minerals like copper, iron, and zinc (Gordon, 2011). (Ndife *et al.* (2020b) reported higher phytate content of 1.80 - 2.60 mg/100g for snacks from maize, soybean and orange fleshed sweet potato flour blends. High ingestion of anti-nutritional compounds like phytate can be of significant health concern. Regression analysis showed that the only insignificant ($p \leq 0.01$) term is combined effect of factor AC (wheat -*akidi* flour) p=0.452. Quadratic and linear interactions of the mixture variables resulted high R^2 of 99.74% and adequate precision ratio of 53.33 which conferred goodness of fit to the model (Adindu-Linus *et al*., 2024). Response surface curve (Figure 6) and equation 14 showed factors' interaction of significant terms.

Final Equation for Phytate = +0.018404+0.35931+0.23422+1.14741+0.15878+1.07923 (8)

Trypsin inhibitor

Result of trypsin inhibitor content of the cookies revealed a range of 0.05 to 0.41mg/100g. The cookies showed significant ($p \leq 0.05$) difference in Trypsin inhibitor content. Highest trypsin inhibitor was recorded for cookies FDE 0.41 mg/100g, while cookies ABA had the lowest trypsin inhibitor content of 0.05 mg/100g. It is important to note that trypsin inhibitors exert their influence by impeding protein digestion. Low levels of this anti-nutrient recorded in this research is an indication that consumption of these cookies irrespective of flour ratio used would not interfere with protein digestion associated with trypsin inhibitor effect. Interaction of the studied variables revealed a strong correlation coefficient of 99.38% and adequate precision ratio of 32.11 which are adequate to confer goodness of fit to the predicted model (Adindu-Linus *et al*., 2024).

Final Equation for Trypsin=-0.19063+0.24232+0.11581+2.22790-0.073232+0.76199 (9)

Tannin content

The result of anti-nutrient showed that tannin content of the cookies ranged from 0.17 to 0.50 mg/100g. Similar result (0.36 to 0.51 mg/100g) was reported by Okpala and Okoli (2011) for cookies produced from pigeon pea, cocoyam, and sorghum flour blends while Adeola and Ohizua (2018) reported higher values of 11.55 to 40.19 mg/100g for biscuits prepared from flour blends of unripe cooking banana, pigeon pea, and sweet potato. Tannins are well-known polyphenolic compounds, distinguished by their astringent taste and potential anti-nutrient effects. They are polyhydric phenols which are present in virtually all parts of plants and have been found to inhibit trypsin, chymotrypsin, amylase, and lipase activities (Inyang and Ekop, (2015). Interaction of the mixture variables accounted to good correlation coefficient of 97.27 and adequate precision ratio of 14.86 which conferred goodness of fit to the model.

Final Equation for Tannin=+0.62942+0.45442+0.54826+1.47191-2.20991+0.040088 (10)

IV. CONCLUSION

High fibre cookies were made from composite flour of wheat, *akidi* and whole yellow corn flour. The result of proximate analysis revealed variations in parameters examined. Low moisture content the cookies indicates higher stability during storage while high fibre content of the cookies showed they will be useful in maintaining gut and gastrointestinal health. Improved protein content of the cookies brought about by inclusion of *akidi* flour (a lesser known and underutilized legume) revealed that consumption of products from added *akidi* beans could also help to alleviate protein malnutrition in Nigeria and Africa where high cost of animal protein has increased occurrence of protein energy malnutrition (PEM). This legume requires lesser attention during cultivation and

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could withstand drought and other harsh conditions, making it a cheaper source of protein for teeming populace that is in need of alternative and cheaper source of protein. Results of anti-nutrients also revealed minimum levels of examined anti-nutrients which is an indication that the cookies are safe for humans.

The use of mixture design of response surface methodology resulted in a design that provided insight on the interactions of the mixture variables (wheat, *akidi* and whole yellow corn flour) in composite cookies formulations. Optimization of the mixture variables showed that optimal responses were generated for flour mixtures comprising 10% wheat, 10.89% *akidi* and 79.11% whole yellow corn with desirability of 55.80%.

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The authors declared no conflict of interest.

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APPENDICES

Wheat grains *Black bean (Akidi)* *Yellow corn*

Figure 1: Pictorial presentation of raw materials

TABLE 1: Formulation of composite flour (%)

Experimental Runs	Wheat Flour	*Akidi* flour	Corn flour
BBM	50.00	10.00	40.00
ABC	16.67	16.67	66.67
BDA	16.67	36.67	46.67
CCD	30.00	30.00	40.00
FDE	10.00	50.00	40.00
ABE	30.00	10.00	60.00
AAJ	36.67	16.67	46.67
CAB	23.33	23.33	53.33
ABJ	10.00	10.00	80.00
GIK	10.00	30.00	60.00
ABA (CONTROL)	100	0.00	0.00

Table 2: Ingredients for making cookies

Ingredient	Quantity
composite flour (wheat, *akidi* and yellow corn)	400
Margarine	160 g
Sugar	96 g
whole egg	100ml
Water	75ml
Salt	4g
baking powder	4 g
milk flavor	4ml

Source: Peter-Ikechukwu *et al.* (2017)

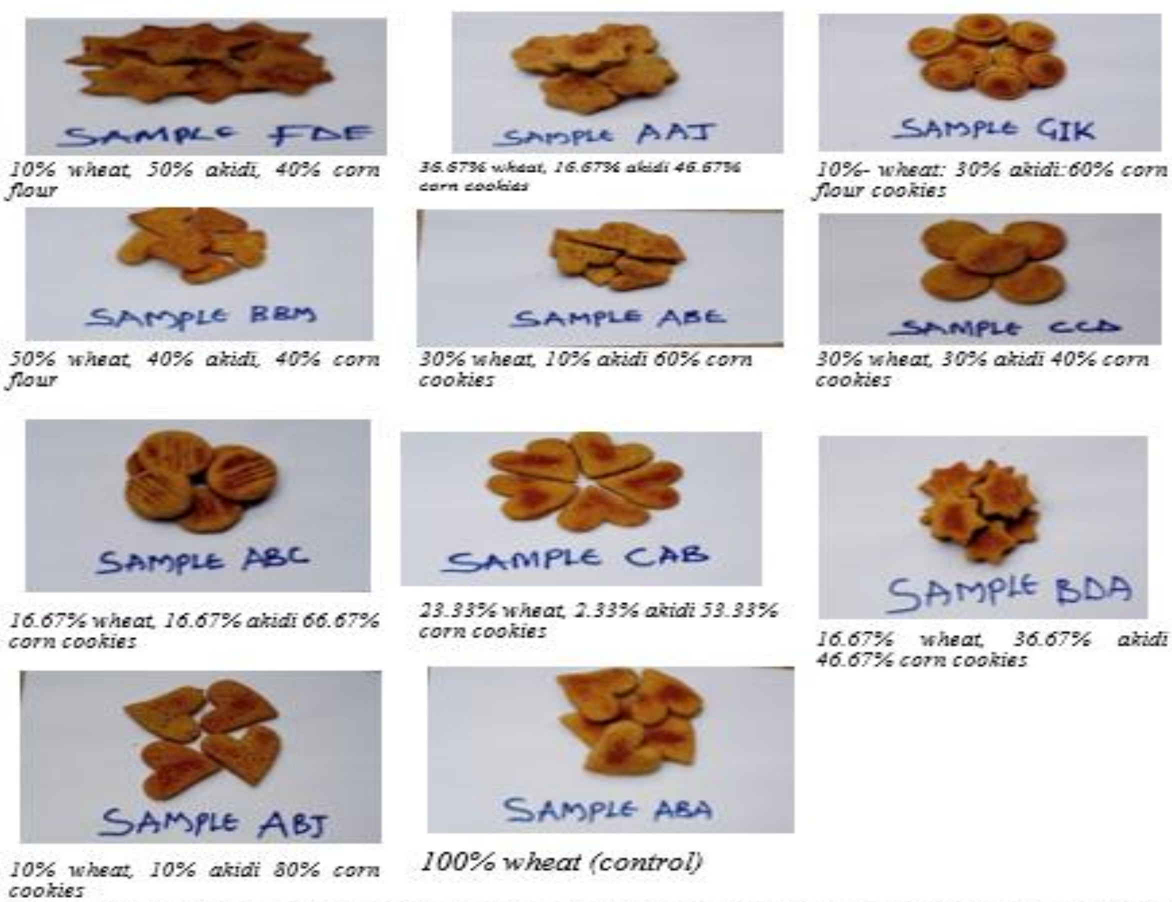

Figure 2: Cookies produced from wheat, akidi and whole yellow corn flour blend

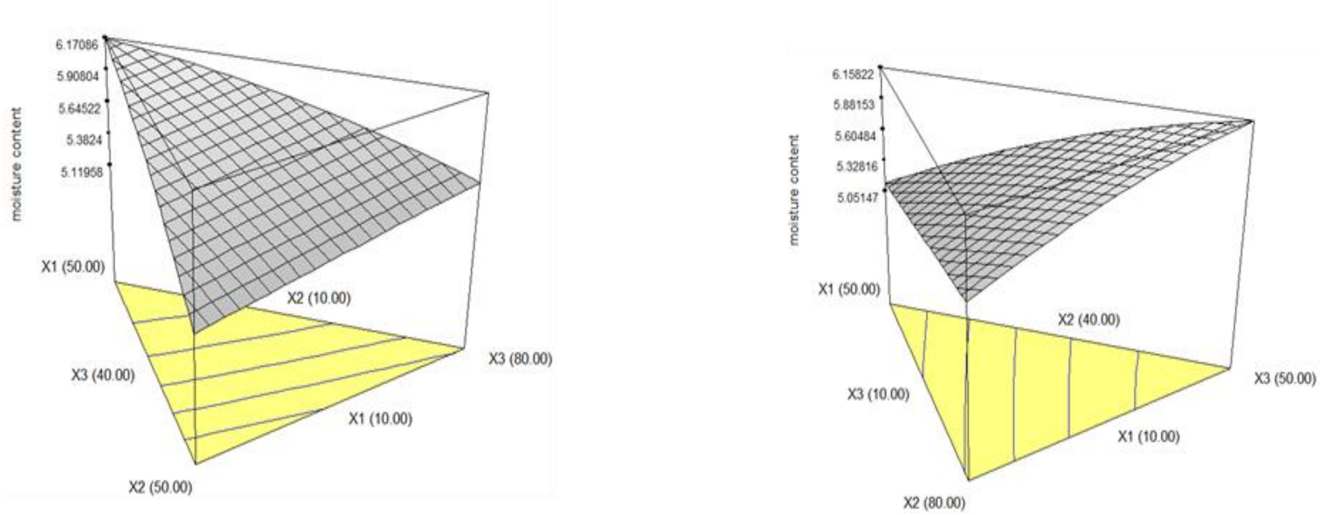

Figure 3: Effect of factors AB and BC on moisture content of cookies

Table 3. Result of proximate composition (%) of cookies from wheat *akidi* and whole yellow corn flour blends

EXPERIMENTAL Runs	Wheat flour	*akidi* flour	corn flour	Sample code	Moisture	Ash	Fibre	Fat	Protein	Carbohydrates
1	50.00	10.00	40.00	BBM	$6.14^{b}\pm0.01$	$4.08^{g}\pm0.04$	$4.09^{i}\pm0.02$	$10.24^{ab}\pm0.05$	$13.74^{f}\pm0.13$	$61.73^{c}\pm0.07$
2	16.67	16.67	66.67	ABC	$5.55^{g}\pm0.01$	$4.33^{f}\pm0.04$	$4.67^{e}\pm0.05$	$10.75^{ab}\pm0.07$	$12.87^{g}\pm0.12$	$61.85^{c}\pm0.02$
3	16.67	36.67	46.67	BDA	$5.42^{i}\pm0.02$	$4.93^{b}\pm0.04$	$5.27^{b}\pm0.05$	$11.32^{ab}\pm0.07$	$17.59^{b}\pm0.13$	$55.49^{g}\pm0.01$
4	30.00	30.00	40.00	CCD	$5.76^{e}\pm0.02$	$4.68^{d}\pm0.04$	$4.97^{d}\pm0.05$	$11.12^{ab}\pm0.02$	$16.47^{c}\pm0.23$	$56.78^{g}\pm0.13$
5	10.00	50.00	40.00	FDE	$5.12^{k}\pm0.28$	$5.08^{a}\pm0.04$	$5.44^{a}\pm0.05$	$11.69^{ab}\pm0.02$	$18.47^{a}\pm0.12$	$54.28^{h}\pm0.06$
6	30.00	10.00	60.00	ABE	$5.92^{d}\pm0.01$	$4.18^{g}\pm0.04$	$4.25^{h}\pm0.03$	$15.37^{a}\pm0.17$	$13.39^{e}\pm0.13$	$61.90^{c}\pm0.03$
7	36.67	16.67	46.67	AAJ	$6.02^{c}\pm0.28$	$4.30^{f}\pm0.07$	$4.54^{f}\pm0.05$	$10.49^{ab}\pm0.02$	$14.62^{d}\pm0.12$	$60.05^{d}\pm0.11$
8	23.33	23.33	53.33	CAB	$5.67^{f}\pm0.01$	$4.58^{d}\pm0.04$	$4.33^{gh}\pm0.04$	$10.95^{ab}\pm0.03$	$10.32^{j}\pm0.13$	$58.66^{e}\pm0.03$
9	10.00	10.00	80.00	ABJ	$5.49^{h}\pm0.01$	$4.45^{e}\pm0.08$	$4.37^{g}\pm0.05$	$10.57^{ab}\pm0.05$	$12.34^{h}\pm0.13$	$62.74^{b}\pm0.00$
10	10.00	30.00	60.00	GIK	$5.33^{j}\pm0.01$	$4.80^{c}\pm0.08$	$5.12^{c}\pm0.02$	$11.47^{ab}\pm0.09$	$16.02^{c}\pm0.12$	$57.28^{f}\pm0.01$
CONTROL	100	0.00	0.00	ABA	$6.49^{a}\pm0.01$	$3.53^{h}\pm0.04$	$3.82^{j}\pm0.07$	$9.84^{b}\pm0.05$	$11.29^{i}\pm0.13$	$65.04^{a}\pm0.03$
				LSD	1.00	0.06	0.11	0.44	1.00	0.13
Main effects										
Model					0.0002***	0.0026***	0.0452**	0.1374NS	0.0321**	0.0021***
Linear Mixture					<0.0001***	0.0006***	0.0118***	1.0000NS	0.0321**	0.0005***
AB					0.0417*	0.2701NS	0.7236NS	0.8785NS	-	0.2027NS
AC					0.0799*	0.2800NS	0.7588NS	0.0186**	-	0.9863NS
BC					0.0583*	0.7571NS	0.7017NS	0.7477NS	-	0.2652NS
ABC					-	-	-	0.0743*	-	
R^2					0.9940	0.9754	0.8925	0.8911	0.6256	0.9781
Adjusted R^2					0.9865	0.9447	0.7581	0.6734	0.5186	0.9507
Adequate precision					35.76	16.995	7.581	7.021	6.925	17.294

*Values are means ± standard deviation of triplicate determinations. $^{a-j}$ means bearing different superscripts on the same column are significantly ($p\leq0.05$) different.

KEY: ***significant at $p\leq0.01$; ** significant at $p\leq0.05$; * significant at $p\leq0.10$ while NS = Not Significant

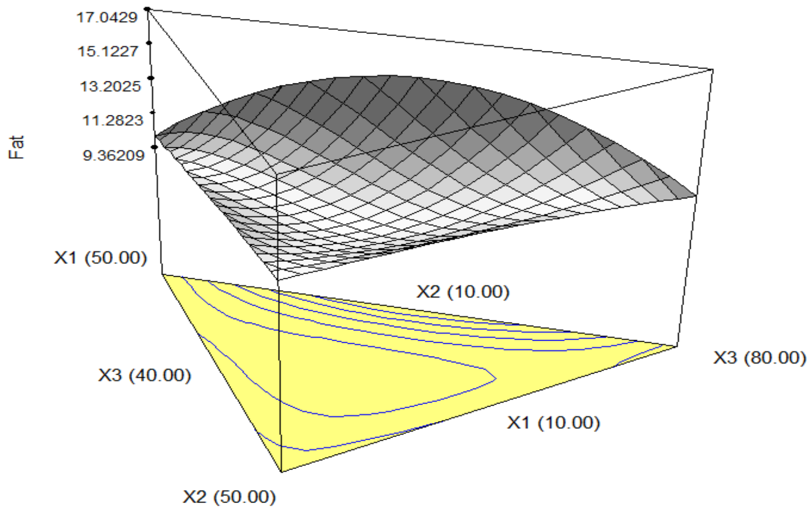

Figure 4: Interaction of mixture variable on fat content

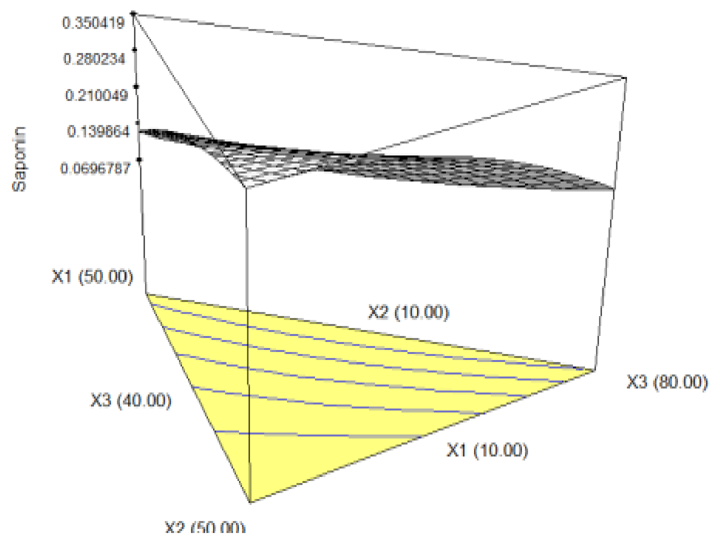

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Figure 5: Response surface curve for Saponin

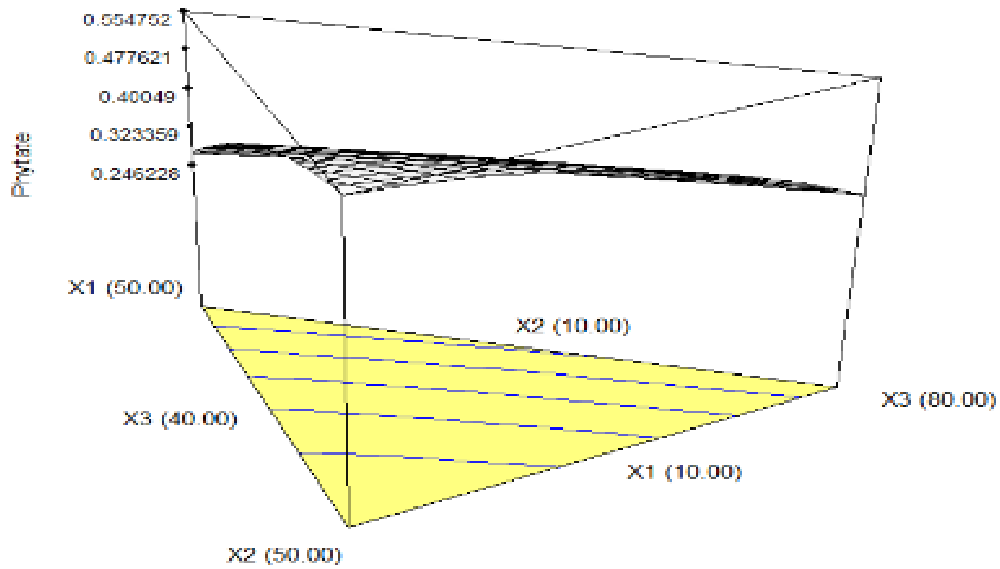

Figure 6 Response surface curve for phytate

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Table 4 Antinutrient composition(mg/100g) of cookies

EXPERIMENTAL Runs	wheat flour	*akidi* flour	corn flour	Treatment	Saponin	Phytate	Trypsin inhibitor	Tannin
1	50.00	10.00	40.00	BBM	0.13^{h}±0.01	0.27^{j}±0.01	0.11^{i}±0.01	0.21^{h}±0.01
2	16.67	16.67	66.67	ABC	0.20^{f}±0.01	0.40^{f}±0.01	0.25^{f}±0.01	0.31^{e}±0.00
3	16.67	36.67	46.67	BDA	0.33^{b}±0.01	0.52^{b}±0.01	0.38^{b}±0.01	0.46^{b}±0.01
4	30.00	30.00	40.00	CCD	0.26^{d}±0.01	0.46^{d}±0.01	0.35^{c}±0.01	0.43^{c}±0.01
5	10.00	50.00	40.00	FDE	0.35^{a}±0.00	0.56^{a}±0.01	0.41^{a}±0.01	0.50^{a}±0.01
6	30.00	10.00	60.00	ABE	0.10^{i}±0.01	0.31^{i}±0.01	0.13^{j}±0.01	0.24^{g}±0.01
7	36.67	16.67	46.67	AAJ	0.19^{f}±0.01	0.37^{g}±0.01	0.21^{g}±0.01	0.27^{f}±0.01
8	23.33	23.33	53.33	CAB	0.24^{e}±0.01	0.43^{e}±0.01	0.28^{e}±0.01	0.35^{c}±0.01
9	10.00	10.00	80.00	ABJ	0.15^{g}±0.01	0.34^{h}±0.01	0.17^{h}±0.01	0.40^{b}±0.01
10	10.00	30.00	60.00	GIK	0.30^{c}±0.01	0.49^{c}±0.01	0.32^{d}±0.01	0.47^{b}±0.01
CONTROL	100	0.00	0.00	ABA	0.07^{j}±0.01	0.21^{k}±0.01	0.05^{k}±0.01	0.17^{i}±0.01
				LSD	0.14	1.00	1.00	0.61
Main effects								
Model					0.0004***	<0.0001***	0.0002***	0.0032***
Linear Mixture					<0.0001***	<0.0001***	<0.0001***	0.0009***
AB					0.0721*	0.0038***	0.0029***	0.1039*
AC					0.0807*	0.4520NS	0.8412NS	0.0345**
BC					0.0160***	0.0048***	0.0902*	0.9572NS
ABC					-	-	-	-
R^2					0.9908	0.9976	0.9938	0.9728
Adjusted R^2					0.9792	0.9946	0.9861	0.9387
Adequate precision					25.549	53.325	32.111	14.861

*Values are means ± standard deviation of triplicate determinations. a-j means bearing different superscripts on the same column are significantly (p≤0.05) different.

KEY: ***significant at p≤0.01; ** significant at p≤0.05; * significant at p≤0.10 while NS = Not Significant

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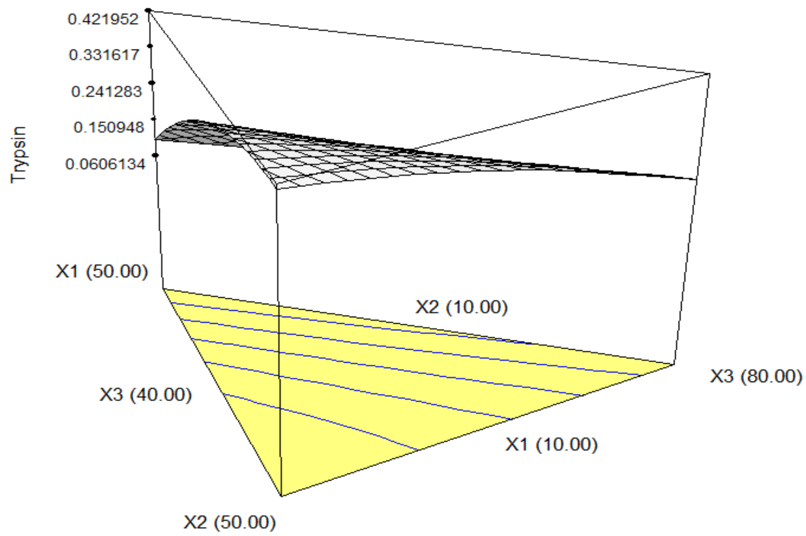

Figure 7 Response surface curve for Trypsin inhibitor

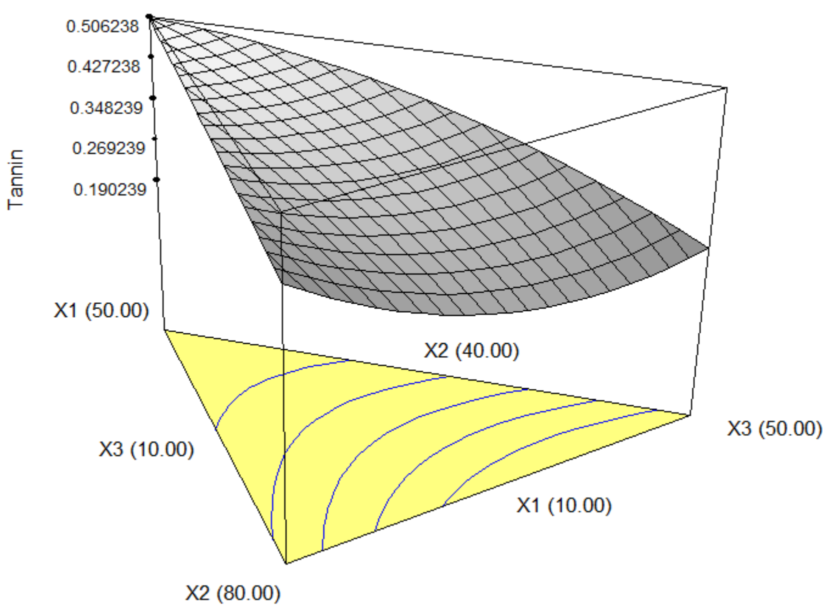

Figure 8 Response surface curve for Tannin