



## MODELLING OF PROXIMATE COMPOSITION OF AMARANTH, SORGHUM, PUMPKIN AND SUNFLOWER FLOUR BLENDS USING RESPONSE SURFACE METHODOLOGY

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**ABSTRACT:** *Blends of cereals and legumes have gained attention especially in complementary nutrition. Optimization of the production of composite flour from defatted amaranth, sorghum, defatted pumpkin and defatted sunflower flour using D-optimal mixture design of Design Expert software with the levels for amaranth (40-60 %), sorghum (10-30 %), pumpkin (20-40 %), and sunflower flour (3-10 %), respectively was carried out. The responses were proximate composition. Run 5 and 14 recorded the highest crude protein (4.08 %) and total ash content (5.71 %) while run 7 and 10 had the highest fibre content (4.08 %), respectively. The model terms were significant ( $p < 0.05$ ) for the proximate composition of the blends with  $R^2$  values of 0.93, 0.92, 0.96, 0.90, 0.91, and 0.90 respectively for moisture, protein, fat, ash, fibre, and carbohydrate. The optimal blend from the numerical optimization through the desirability function approach were 42.46% amaranth, 10.00% sorghum, 40.00% pumpkin, and 7.54%. In conclusion, composite flour from amaranth, sorghum, pumpkin and sunflower flour have acceptable proximate composition in terms of nutritional quality necessary for the production of nutrient-dense food products capable of addressing issues of malnutrition.*

**KEYWORDS:** Blends, amaranth, sorghum, pumpkin, sunflower flour, D-optimal design.



## INTRODUCTION

Cereal and legume blends have received a lot of attention in the nutrition field, especially when it comes to complementary feeding. This method entails combining various cereals and legumes to produce a complementary nutritional profile that increases the overall content of essential nutrients, particularly proteins (Kumari & Sangeetha, 2016). These food groups work together to provide a more balanced and comprehensive nutritional intake, making them invaluable components in addressing long-term food insecurity, malnutrition, and certain diseases in infants, children, and adults (Kumari & Sangeetha; Olson et al., 2021). Cereals and legumes work together to create a nutritionally complementary effect. Cereals are high in energy-giving carbohydrates, whereas legumes are high in protein, fibre, and micronutrients (Olson et al., 2021). Combining these two food groups creates a more complete nutritional profile that meets both energy and protein requirements. This strategy is especially important during the complementary feeding stage when infants transition from exclusively breastfeeding to a more varied diet. Amaranth, a pseudocereal known for its high nutritional value, has emerged as an exciting ingredient in cereal-legume blends (Kaur et al., 2023). Amaranth grain is high in vitamins such as  $\beta$ -carotene, vitamin B6, vitamin C, riboflavin, folate, as well as essential amino acids and minerals like Ca, P, Fe, Mg, K, Cu, Zn, and Mn (Neelesh & Pratibha 2018; Mampholo et al., 2015). Amaranth grain contains approximately 15% protein and is high in lysine and sulphur amino acids (Vasundhara & Amrita, 2023). When mixed with sorghum, pumpkin seed flour, and sunflower flour, these blends can provide a well-rounded nutritional profile. Sorghum provides valuable carbohydrates (Abah et al., 2020), whereas pumpkin seed flour and sunflower flour contain healthy fats, proteins, and essential micronutrients. The addition of amaranth enhances the blends' nutritional value by providing essential amino acids, vitamins, and minerals. Advanced experimental design methodologies are essential for achieving optimal nutritional blends. The D-optimal design of Response Surface Methodology (RSM) is an effective tool for developing and optimising food processes (Nahemiah et al., 2015). In the context of cereal-legume blend formulation, RSM helps to systematically investigate the effects of various factors, such as ingredient proportions and processing conditions, on the nutritional content of the final product (Griffith et al., 1998).

The D-optimal design enables researchers to deliberately choose experimental conditions that maximise the amount of information gained from each experiment. This efficiency is critical in optimising the formulation of cereal-legume blends to achieve the desired nutritional benefits. As a result, the current study seeks to optimise the proximate composition of composite flour made from amaranth, sorghum, pumpkin, and sunflower flours using response surface methodology.

## MATERIALS AND METHODS

### Collection of Materials

Amaranth seed (*Amaranthus hypochondriacus*) and Sorghum seed (*Sorghum bicolor L*) with accession number NGB00544 and NGB03143 respectively were obtained from the National Center for Genetic Resources and Biotechnology (NACGRAB), Nigeria while pumpkin seed (*Cucurbita pepo*) and sunflower seed (*Helianthus annuus*) were obtained from Ahmadu Bello University Experimental Farm, Zaria, Nigeria.



## Experimental Design

A four-component constrained D-optimal mixture process experimental design, with 14 randomized experimental runs, was employed. The formulation design constraints were process design. The raw materials and their ranges were: amaranth flour (40-60 %), sorghum (10-30 %), pumpkin seed flour (20-40 %) and sunflower flour (3-10 %). The formulation of the composite blend were based on the D-Optimal mixture:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \epsilon \quad 1$$

where  $Y$  represents the response variables to be modeled,  $\beta_0$  is the value of the fitted response at the center point of the design, and 1, 2, 3 and 4 are the coefficients of the input variables  $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$ , and  $\epsilon$  is the random error of the model.

## Preparation of Defatted Amaranth Flour, Sorghum, Defatted Sunflower Flour, and Defatted Pumpkin Flour

### Preparation of Defatted Amaranth Seed Flour

Defatted amaranth seed was prepared by the modified method of Tanimola *et al.* (2016). The amaranth seeds were properly cleaned, winnowed, and sorted to remove stones, sand, and all forms of dirt. The grains were finely ground using a disc attrition mill and then defatted with n-hexane in Soxhlet apparatus for 6 h to a residual lipid concentration of less than 1 %. The flour was oven dried at 60°C and stored in an air-tight plastic container at room temperature until further use.

### Preparation of Sorghum Seed Flour

Sorghum grains were sorted and winnowed to remove foreign matter such as dust particles, seeds of other grains/crops, and other impurities such as stones and weeds. They were washed and dried at 60°C in the oven. The dried sorghum seed was milled using a disc attrition mill, and the resulting product was passed through a 100  $\mu\text{m}$  sieve. Thereafter, the flour was packaged in a well-sealed low-density polyethylene bag (Olurin *et al.*, 2021).

### Preparation of Defatted Pumpkin Seed Flour

Defatted pumpkin seed flour was prepared by the modified method of Atuonwu and Akobundu (2010). Matured pumpkin fruits were cut open to obtain the seeds which were then separated from the pulp. Seeds with intact seed coats were washed and the cleaned seeds were spread thinly on a tray and sundried. The dried slices were ground using an attrition mill and then sieved through a 500  $\mu\text{m}$  British standard sieve (model BS 410, Endecotts Ltd, London, UK). The obtained flour was defatted using n-hexane for 6 h. The defatted flour was subsequently oven-dried at 50°C for 30 min to remove the residual n-hexane. The flour was milled again and stored in an air-tight plastic container at room temperature (37°C) until it was used.

### Preparation of Defatted Sunflower Seed Flour

Sunflower seeds were cleaned, graded, and dehulled manually. The dehulled seeds were flaked to a thickness of 0.3 mm using a three-roller flaking machine (SZLH-300). The flour obtained was defatted using n-hexane for 24 h. The defatted flour was subsequently oven-dried at 60°C for 30 min to remove the residual n-hexane. The flour was stored in an air-tight



plastic container at room temperature until it was used (Ghandi *et al.*, 2008). The flour was packaged in a well-sealed low-density polyethylene bag.

### Preparation of Composite Flour

Composite flours consisting of amaranth, sorghum, pumpkin and sunflower flours were prepared based on the combination obtained from the experimental design, as shown in Table 2. The mixtures were carefully blended in a mixer (model K5SS, KitchenAid Inc., St. Joseph, MI) in order to achieve uniform mixing.

### Proximate Analysis

The samples were analyzed for moisture, crude protein, crude fat, total ash and crude fibre content, according to the method described by AOAC (2012), and carbohydrate was calculated by difference.

### Statistical Analysis

The results of the experiment were analyzed, and appropriate Scheffe canonical models were applied to the average proximate property data. Each term in the Scheffe canonical regression models underwent ANOVA to assess its statistical significance for every response variable. The models' adequacy was assessed through the coefficient of determination, F-value, and model p-values at a significance level of 0.05. The models were checked for coefficients of determination ( $R^2$ ), adjusted R-squared (Adj  $R^2$ ), predicted R-squared values and coefficient of variation (C.V), and only the statistically significant model parameters were retained in the final fitted Scheffe canonical models. By utilizing the DESIGN EXPERT statistical software, 3-D response surfaces plots were generated based on the fitted models for all proximate constituents.

### Optimization

Numerical optimization technique of the Design Expert software was used for simultaneous optimization of multiple responses. The desired goal for each of the responses was chosen. The different flours (A, S, P and SF) were kept within range; fat and carbohydrate were minimized, ash and fibre were in range, while protein was maximized.

**Table 1: Design Matrix for Blends of Amaranth, Sorghum, Pumpkin and Sunflower flour Blends (Factors and Levels)**

Component	Unit	Low	High
Amaranth flour	%	40	60
Sorghum flour	%	10	30
Pumpkin seed flour	%	20	40
Sunflower seed flour	%	3	10



**Table 2: Experimental Design for the production of Amaranth, Sorghum, Pumpkin and Sunflower Flour Blends**

S/N	A	S	P	SF
1	40.00	30.00	27.00	3.00
2	40.00	30.00	20.00	10.00
3	40.00	30.00	20.00	10.00
4	40.00	10.00	40.00	10.00
5	40.00	17.00	40.00	3.00
6	60.00	10.00	27.00	3.00
7	60.00	10.00	20.00	10.00
8	47.00	30.00	20.00	3.00
9	40.00	30.00	20.00	10.00
10	60.00	10.00	20.00	10.00
11	60.00	17.00	20.00	3.00
12	48.22	18.22	28.22	5.33
13	40.00	10.00	40.00	10.00
14	40.00	17.00	40.00	3.00

A = X1 = Amaranth flour, S = X2 = Sorghum flour

P = X3 = Pumpkin seed flour, SF = X4 = Sunflower flour.

## RESULTS AND DISCUSSIONS

### Design Summary for the Proximate Composition of Amaranth-Based Blends

The results of the design summary for the proximate composition of amaranth-based blends are presented in Table 3. The values revealed that moisture, protein, fat, total ash, fibre and carbohydrate showed 'None,' indicating that no mathematical transformation was performed on the data under the design. The transformation model applied to all the variables was "None," indicating that no mathematical transformation was performed on the data. The minimum and maximum values are 6.53 % and 7.64 %, 25.17 % and 37.02 %, 0.91 % and 1.51 %, 3.71 % and 5.71 %, 3.38 % and 4.08 %, and 44.79 % and 58.51 % for moisture, protein, fat, fibre, total ash, and carbohydrate, respectively. This work supported the view of Ahmed *et al.* (2023) who worked on the optimization of soaking conditions (temperature and time) on the physicochemical properties of selected parboiled rice varieties.

**Table 3: Design Summary for the Proximate Composition of Amaranth-Based Blends**

Response	Name	Unit	Transformation	Model	Minimum	Maximum
Y1	Moisture	%	None	Linear	6.53	7.64
Y2	Protein	%	None	Linear	25.17	37.02
Y3	Fat	%	None	Linear	0.91	1.51
Y4	Ash	%	None	Linear	3.71	5.71
Y5	Fibre	%	None	Linear	3.38	4.08



Y6	CHO	%	None	Linear	44.79	58.51
Y = response variable						

### Proximate Composition of the Amaranth-Based Blends

The result of the proximate composition of amaranth-based flour blends is presented in Table 4. The moisture content of the flour formulated ranged from 7.02 - 7.64 %. Run 12 had the least moisture content with the mean value of 7.02 % while Run 8 had the highest moisture content of 7.64 %. Run 11 and 12, both with the moisture content of 7.02 %, were not significantly different from each other. Similar significant effects occurred between Run 2, 3, 9 and Run 8 with the mean concentration of 7.63 and 7.64 %. The values obtained in this study were higher than the values (4.68 to 7.52) reported by Arise *et al.* (2017) for wheat, plantain and bambara groundnut composite flour but lower than the moisture content (10%) in flour that inhibits the growth of mould and other biochemical reactions while improving its shelf life (Al-Defiery & Merjan, 2015).

The protein content of the flour blend ranged from 25.17 - 37.02 %. The lowest protein content was found in Run 8 with mean value of 25.17 % while the highest protein content was found in Run 5, 14 with a mean value of 37.02 %. The high protein content recorded in Run 8 could be attributed to increasing pumpkin, sunflower and amaranth flour substitution, with pumpkin exerting the most effect. Similarly, Mitiku and Bereka (2021) reported that increasing substitution of wheat flour with soybean increased the protein content of the blends. Significant difference was observed among the various flour blends. The range of the protein content recorded in the blends were higher than the range (9.02-25.85 %) reported by Simwaka *et al.* (2017) who worked on millet, sorghum, pumpkin and amaranth seed flours. Increase in protein content of products containing pumpkin seed flour has also been reported by several authors (Norfezah *et al.*, 2011; Giami *et al.*, 2003; El-Soukkary, 2001). Protein is required for growth, repair, and maintenance of the body.

The fat contents of the blends ranged from 0.91 - 1.51. The lowest crude fat content was found in Run 6 with a mean value of  $0.91 \pm 0.02$  while the highest was found in Run 2, 3, and 9 with a mean value of  $1.51 \pm 0.02$ . Run 2, 3, and 9 exhibited significant differences ( $p < 0.05$ ) when compared to all other runs, except for Run 1, which displayed a mean value of 1.49 %. There was no significant difference between Run 6, 7 and 10. The crude fat contents reported were lower than the 1.95 % reported by Babatunde and Saka (2020) for defatted amaranth flour. The low value of fat recorded in the blend could be associated with the proportion of the defatted amaranth, pumpkin and sunflower flour in the blends. Low contents of fat helps in reducing the overall calorie content of foods which is vital in weight management. Fat provides essential fatty acids for growth, serves as a source of energy and fat-soluble vitamins, and plays a crucial role in infant nutrition. Exceeding the desired level of fat could negatively impact product stability, primarily because unsaturated fatty acids become more susceptible to oxidative rancidity (Tenagashaw *et al.*, 2015).

Ash content, a reflection of the mineral element present in the samples, ranged from 3.93 - 5.71 % with Run 11 having the lowest ash content and Run 5 and 14 the highest. Significance differences ( $p < 0.05$ ) were observed among various blends except for Run 6 and Run 12 with mean values of 4.48 % and 4.52 % respectively. Defatted pumpkin contributed a great deal to the ash content due to their inherent ash content and proportion in the mixes. The values in this study were more than the values (1.01 - 2.87 %) reported by Twinomuhwezi *et al.* (2020)



for amaranth, millet, rice and soybean flour. This could be due to inherent ash content of pumpkin seed which was reported to be very high (Elinge *et al.*, 2012). The ash contents in the flour blend hold great potential for providing significant minerals to the body.

The crude fibre content ranged from 3.38 - 4.08 %. Significant differences existed among various samples in the blends. The highest fibre content which was observed in Run 7 and 10 could be attributed to the high proportion of amaranth flour in the blends. This also supports the work of Muluken and Shimelis (2021), on their work on *Amaranthus* (*Genus amaranthus* L.) based composite flour blends for preparation of gluten-free biscuits, who reported an appreciable increased in the fibre content (1.31 - 2.22 %) as the level of amaranth grain flour substitution increased. Crude fibre helps to prevent heart diseases, colon cancer and diabetes among other functions (Awuchi, 2019). Crude fibre reduces the rate of release of glucose into the bloodstream and also reduces intracolonic pressure, thereby reducing the risk of colon cancer (Awuchi, 2019).

The carbohydrate contents of the flour blends ranged from 44.79 - 58.52 %. Run 8 recorded the highest carbohydrate content while Run 5 and 14 recorded the lowest. Significant difference ( $p < 0.05$ ) was observed in the carbohydrate values of the various flour blends. Carbohydrates serve as the main energy source for cells, particularly those found in the central nervous system (CNS) and red blood cells (Hannington *et al.*, 2020). The substantial carbohydrate content of the flours indicated their potential in addressing protein-energy malnutrition (PEM), as they can provide energy to the body and help spare protein.

**Table 4: Proximate Composition of the Amaranth-Based Blends (%)**

S/N	Moisture content	Crude protein	Crude fat	Total ash	Crude fibre	CHO
1	7.58 ± 0.03f	27.96 ± 0.03c	1.49 ± 0.04ef	4.15 ± 0.03d	3.38 ± 0.03a	55.44 ± 0.07e
2	7.63 ± 0.03g	26.29 ± 0.03b	1.51 ± 0.02f	4.05 ± 0.03c	3.41 ± 0.03a	57.11 ± 0.10g
3	7.63 ± 0.03g	26.29 ± 0.03b	1.51 ± 0.02f	4.05 ± 0.03c	3.41 ± 0.03a	57.11 ± 0.10g
4	6.53 ± 0.04a	36.30 ± 0.04g	1.12 ± 0.04b	5.63 ± 0.03g	3.71 ± 0.02c	46.71 ± 0.07b
5	7.30 ± 0.03e	37.02 ± 0.03h	1.36 ± 0.03d	5.71 ± 0.03h	3.82 ± 0.03d	44.79 ± 0.07a
6	6.68 ± 0.03b	29.83 ± 0.03e	0.91 ± 0.02a	4.48 ± 0.02f	4.05 ± 0.03f	54.05 ± 0.06d
7	6.73 ± 0.03c	28.05 ± 0.04d	0.92 ± 0.02a	4.38 ± 0.03e	4.08 ± 0.02f	55.84 ± 0.09f
8	7.64 ± 0.02g	25.17 ± 0.03a	1.45 ± 0.03e	3.71 ± 0.02a	3.52 ± 0.03b	58.52 ± 0.09i
9	7.63 ± 0.03g	26.29 ± 0.03b	1.51 ± 0.02f	4.05 ± 0.03c	3.41 ± 0.03a	57.11 ± 0.10g
10	6.73 ± 0.03c	28.05 ± 0.04d	0.92 ± 0.02a	4.38 ± 0.03e	4.08 ± 0.02f	55.84 ± 0.09f
11	7.06 ± 0.03d	26.33 ± 0.03b	1.08 ± 0.03b	3.93 ± 0.03b	3.95 ± 0.03e	57.65 ± 0.10h
12	7.02 ± 0.03d	29.98 ± 0.02f	1.22 ± 0.03c	4.52 ± 0.02f	3.72 ± 0.02c	53.54 ± 0.06c
13	6.53 ± 0.04a	36.30 ± 0.04g	1.12 ± 0.04b	5.63 ± 0.03g	3.71 ± 0.02c	46.71 ± 0.07b
14	7.30 ± 0.03e	37.02 ± 0.03h	1.36 ± 0.03d	5.71 ± 0.03h	3.82 ± 0.03d	44.79 ± 0.07a

Values are means of triplicate determinations ± SD

Means followed by the different alphabet within the same column are significantly different ( $p < 0.05$ )



## Analysis of Variance (ANOVA) for Response Surface Mixture Linear Model for Proximate Composition of the Amaranth-Based Blends

The analysis of variance (ANOVA) model result for response surface for mixture linear model for proximate composition of the amaranth-based blends is presented in Table 5. From the table, it was revealed that the model terms and linear terms were all significant ( $p < 0.05$ ) respectively. A p-value of less than 0.0001 indicates high statistical significance. In this table, all p-values were less than 0.0001, suggesting that both the model and the linear mixture term significantly contribute to the variation in the proximate composition of the amaranth-based blends. Coefficients of determination ( $R^2$ ) measure the proportion of variance in the response variables (proximate composition components) explained by the model; adjusted (Adj)  $R^2$  values take into account the number of predictor variables in the model and the "Pred  $R^2$ " column represents the predicted  $R^2$  values, assessing the model's predictive ability for new data. The  $R^2$  values ranged from 0.9043 - 0.9608 suggesting a good fit. Crude fat content had the highest  $R^2$  value of 0.9608 while carbohydrate contents displayed least  $R^2$  values. The model with  $R^2 > 0.75$  was considered acceptable according to Yang *et al.* (2010). The closer the  $R^2$  value to unity, the better and more significant an empirical model fits the actual data. The smaller  $R^2$  is, the less important the dependent variables in the model have in explaining the behavior of variation (Myers & Montgomery, 1995). This indicates that the model explains a substantial amount of the variation in the proximate composition components. The "adequate precision" measures the signal-to-noise ratio, representing the adequacy of the model. Higher values indicate a better signal-to-noise ratio and a more reliable model. The adequate precision values range from 12.727 - 21.947, suggesting that the model is adequate for predicting the proximate composition of amaranth-based blends. Coefficient of variation (C.V) indicates the precision or reliability of the estimates obtained from the model. Lower values signify higher precision. The coefficients of variation for the proximate composition components ranged from 1.81 to 5.85, indicating reasonably precise estimates from the model. The high  $R^2$  values and low coefficients of variation suggest that the model is reliable for predicting the proximate composition of amaranth-based blends. This corroborates the work of Awolu *et al.* (2017), Laura *et al.* (2012) and Oluwasina *et al.* (2020). Final equations in terms of actual factor for proximate composition of the amaranth-based blends are:

$$\text{Moisture} = 0.063120A + 0.11059S + 0.064142P + 0.046526SF \quad (1)$$

$$\text{Protein} = 0.1743A + 0.10425S + 0.64917P + 0.28711SF \quad (2)$$

$$\text{Crude fat} = 2.76851E - 003A + 0.032795S + 0.015049P + 0.010124SF \quad (3)$$

$$\text{Ash} = 0.024820A + 0.012197S + 0.10089P + 0.060889SF \quad (4)$$

$$\text{Crude fibre} = 0.048506A + 0.016278S + 0.036191P + 0.024451SF \quad (5)$$

$$\text{Carbohydrate} = 0.68620A + 0.72390S + 0.13462P + 0.57165SF \quad (6)$$



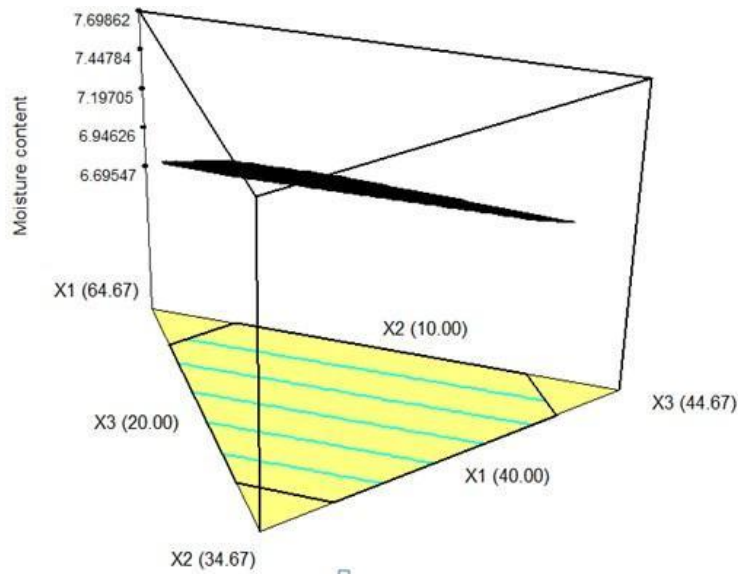


where A, S, P, and SF represent the proportion of defatted amaranth, sorghum, defatted pumpkin and defatted sunflower flower, respectively. The positive (+) sign in the equations means that the response value increased with increase of the variables. The 3D plot, showing the effect of the composite flours on the moisture content (Figure 1), showed that sorghum had more effect on the moisture content followed by amaranth flour. The moisture content decreases as the amaranth and sorghum decreases. Pumpkin flour produced the highest increase in protein followed by sunflower and amaranth, respectively. The effect of the composite flour on the protein contents is shown in Figure 2. The 3D plot showing the effect of composite flour on the crude fat content (Figure 3) showed that sorghum had more effect on the increase in fat content. As the sorghum flour content increased, the crude fat content increased and vice versa. The increase in pumpkin flour contributed more to the increase in ash content of the blend. Ash content increases with regard to increase in the addition of pumpkin flour (Figure 4). The 3D plot, displaying the effect of the composite flour on the crude fibre, is shown in Figure 5. The crude fibre increased as the levels of each component increased with amaranth having the highest effect. Among the component mixtures, sorghum produced the highest increase in carbohydrate followed by defatted amaranth, defatted sunflower flour and defatted pumpkin flour, respectively (Figure 6).

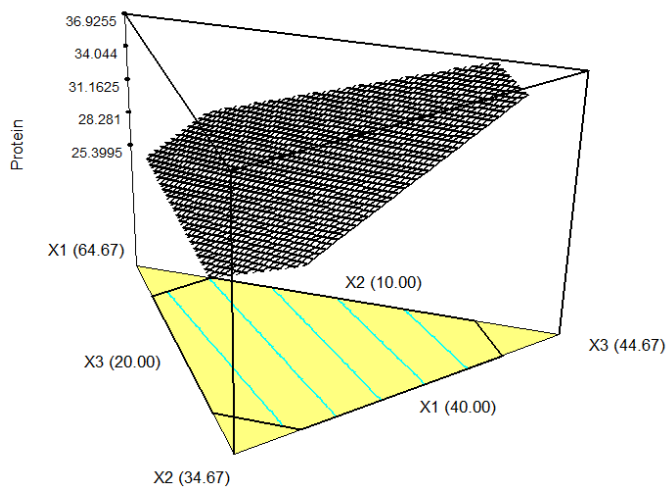
**Table 5: Summary of Analysis of Variance (ANOVA) for Response Surface Mixture Linear Model for Proximate Composition of the Amaranth-Based Blends**

Source	Moisture content	Crude Protein	Crude Fat	Ash content	Crude Fibre	Carbohydrate
Model (Prob>F)	<0.0001	<0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001
Linear mixture (Prob >F)	<0.0001	<0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001
R <sup>2</sup>	0.9339	0.9248	0.9608	0.9050	0.9157	0.9043
Adj R <sup>2</sup>	0.9141	0.9023	0.9491	0.8765	0.8904	0.8755
Pred R <sup>2</sup>	0.8666	0.8912	0.9397	0.8556	0.8494	0.8532
Adeq precision	15.525	15.053	21.947	14.227	15.384	12.727
C.V	1.81	4.87	4.37	5.85	2.37	3.46

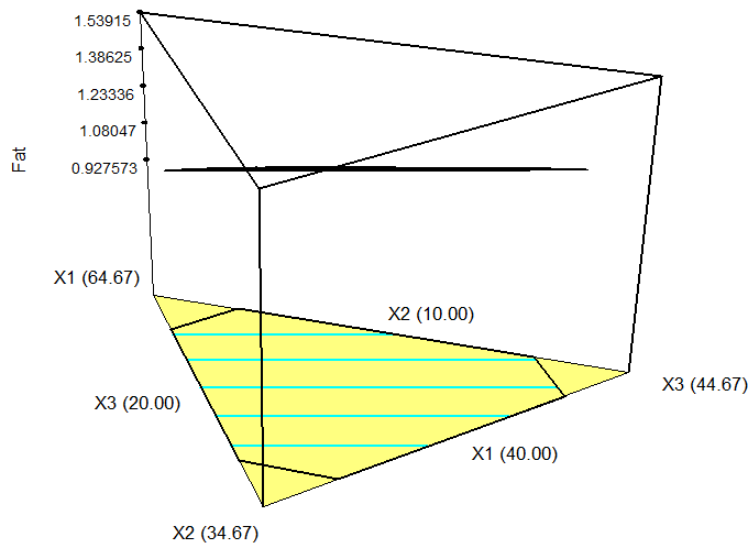
Values of “Prob>F” less than 0.0500 indicate model terms are significant



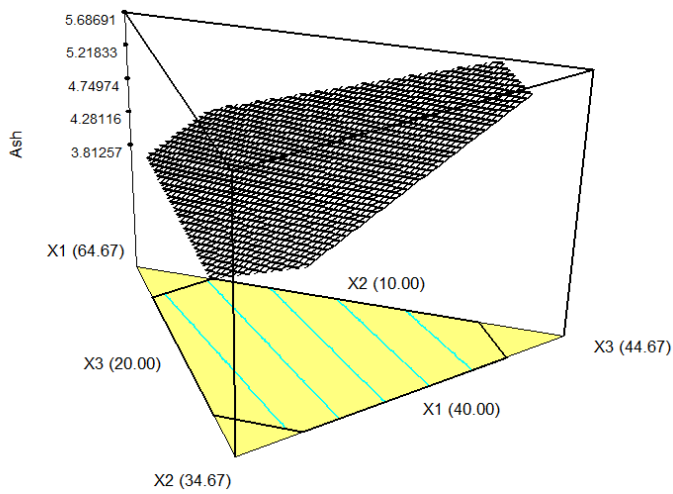
**Figure 1: 3D plot showing the effect of amaranth-based blends on moisture content**



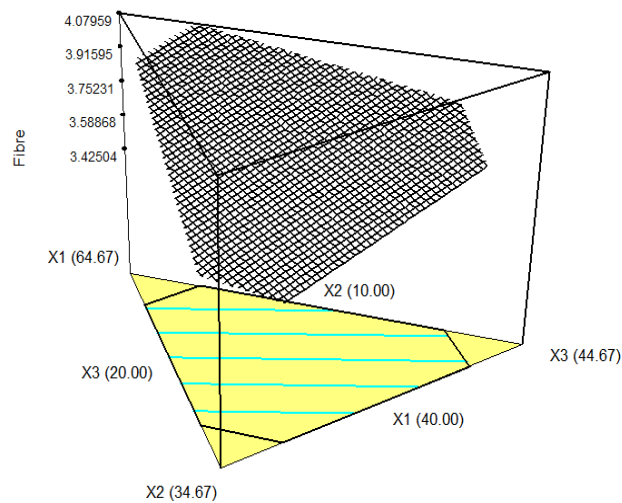
**Figure 1: 3D plot showing the effect of amaranth-based blends on Protein content**



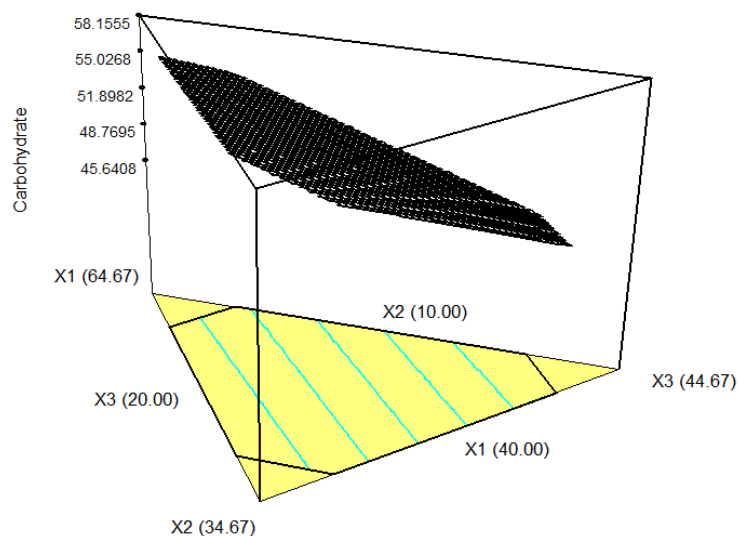
**Figure 1: 3D plot showing the effect of amaranth-based blends on fat content**



**Figure 1: 3D plot showing the effect of amaranth-based blends on the ash content**



**Figure 1: 3D plot showing the effect of amaranth-based blends on fibre content**



**Figure 1: 3D plot showing the effect of amaranth-based blends on carbohydrate content**

### Diagnostic Case Statistics for Proximate Composition of the Amaranth-Based Blends

The result of diagnostic case statistics for proximate composition of the amaranth-based blends is presented in Table 6. The actual values in the table represent the experimental results for proximate composition of amaranth-based flour blends. The predicted values signify the standard generated by the design expert software applied. The residual values reveal the closeness of the actual value to the predicted value. A negative value of the residual value indicates that the predicted value is greater than the actual value while a



predicted value of zero means that the actual value is equivalent to the standard value against which it was compared. A zero value suggests that the model accurately estimated a response variable. The table revealed that residual values for crude fat, total ash and crude fibre were all negative while zero value was recorded for protein. Moisture contents and carbohydrates recorded positive values which indicated that the actual values were more than the predicted values. This corroborates the work of Awolu *et al.* (2017) and Laura *et al.* (2012).

**Table 6: Diagnostic Case Statistics for Proximate Composition of the Amaranth-Based Blends**

Name	Standard Order	Actual Value	Predicted Value	Residual Value
Moisture content	1	7.142143	7.141429	0.000714
Crude protein	2	29.80	29.80	0.00
Crude fat	3	1.238571	1.239286	-0.00071
Ash content	4	4.556429	4.558571	-0.00214
Crude fibre	5	3.734286	3.735714	-0.00143
Carbohydrate	6	53.52214	53.52143	0.000714

Values represent average of 14 runs for each response

### Optimization of Blend Formulation

From the numerical optimization through the desirability function (Table 7), the blend that produced the highest desirability index of 0.825 while maximizing protein was: 42.46% amaranth, 10.00% sorghum, 40.00% pumpkin, and 7.54% sunflower flour. The experimental values at this blend (42.46A, 10.00S, 40.00P and 7.54SF) resulted in: moisture (6.91%), protein (36.86%), fat (1.05%), total ash (5.61), crude fibre (3.90), and CHO (45.67).

**Table 7: Numerical Optimization of Amaranth-Based Blends using Proximate Composition**

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Amaranth flour	Is in range	40	60	1	1	3
Sorghum flour	Is in range	10	30	1	1	3
Pumpkin flour	Is in range	20	40	1	1	3
Sunflower Flour	Is in range	3	10	1	1	3
Moisture content	Is in range	6.63	7.64	1	1	3
Protein	Maximize	25.17	37.02	1	1	3
Fat	Minimize	0.91	1.51	1	1	3
Ash	Maximize	3.71	5.71	1	1	3



Fibre	Is in range	3.38	4.08	1	1	3
CHO	Minimize	44.79	58.51	1	1	3

## CONCLUSION

The study demonstrated significant variations in proximate composition among the blends, emphasizing the importance of strategic formulation. There were high  $R^2$  values and low coefficients of variation for the proximate composition of the blends, indicating the reliability of the experimental design in optimizing nutritional properties of the blends. Through numerical optimization using the desirability function, the optimal blend composition was 42.46% amaranth, 10.00% sorghum, 40.00% pumpkin, and 7.54% sunflower flour. Composite blends of amaranth, sorghum, pumpkin seed flour, and sunflower flour through advanced experimental design methodologies hold potential for the production of nutrient-dense food products capable of addressing issues of malnutrition.

## CONFLICT OF INTEREST

In the research work presented in this manuscript, the authors declare no conflict of interest.

## REFERENCES

- Abah, C. R., Ishiwu, C. N., Obiegbuna, J. E., & Oladejo, A. A. (2020). Sorghum Grains: Nutritional Composition, Functional Properties and Its Food Applications. *European Journal of Nutrition and Food Safety*, 12(5), 101-111.
- Ahmed, Z., Forsido, S. F., Kuyu, C. G., & Keyata, E. O. (2023). Optimization of soaking conditions (temperature and time) on physicochemical properties of selected parboiled rice varieties grown in Eastern Ethiopia, *Food Science and Nutrition*, 11(6): 3171-3183. <https://doi.org/10.1002/fsn3.3298>.
- Al-Defiery, M. E., & Merjan, A. F. (2015). Mycoflora of mold contamination in wheat flour and storage wheat flour. *Mesop. Environ. J*, 1(2), 18-25.
- AOAC (2012). Association of Official Analytical Chemists international. 20th ed. Gaithersburg, MD, USA: AOAC International, p. 3172.
- Arise, A. K., Dauda, A. O., & Awolola, G. V. (2017). Physico-chemical, functional and pasting properties of composite flour made from wheat, plantain, and bambara for biscuit production. *Annals of Food Science and Technology*, 18(4).
- Awolu, O.O., Oyebanji, O.V, & Modupelola, S (2017). Optimization of proximate composition and functional properties of composite flours consisting wheat, cocoyam (*Colocasia esculenta*) and bambara groundnut (*Vigna subterranea*). *International Food Research Journal* 24(1), 268-274.
- Awuchi, C. G. (2019a). Proximate Composition and Functional Properties of Different Grain Flour Composites for Industrial Applications. *International Journal of Food Sciences*, 2(1), 43-64.
- Babatunde, O, & Gbadamosi, S.O. (2020). Influence of Processing on the Physicochemical, Functional and Pasting Properties of Nigerian *Amaranthus viridis* seed flour: a multivariate analysis approach. *SN Applied Sciences*. <https://doi.org/10.1007/s42452-020-2418-8>.



- Elinge, C. M., Muhammad, A., Atiku, F. A., Itodo, A. U., PeniI, J., Sanni, O. M., & Mbongo, A. N. (2012). Proximate, Mineral, and Anti-nutrient Composition of Pumpkin (*Cucurbita pepo* L) Seeds Extract. *International Journal of Plant Research*, 2(5), 146-150. <https://doi.org/10.5923/j.plant.20120205.02>.
- El-Soukkary, F. A. (2001). Evaluation of Pumpkin Seed Products for Bread Fortification. *Plant Foods Human Nutrition* 56(4): 365-384.
- Fadimu, G. J., Sanni, L. O., Adebowale, A. A., Kareem, S. O., Sobukola, O. P., Kajihausa, O. E., and Adenekan, M. K. (2018). Optimisation of pre-treatment conditions for plantain (*Musa parasidiaca*) flour using Box-Behnken design. *Quality Assurance and Safety of Crops and Foods*, 10 (3), 223-232.
- Gandhi, A. P, Jha, K. & Gupta, V. (2008). Studies on the Production of Defatted Sunflower Meal with Low Polyphenol and Phytate Contents and its Nutritional Profile, *ASEAN Food Journal*, 15 (1), 97-100.
- Giami, S. Y., & Barber, L. I. (2004). Utilization of Protein Concentrates from Ungerminated and Germinated Fluted Pumpkin (*Telfairia occidentalis*). Hook Seeds in Cookies Formulation. *Journal of the Science of Food and Agriculture*, 84(14), 1901-1907.
- Giami, S. Y., Merba, H. D., Kiin-Kabari, D. B. & Achinewhu, S. C. (2003). Evaluation of the Nutritional Quality of Breads Prepared from Wheat-Fluted Pumpkin (*Telfairia occidentalis* Hook) Seed Flour Blends. *Plant Foods for Human Nutrition* 58, 1-8.
- Jawan, R., Abbasiliasi, S., Tan, J. S., Kapri, M. R., Mustafa, S., Halim, M., & Ariff, A. B. (2021). Evaluation of the Estimation Capability of Response Surface Methodology and Artificial Neural Network for the Optimization of Bacteriocin-Like Inhibitory Substances Production by *Lactococcus lactis* *Microorganisms*, 9(3), 579. <https://doi.org/10.3390/microorganisms9030579>.
- Kaur, H., Shams, R., Dash, K. K., & Dar, A. H. (2023). A comprehensive review of pseudo-cereals: Nutritional profile, phytochemicals constituents and potential health promoting benefits. *Applied Food Research*, 3(2), 100351. <https://doi.org/10.1016/j.afres.2023.100351>.
- Kumari, P.V., & Sangeetha, N (2016). Nutritional significance of cereals and legumes based food mix- A review *International Journal of Agricultural and Life Sciences*- 3 (1), 115-122.
- L. D. Griffith, M. E. Castell-Perez, & M. E. Griffith. *Cereal Chemistry*, 75(1) 1998 105
- Tanimola, A. R., Otegbayo, B., & Akinoso, R. (2016). Chemical, functional, rheological and sensory properties of amaranth flour and amaranth flour based paste. *African Journal of food science* 10(11), 313-319. <https://doi.org/10.5897/AJFS2016.1422>.
- Mampholo, M. B., Sivakumar, D., & Van, J. (2015). Variation in Bioactive Compounds and Quality Parameters in different Modified Atmosphere Packaging during Postharvest Storage of Traditional Leafy Vegetables (*Amaranthus cruentus* L and *Solanum retroflexum*). *Journal of Food Quality*, 38(1), 1-12.
- Mitiku, D. H., & Bereka, T. Y. (2021). Effects of Pumpkin (*Cucurbita moschata*)/Soybean (*Glycine max*) Flour Blends on Functional, Physico-Chemical Properties and Sensory Attributes of Breads Produced From Whole Wheat (*Triticum aestivum* L). *Carpathian Journal of Food Science and Technol*, 13(1), 2021.
- Mulleken, K.K., & Shimelis A.E (2021). Evaluation of Various Properties of Amaranthus (Genus *Amaranthus* L.) Based Composite Flour Blends for Preparation of Gluten-Free Biscuits. *Croatian Journal of Food Science and Technology*, 13(1), 57-68.



- Myers, R. H., & Montgomery, D. C. (1995). *Response Surface Methodology: Process and Product Optimization Using Design Experiment* (2nd ed.). John Wiley and Sons, Inc.
- Nahemiah, D, Iro, N., & Mamudu, H.B (2015). Application of Response Surface Methodology (RSM) and Central Composite Design (CCD) to Optimize Minerals Composition of Rice-Cowpea Composite Blends during Extrusion Cooking, *International Journal of Food Science and Nutrition Engineering*, 5(1) : <https://doi.org/10.5923/j.food.20150501.06>.
- Neelesh, K., M & Pratibha A. (2018). Amaranthus Grain Nutritional Benefits: A Review. *Journal of Pharmacognosy and Phytochemistry*, 7(2): 2258-2262.
- Norfezah, M. N., Hardacre, A. & Brennan, C. S. 2011. Comparison of Waste Pumpkin Material and its Potential use in Extruded Snack Foods. *Food Science and Technology International* 17(4), 367-373.
- Ogunlakin, G. O., Ajala, F. O., & Olajire, A. S. (2022). Nutritional Composition of Wheat, Mushroom (*Pleurotus ostreatus*) and Unripe Plantain (*Musa paradisiaca*) Flour Blends. *Asian Food Journal*, 21(7), 14-23.
- Olson, R., Gavin-Smith, B., Ferraboschi, C. & Kraemer, K. (2021). Food Fortification: The Advantages, Disadvantages and Lessons from Sight and Life Programs. *Nutrients*, 13(4), 1118. <https://doi.org/10.3390/nu13041118>.
- Olurin T.O., Ogunmoyela O.A.B., Dudu O.E., Adubi T.A. (2021). Cookies-making potentials of sorghum-wheat flour blends. *Anchor University Journal of Science and Technology* 1(2), 44-51.
- Oluwasina, O. O., Demehin, B. F., Awolu, O. O., & Igbe, F. O. (2020). Optimization of starch-based candy supplemented with date palm (*Phoenix dactylifera*) and tamarind (*Tamarindus indica* L.). *Arabian Journal of Chemistry*, 13(11), 8039-8050. <https://doi.org/10.1016/j.arabjc.2020.09.033>.
- Simwaka, J.E., Chamba, M.V.M., Huiming, Z., Masamba, K.G. & Luo, Y (2017). Effect of Fermentation on Physicochemical and Antinutritional Factors of Complementary Foods from Millet, Sorghum, Pumpkin and Amaranth Seed Flour. *International Food Research Journal* 24(5), 1869-1879.
- Tenagashaw, M. W., Kenji, G. M., Melaku, E. T., Huyskens-Keil, S., & Kinyuru, J. N. (2015). Proximate composition and selected functional properties of complementary foods from teff fortified with soybean and orange-fleshed sweet potato. *RUFORUM Working Document Series* 14 (1), 953-965.
- Twinomuhwezi, H., Awuchi, C. G., & Rachael, M. (2020). Comparative Study of the Proximate Composition and Functional Properties of Composite Flours of Amaranth, Rice, Millet, and Soybean. *American Journal of Food Science and Nutrition*, 6(1), 6-19.
- Vasundhara Rao & Amrita Poonia. (2023). Protein Characteristics, Amino Acid Profile, Health Benefits and Methods of Extraction and Isolation of Proteins from some Pseudocereals—a review. *Food Production, Processing and Nutrition*. 5 (37), 1-17.
- Yang, L., Cao, Y. L., Jiang, J. G., Lin, O. S., Chen, J., & Zhu, L. (2010). Response Surface Optimization of Ultrasound-assisted Flavonoids Extraction from the Flower of *Citrus Aurantium* L. var. *amara* Engl. *Journal of Separation Science*, 33, 1349-1355.