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D-Optimal Mixture Design Applications in the Assessment of Mineral and Antinutritional Contents of Amaranth-Based Blends

Adewole S. A[1]., Abiona, O. O[2]., Akande, E. A[3]., Oke, M. O[4]., Adewole, O. A[5]. and Ologunde, M. O[6]

[1]Department of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Nigeria
[2]Department of Food Science and Technology, Osun State University, Osogbo, Nigeria
[3,6] Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[4]Department of Food Engineering, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[5]Department of Biochemistry, School of Basic Medical Sciences, Benjamin S. Carson (Snr.) College of Health and Medical Sciences, Babcock University, Ilishan-Remo, Nigeria.

Corresponding Author's Email: gokesax4success@gmail.com

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ABSTRACT

Malnutrition is becoming more endemic especially in war-ton and highly deprived area of Africa. The amaranth, pumpkin and sunflower flour seed were cleaned, sorted, milled and defatted to obtain defatted flour samples. The amaranth, pumpkin and sunflower seeds were cleaned, sorted, milled and defatted using n-hexane. 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. Using D-optimal design, Fourteen (14) runs were generated and the blends were analyzed for mineral composition (sodium, potassium, iron, calcium, and phosphorus) and anti-nutritional factors (phytate, tannin, oxalate, saponin and trypsin), respectively. The response variables were modeled using a linear regression equation. The mineral composition ranged from 5.50-9.82, 335.01-340.84, 10.80-12.15, 73.56-89.16, and 276.82-284.90 mg/100g for sodium, potassium, iron, calcium, and phosphorus. Phytate and tannin content ranged from 0.1-0.22 and 2.70-2.90 mg/100g, respectively. The linear model terms for both mineral and antinutritional factors were significant ($p \leq 0.05$). In conclusion, composite flour from amaranth, sorghum, pumpkin and sunflower flour have substantial mineral profile and controlled antinutritional factors and therefore can be used in the development of nutrient-densed food product such as snack.

Keywords: Mineral, antinutritional, D-optimal design, amaranth, sorghum, malnutrition

1.0 INTRODUCTION

Malnutrition is a global health concern that arises when there is an imbalance between the intake of nutrients needed for body development and the inability of the body to absorb or use these nutrients, leading to adverse effects on physical and mental well-being (Ghodsi *et al*., 2023). While malnutrition encompasses deficiencies in macronutrients like proteins, fats, and carbohydrates, it also involves insufficient intake of micronutrients; minerals and vitamins. Despite the nutritional benefits of amaranth, its incorporation into blends necessitates consideration of its mineral composition and potential antinutritional factors. Currently, there is a lack of comprehensive research utilizing optimal mixture design to assess the mineral and antinutritional composition of blends containing amaranth. This knowledge gap hinders the development of optimized blends that maximize nutritional value while minimizing antinutritional factors, impeding efforts to promote the consumption of nutritious and balanced food products. The role of minerals is paramount as they play a vital role in various physiological functions including bone health, muscle and nerve functions, regulating body's water balance and enzymatic reactions (Weyh *et al*., 2022). Minerals such as iron, zinc, calcium, phosphorus and magnesium are essential for the proper functioning of the human body. In contrast to micronutrient deficiencies, which result in noticeable clinical symptoms, micronutrient insufficiencies may produce subtle symptoms that are challenging to detect (Kiani *et al*., 2022). Furthermore, an energy-rich and nutrient-poor diet might lead to "hidden hunger" condition, which is characterized by micronutrient inadequacies despite having sufficient or excessive amount of calories (Kiani *et al*., 2022). These micronutrient inadequacies could ultimately cause various health issues like cancer, osteoporosis, cardiovascular diseases, anemia, impaired immune function, and developmental abnormalities (Kiani *et al*., 2022, Awuchi *et al*., 2020). However, malnutrition is not solely about inadequate intake; it can also be influenced by antinutritional factors present in certain foods. Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients (Cammack *et al*., 2020) such as vitamins, minerals, and proteins in addition to inhibiting enzyme activities (Salim *et al*., 2023). These factors can negatively impact the bioavailability of essential minerals, worsening malnutrition issues. Understanding the presence and effects of antinutritional factors is vital for developing strategies to alleviate their impact on nutritional health (Samtiya *et al.*, 2020). The best option for treating malnutrition is to enhance normal eating and drinking. A "food first" approach encourages eating frequent, small, and high energy protein meals. Many cereals, legumes and root crops can be employed as base raw materials for dietary product capable of addressing the problem of malnutrition (Awuchi *et al*., 2020). Amaranth, a pseudocereal, is grown for its edible starchy seeds, just like cereals, but it is not a member of the cereal family like wheat and rice. Amaranth seeds, known for their high nutritional content, have gained attention as a potential solution to malnutrition. Amaranth is rich in essential nutrients such as protein, fiber, vitamins, and minerals, making it a valuable addition to a balanced diet for promoting overall health and well-being (Aditya *et al*., 2019). Due to its diverse nutritional advantages, recent findings suggest that amaranth possesses a dual nature as a pseudocereal, exhibiting both food-like properties and health-enhancing capabilities (Baraniack *et al*., 2022). Because of its high content of quality proteins (12.5%), and soluble fibre of about 4.2%, grain amaranth is highly recommended for complementary feeding (Bender *et al*., 2021). Moreover, blending amaranth with other nutrient-dense flours, such as sorghum, pumpkin, and sunflower, offers a synergistic approach to enhance the overall nutritional quality of the diet. Sorghum contributes proteins and carbohydrates, pumpkin provides protein, essential vitamins and minerals like vitamin A and potassium, while sunflower flour adds healthy fats and additional proteins. Combining these ingredients with amaranth not only diversifies the nutrient profile but also addresses the challenges posed by antinutritional factors, promoting better nutrient absorption and utilization. D-optimal mixture design is a specific type of experimental design that is used in response surface methodology (RSM). D-optimal design aims to maximize the precision of parameter estimates in the statistical model. In the context of mixture experiments, D-optimal mixture design is used to efficiently allocate experimental runs in such a way that the resulting model is optimal for prediction and optimization (Rahayu *et al*., 2019).

Therefore, the objectives of this present study are to apply optimal mixture design methodologies to formulate blends containing amaranth along with other ingredients and assess some mineral composition and antinutritional factors of the blends.

2.0 MATERIALS AND METHODS

2.1 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), Ibadan, Nigeria while pumpkin seed and sunflower seed were obtained from Ahmadu Bello University Experimental Farm, Zaria, Nigeria.

2.2 Experimental Design

A four-component constrained D-optimal mixture process experimental design, with 14 randomized experimental runs, was employed. 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

$$Y = \beta o + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 4X4 + € \quad (1)$$

Where *Y* represents the response variables to be modeled, βo is the value of the fitted response at the center point of the design, and β1, β2, β3 and β4 are the coefficient of the input variables X1 X2, X3 and X4, and € the random error of the model.

2.3 Preparation of Defatted Amaranth Flour, Sorghum, Defatted Sunflower Flour, And Defatted Pumpkin Flour

2.4 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 ℃ and stored in an air-tight plastic container at room temperature until further use.

2.5 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 µm sieve. Thereafter, the flour was packaged in a well-sealed low-density polyethylene bag (Olurin *et al*., 2021).

2.6 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 a attrition mill and then sieved through a 500 µm 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 ℃ 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 ℃) until it was used.

2.7 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 oil-extracted flour was dried at room temperature for 24 h. The defatted flour was subsequently oven-dried at 60 ℃ for 30 min to remove the residual n-hexane. The flour was stored in an air-tight plastic container at room temperature (37 ℃) until it was used (Gandhi *et al*., 2008). The flour was packaged in a well-sealed low-density polyethylene bag.

2.8 Blend Preparations

Composite flours consisting 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.

2.9 Sample Digestion

Determination of the Mineral Content

The ashed sample was subjected to digestion in a digestion flask with 1M HCl at 90 ℃ for 5 min. The contents were thoroughly mixed before being transferred into a 100 mL volumetric flask and diluted to the mark with 1M HCl. The solution was subsequently filtered and stored in a polythene bottle. The prepared digest was taken to an Atomic Absorption Spectrophotometer (AAS), where the mineral elements were automatically quantified.

2.10 Phytate Content

The phytate content of the sample was determined using the method described by Inuwa *et al*. (2011). Two grams (2 g) of each finely ground flour sample were soaked in 20 mL of 0.2M HCl and subsequently filtered. After filtration, 0.5 mL of the filtrate was combined with 1 mL of Ferric ammonium sulfate solution in a test tube. The mixture was then boiled for 30 min in a water bath, cooled in ice for 15 min, and centrifuged for 15 min. One mL of the supernatant was mixed with 1.5 mL of a 2.2-pyridine solution, and the absorbance was measured using a spectrophotometer at 519 nm. The concentration of phytic acid was determined by extrapolating from a standard curve using a standard phytic acid solution (Inuwa *et al*., 2011).

2.11 Tannin Content

Tannin content was determined by adding 10 mL of 70 % aqueous acetone to 200 mg of finely ground flour sample in a bottle, which was then properly covered. The bottle was placed in an ice bath shaker for 2 h at 30 ℃. Afterward, the solution was centrifuged, and the supernatant was stored on ice. Thereafter, 0.2 mL of the supernatant was pipetted into 0.8 mL of distilled water. A standard tannic acid solution was prepared. To both the samples and the standard, 0.5 mL of Follin reagent was added, followed by 2.5 mL of 20 % Na_2CO_3. The solutions were vortexed and allowed to incubate for 40 min at room temperature. Subsequently, absorbance was read at 725 nm. The concentration of tannin in the sample was estimated from the standard tannic acid curve, following the methodology of Makkar & Goodchild (1996).

2.12 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 undergone ANOVA to assess its statistical significance for every response variables. 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), 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.

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 Mineral and Antinutritional properties of Amaranth based 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

Table 3: Design Summary for the Production of Amaranth, Sorghum, Pumpkin and Sunflower flour Blends

Response	Name	Unit	Transformation	Model	Minimum	Maximum
Y1	Na	mg/100g	None	Linear	6.94	9.82
Y2	K	mg/100g	None	Linear	335.01	340.84
Y3	Fe	mg/100g	None	Linear	10.80	12.15
Y4	Ca	mg/100g	None	Linear	73.56	89.16
Y5	P	mg/100g	None	Linear	276.82	284.90
Y6	Phytate	mg/100 g	None	Linear	0.11	0.22
Y7	Tannin	mg/100 g	None	Linear	2.70	2.90
Y8	Saponin	%	None	Linear	0.48	0.67

3.0 DISCUSSIONS AND RESULTS

Table 4 displays the results of the amaranth-based blends' Between 5.50 and 9.82 mg/100 g of sodium were present. Sample run 6 had the greatest mean sodium level of 9.82 mg/100 g when compared to all other samples, whereas run 8 had the lowest mean sodium content of 5.50 mg/100 g. The increased value of pumpkin and the rising percentage of amaranth seed flour may be the cause of the high Na content in XF6. Sodium represents the principal cation of extracellular fluid that maintains the osmotic pressure of the fluid and activities of some enzymes such as amylase (Chen *et al*., 2020). The body's requirement for sodium is relatively small, and it is advised to consume it in limited amounts. A daily sodium intake of 5 g/day of salt has been suggested according to the World Health Organization (WHO, 2012). Consuming excessive sodium in the diet can lead to hypertension, particularly in individuals with a genetic predisposition (Grillo *et al*., 2019).

The potassium content obtained from the sample ranged between 335.01 and 340.84 mg/100 g. The lowest potassium content was observed in run 1 with mean value of 335.01 mg/100 g while the highest potassium content was observed in run 6 with the mean value of 340.84

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mg/100 g. Run 6 was significantly higher (P≤0.05) than all other samples which could be due to the increasing addition of amaranth seed flour similar to the report of Coțovanu *et al*. (2022) who also recorded an increased in potassium from 126 - 356 mg/100 g for amaranth-wheat flour blends. The increased in the addition of amaranth flour could have led to increase in potassium content of the blends. Potassium, in combination with sodium, plays a vital role in upholding osmotic equilibrium, thus providing a safeguard against the development of arterial hypertension (Yamada & Inaba, 2021).

The iron content obtained from the samples ranged from 10.80 - 12.15 mg/100 g. The highest iron content was recorded in run 6 with a mean value of 12.15 mg/100g while the lowest mean score of 10.80 mg/100 g was recorded for run 1. Noticeable variations were evident across all samples, except for run 11 and 7, 10 which exhibited mean values of 12.00 and 11.98 mg/100 g respectively. The trend of increasing sunflower flour proportion could be linked to arise in iron concentration. Iron is a crucial element that plays a vital role in numerous physiological processes within the body (Cronin *et al*., 2021). It is essential for erythropoiesis, the production of red blood cells, which is necessary for oxygen transport. Highest amount of calcium was found in run 2, 3, 9 with a mean value of 89.16 mg/100 g followed by run 4, 13 with a mean value of 84.73 mg/100 g while run 1 recorded the lowest value of 77.42 mg/100 g. The phosphorus contents ranged from 276.82 - 284.67 mg/100 g. Run 7, 10 recorded the highest mean value of 284.67 mg/100 g while lowest mean value of 276.82 mg/100 g was recorded by run 5, and 14. Run 7, 10 is significantly different (p≤0.05) from other samples.

Phosphorus is a vital component of hydroxyapatite, the mineral complex that gives strength and structure to bones and teeth. Along with calcium, phosphorus helps maintain their strength and integrity (Kashan, 2023). Phytate is an antinutrient known for its ability to bind minerals such as iron, zinc, and calcium and reduce their bioavailability. The phytate levels within the samples ranged from 0.11 - 0.22 mg/100 g. Run 4, 13 displayed the lowest mean value of 0.11 mg/100 g, whereas run 6 had the highest phytate content with a mean value of 0.22 mg/100 g. Sample run 6 is significantly different (p≤0.05) from all other samples. Run 7, 10, 11 and 12 showed no significant difference. These findings suggested that sample of run 4 and 13 may lead to an increase in the bioavailability of mineral elements more than other samples due to a lower content of phytate in the blend. The phytate content obtained in this study was below the permissible limit reported by Mada *et al*. (2012). Phytate in the raw blend can chelate/bind with divalent and trivalent mineral ions such as Ca^{2+} , Mg^{2+} , Zn^{2+}, Cu^{2+} and Al^{3+} and render these ions unavailable. Tannins are polyphenolic compounds that can interfere with protein digestion and reduce the bioavailability of certain nutrients.

The tannin levels in the samples ranged from 2.70-2.90 mg/100g. Run 4, 13 displayed the lowest tannin content with a mean value of 2.70 mg/100 g while run 7, 10 and 11 have the highest with a mean value of 2.90 mg/100g. Significant differences were found among many samples. There were no significant difference between run 4, 12 and 13. The run 4 and 12 are duplicate samples. These results indicated that run 7, 10 and 11 may have a lower potential to hinder iron bioavailability. Lower saponin concentration may also indicate a low level of its low affinity in binding with macronutrients fat and protein. The range of the tannin content obtained in this study was found to be lower than those reported by Adesina (2019). Saponins are glycosides known for their potential negative effects on nutrient absorption and digestion. The saponin levels in the samples ranged between 0.48% and 0.67%. Run 2, 3, 9 displayed the lowest saponin content with a mean value of 0.48 % while Run 5, 14 recorded the highest mean value of 0.67%.

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Table 4: Mineral and Antinutritional Composition of the Amaranth-based blends (mg/100g)

Run order	Na	K	Fe	Ca	P	Phytate	Tannin	Saponin
1	7.56 ± 0.03[d]	335.01±0.02[a]	10.80±0.04[a]	77.42±0.03[b]	280.00 ±0.04[c]	0.17±0.03[bcd]	2.79 ± 0.03[bc]	10.16 ± 0.03[b]
2	6.94 ± 0.02[b]	337.00±0.02[b]	11.12±0.03[d]	89.16±0.02[g]	282.53± 0.04[e]	0.16 ± 0.04[bc]	2.83 ± 0.03[cd]	10.19 ± 0.03[b]
3	6.94 ± 0.02[b]	337.00±0.02[b]	11.12±0.03[d]	89.16±0.02[g]	282.53 ±0.04[e]	0.16 ± 0.04[bc]	2.83 ± 0.03[cd]	10.19 ± 0.03[b]
4	6.99 ±0.04[c]	337.94±0.03[c]	11.67±0.03[e]	84.73 ±0.04[f]	279.01 ±0.01[b]	0.11 ± 0.03[a]	2.70 ± 0.04[a]	12.15 ± 0.01[e]
5	9.50 ±0.03[g]	340.05±0.03[f]	11.05±0.04[c]	77.80±0.04[c]	276.82 ±0.03[a]	0.13 ± 0.03[ab]	2.74 ± 0.03[ab]	12.23 ± 0.05[d]
6	9.82 ± 0.03[i]	340.84±0.02[i]	12.15±0.05[g]	73.86±0.03[a]	284.10± 0.03[g]	0.22 ± 0.02[e]	2.86 ± 0.01[de]	10.19 ± 0.03[b]
7	9.67 ± 0.04[h]	340.20±0.02[g]	12.00±0.03[f]	80.14±0.05[e]	284.67± 0.03[h]	0.19±0.02[cde]	2.90 ± 0.04[e]	10.17 ± 0.02[b]
8	5.50 ±0.03[a]	339.12±0.03[e]	11.00±0.04[c]	80.14±0.05[e]	284.67± 0.03[h]	0.21 ±0.02[de]	2.77 ± 0.03b	10.11 ± 0.02[a]
9	6.94 ±0.02[b]	337.00±0.02[b]	11.12±0.03[d]	89.16±0.02[g]	282.53 ±0.04[e]	0.16 ± 0.04[bc]	2.83 ± 0.03[cd]	10.19 ± 0.03[b]
10	9.67 ±0.04[h]	340.20±0.02[g]	12.00±0.03[f]	80.14±0.05[e]	284.67±0.03[h]	0.19±0.02[cde]	2.90 ± 0.04[e]	10.17 ± 0.02[b]
11	8.11 ± 0.02[f]	340.30±0.03[h]	11.98±0.03[f]	80.11±0.03[e]	284.70±0.02[h]	0.18±0.03[cde]	2.90 ± 0.04[e]	10.17± 0.02[b]
12	8.02 ±0.03[e]	338.15±0.05[d]	10.90±0.04[b]	78.24±0.04[d]	283.09 ± 0.03[f]	0.19±0.02[cde]	2.71 ± 0.02[a]	10.30 ± 0.04[c]
13	6.99 ±0.04[c]	337.94±0.03[c]	11.67±0.03[e]	84.73 ± .04[f]	279.01 ±0.01[b]	0.11 ± 0.03[a]	2.70 ± 0.04[a]	12.15 ± 0.01[e]
14	9.50 ± 0.03[g]	340.05±0.03[f]	11.05±0.04[c]	77.80±0.04[c]	276.82 ±0.03[a]	0.13 ± 0.03[ab]	2.74 ± 0.03[ab]	12.23 ± 0.05[d]

Mean with different superscript letter within the same column are significantly different ($p \leq 0.05$)

3.1 ANOVA for Response Surface Mixture Linear Model for Mineral Contents of the Amaranth-Based Blends

The ANOVA model results for response surface for mixture linear model for mineral composition of the amaranth-based blends is presented in Table 5. From the table, values of P>F for Na, K, Fe, Ca, P were 0.0193, 0.0079, 0.0003, 0.0001, and < 0.0001 respectively. It was revealed that the model linear terms were all significant. A p-value from this model indicated high statistical significance suggesting that the linear mixture term significantly contributed to the variation in the mineral composition of the amaranth-based blends. The R-squared value for Na, K, Fe, Ca, P are 0.61, 0.69, 0.84, 0.87, and 0.90, respectively**.** Ca recorded the highest R^2 value of 0.90 while sodium contents displayed least R^2 values of 0.61 showing a fitted model. The closer the R^2 value to unity, the better and more significant an empirical model fits the actual data. The adequate precision values range from 0.50-0.87, which further confirm that the model is adequate for predicting the mineral composition of amaranth-based blends. The adjusted R^2 ranged from 0.50-0.87. Phosphorus recorded the highest value for adjusted R^2.

The coefficients of variation for the mineral composition ranged from 0.33-10.33, indicating reasonably precise estimates from the model. The high R-squared values and low coefficients of variation suggest that the model is reliable for predicting the proximate composition of amaranth-based blends. This is in corroboration with the work of Eweama *et al*. (2021) who worked on the modelling and optimization of proximate and antinutritional composition of breakfast cereals produced from blends of millet, mungbean and tigernut flour using response surface methodology. The coefficients of variation for the mineral composition components range between 0.33 and 10.30, indicating reasonably precise estimates from the model. The response surface mixture linear model is highly significant and provides a good fit to the data. Below are the final equations in terms of actual factor for mineral composition of amaranth-based blend.

$$\mathrm{Na} = 0.12869\mathrm{A} + 0.030528\mathrm{S} + 0.076318\mathrm{P} - 0.070305\mathrm{SF} \quad (2)$$
$$\mathrm{K} = 3.45501\mathrm{A} + 3.28322\mathrm{S} + 3.37729\mathrm{P} + 3.23792\mathrm{SF} \quad (3)$$
$$\mathrm{Fe} = 0.13291\mathrm{A} + 0.077421\mathrm{S} + 0.10118\mathrm{P} + 0.13968\mathrm{SF} \quad (4)$$
$$\mathrm{Ca} = 0.64374\mathrm{A} + 0.97510\mathrm{S} + 0.74539\mathrm{P} + 1.85418\mathrm{SF} \quad (5)$$
$$\mathrm{P} = 2.93519\mathrm{A} + 2.78889\mathrm{S} + 2.59500\mathrm{P} + 2.97215\mathrm{SF} \quad (6)$$

The positive significant linear coefficients of the components (defatted amaranth, sorghum, defatted pumpkin and defatted sunflower flour) on K, Fe, Ca and P were based on increases in the levels of amaranth, sorghum, defatted pumpkin and defatted sunflower flour, respectively. That is as the composite flour level increases, the mineral content (K, Fe, Ca and P) increases and vice versa (Figure 2, 3, 4, and 5). Meanwhile, as the level of sunflower flour increases, Na content decreases as shown in Equation 2. The effect of composite flour on the Na contents is shown in Figure 1.

Table 5: ANOVA for Mixture Response Surface Linear Model for Mineral composition of the Amaranth-Based Blends

Source	Na	K	Fe	Ca	P
Model (Prob>F)	0.0193	0.0079	0.0003	<0.0001	<0.0001
R^2	0.61	0.69	0.84	0.87	0.90
Adj R^2	0.50	0.58	0.79	0.83	0.87
Adequate precision	6.60	7.35	11.59	13.19	14.70
C.V	10.30	0.33	1.95	2.50	0.37

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3.2 ANOVA for Mixture Response Surface Linear Model for the Antinutritional Factors of Amaranth-Based Blends

The ANOVA model result for response surface for mixture linear model for antinutritional composition of the amaranth-based blends is as presented in Table 6 From the table, values of P>F for antinutritional content ranged from < 0.0001-0.0035. It could be observed that the model linear terms were all significant. A p-value from this model indicated high statistical significance suggesting that the linear mixture term significantly contribute to the variation in the antinutritional composition of the amaranth-based blends. The R^2 values range from 0.73-0.93 suggesting a good fit. Saponin had the highest R^2 value of 0.93 while tannin contents displayed least R^2 values of 0.73. The closer the R^2 value

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to unity, the better and more significant an empirical model fits the actual data. The smaller the R^2 is, the less important the dependent variables in the model have in explaining the behavior of variation. This indicated that the model explains a substantial amount of the variation in the antinutritional composition components. The adjusted R^2 ranged from 0.64-0.90. The adequate precision values ranged from 6.95-16.32, > 4 indicates the sufficiency of the model to navigate the design space with the high signal to-noise ratio of the results which further confirm that the model is adequate for predicting the composition of amaranth-based blends. The coefficients of variation for the antinutritional composition components ranged between 1.49 and 9.46, indicating reasonably precise estimates from the model. The response surface mixture linear model is highly significant and provides a good fit to the data. The high R-squared values and low coefficients of variation suggest that the model is reliable for predicting the antinutritional contents of amaranth-based blends. Final equations in terms of actual factor for antinutritional factors of amaranth-based blends are shown below

$$\text{Phytate} = 3.33255E-003A + 1.91768E-003S - 5.95096E-004P - 1.73984E-003SF \quad (7)$$

$$\text{Tannin} = 0.030821A + 0.027928S + 0.023079P + 0.027914SF \quad (8)$$

$$\text{Saponin} = 4.49045E-003A + 3.07945E-003S + 0.011199P - 2.59223E-003SF \quad (9)$$

The effects of the composite flour on phytate, tannin and saponin are shown in Figure 6, 7 and 8, respectively. As pumpkin and sunflower flour content increases for phytate decreases (Figure 6). Increases in all the composite flour blends increased the tannin content of the blends and vice versa (Figure 7) while an increased in sunflower flour decreases the saponin content of the blend (Figure 8).

Table 6: ANOVA for Mixture Response Surface Linear Model for the Antinutritional Factors of Amaranth-Based Blends

Source	Phytate	Tannin	Saponin
Model (Prob>F)	0.0002	0.0035	<0.0001
R^2	0.84	0.73	0.93
Adj R^2	0.79	0.64	0.90
Adequate precision	12.48	6.95	16.32
C.V	9.46	1.49	4.15

3.3 Diagnostic Case Statistics for Mineral and Antinutritional Composition of the Blends

The diagnostic case statistics for the blends' mineral and antinutritional content are provided in Table 7. The actual values from the experimental analysis, the expected values produced by the design expert program, and the residual values showing how close the actual and predicted values are to each other are all included in the table. When the residual value is positive, it means that the actual value exceeded the expected values. When the residual value is zero, it means that the actual value is in line with the standard anticipated value, and a negative residual value means that the predicted value is higher than the actual value. It could be observed that the residual values for potassium (K), iron (Fe), calcium (Ca), Phosphorus (P), phytate and tannin were positive. This suggests that the experimental values for these minerals exceeded the predicted values. The residual value for phytate was negative while the residual value for tannin and saponin were positive.

Table 7: Diagnostic Case Statistics for Mineral and Antinutritional Composition of the Blends

Name	Actual Value	Predicted Value	Residual Value
Na	8.225	8.225714	-0.00071
K	338.6286	338.6279	0.000714
Fe	11.40286	11.40214	0.000714
Ca	81.59071	81.58929	0.001429
P	281.6643	281.6664	-0.00214
Phytate	0.165	0.165714	-0.00071
Tannin	2.795714	2.795	0.000714
Saponin	0.557857	0.557143	0.000714

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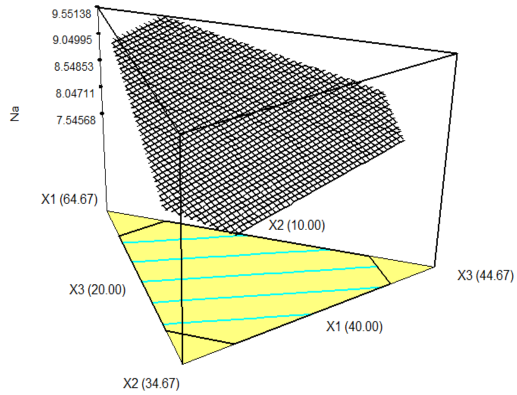

Figure 1: 3D plot showing the effect of amaranth-based blends on Na content

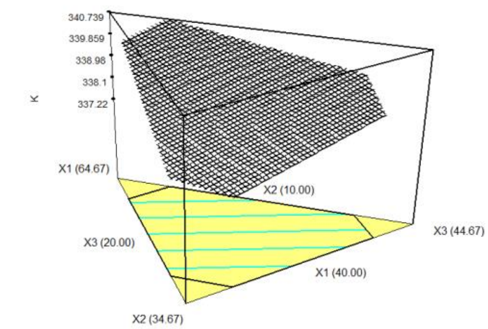

Figure 2: 3D plot showing the effect of amaranth-based blends on K content

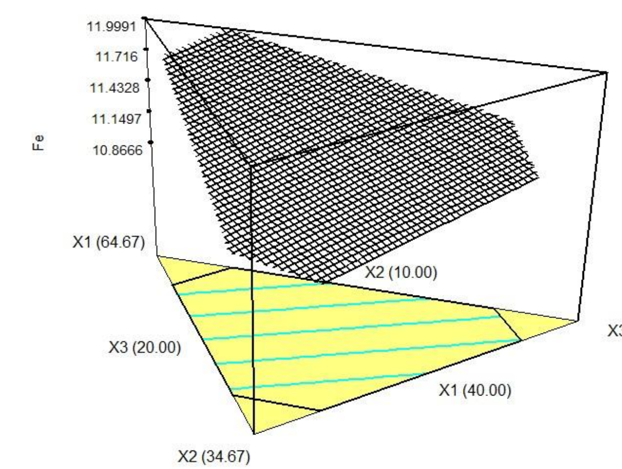

Figure 3: 3D plot showing the effect of amaranth-based blends on Fe content

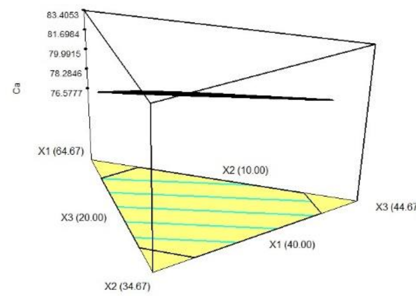

Figure 4: 3D plot showing the effect of amaranth-based blends on Ca content

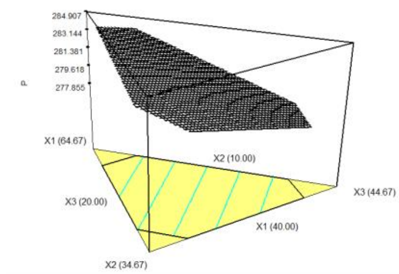

Figure 5: 3D plot showing the effect of amaranth-based blends on P content

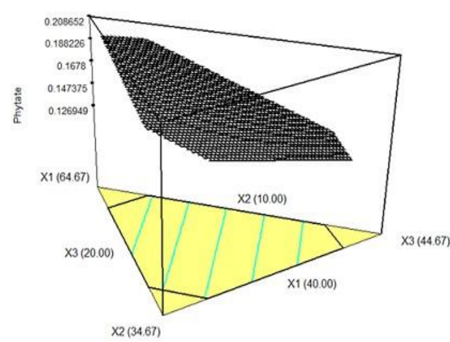

Figure 6: 3D plot showing the effect of amaranth-based blends on phytate content

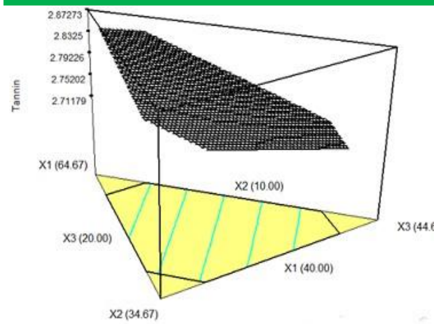

Figure 7: 3D plot showing the effect of Amaranth-based blends on tannin content

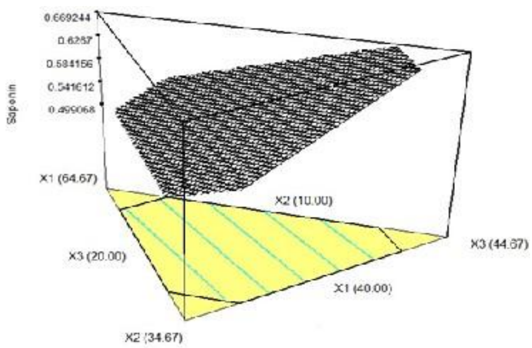

Figure 8: 3D plot showing the effect of Amaranth-based blends on saponin content

4.0 CONCLUSION

D-optimal mixture design was successfully employed to formulate amaranth-based blends with the generation of run with higher mineral content and considerable levels of antinutritional factors. The high R^2 values, highly significant model terms and low coefficients of variation for the mineral and antinutritional composition of the blends, indicated the reliability of the experimental design in optimizing nutritional and antinutritional properties of the blends. Therefore, the composite blends of amaranth, sorghum, pumpkin seed flour, and sunflower flour hold potential for the production of nutrient-dense food products capable of addressing issues of malnutrition.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the research work presented in this manuscript

REFERENCES

Adesina, S. A. (2019). Effects of Conventional Food Processing Methods on the Mineral and AntiNutrient Composition of Sunflower (Helianthus annuus) Seeds. Agricultural and Food Science Journal of Ghana, 12, 1106-1109. https://doi.org/10.4314/as.v17i3.5

Aditya, M., Sen, D., & Bhattacharjee, S. (2019). Amaranth: A reservoir of antioxidant-based phytonutrient for combating degenerative diseases. Studies in Natural Products Chemistry, 67, 81-121. https://doi.org/10.1016/B978-0-12-819483-6.00003-5.

Atonwu, A.C. and Akobundu, E.N.T. (2010). Nutritional and Sensory Quality of Cookies Supplemented with Defatted Pumpkin (Cucurbita pepo) Seed Flour. Pakistan Journal of Nutrition, 9(7): 672-677. https://doi.org/10.3923/pjn.2010.672.677.

Awuchi, C. G., Igwe, V. S., & Amagwula, I. O. (2020). Nutritional diseases and nutrient toxicities: A systematic review of the diets and nutrition for prevention and treatment International Journal of Advanced Research, 6, 1-46.

Baraniak, J., & Kania-Dobrowolska, M. (2022). The dual nature of amaranth—functional food and potential medicine. Foods, 11(4), 618.

Bender, D.; Schönlechner, R. (2021). Recent Developments and Knowledge in Pseudocereals Including Technological Aspects. Acta Aliment. 50(4), 583–609. https://doi.org/10/10.1556/066.2021.00136.

Cammack, R., Atwood, T., Campbell, P., Parish, H., Smith, A., Vella, F., & Stirling, J. (Eds.). (2020). Oxford dictionary of biochemistry and molecular biology (Rev. ed.). Oxford: Oxford University Press.

Coțovanu, I., Stroe, S., Ursachi, F., & Mironeasa, S. (2022). Addition of Amaranth Flour of Different Particle Sizes at Established Doses in Wheat Flour to Achieve a Nutritional Improved Wheat Bread. Foods, 12(1), 133. https://doi.org/10.3390/foods12010133.

Cronin, S. J. F., Woolf, C. J., Weiss, G., & Penninger, J. M. (2021). The Role of Iron Regulation in Immunometabolism and Immune-Related Disease. Frontiers in Molecular Biosciences, 6. https://doi.org/10.3389/fmolb.2019.00116.

Eweama, A. U., Nwosu, J. N., Owuamanam, C. I., & Obeleagu, S. O. (2021). Modelling and Optimization of Proximate and Antinutritional Composition of Breakfast Cereals Produced From Blends of Millet, Mungbean and Tigernut Flour Using Response Surface Methodology. International Journal of Research and Scientific Innovation, 8 (8), 103-118. https://doi.org/10.51244/IJRSI.2021.8804

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.

Ghodsi, D., Rasekhi, H., Yari, Z., Roustaee, R., Nikooyeh, B., Faramarzi, A., and Neyestani, T. R.

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IJSGS FUGUSAU VOL. 10(1) WEBSITE: https://fugus-ijsgs.com.ng

(2023). Prediction and Analysis of Trends in the Nutritional Status of Children under 5 years in Iran: Reanalysis of the Results of National Surveys Conducted between 1998 and 2020. Frontiers in Nutrition, 1-10. https://doi.org/10.3389/fnut.2023.1083318.

Grillo, A., Salvi, L., Coruzzi, P., Salvi, P., and Parati, G. (2019). Sodium Intake and Hypertension. Nutrients, 11(9). https://doi.org/10.3390/nu11091970.

Inuwa, H.M., Aina, V.O., Baba, G., Aimola, I. & Amao, T. (2011). Comparative Determination of Antinutritional factors in Groundnut Oil and Palm Oil. Advanced Journal of Food Science and Technology 3(4): 275-279.

Kashan, J. S. (2023). A review: In vivo studies of bioceramics as bone substitute materials. Nano Select, 4(2): 123-144. https://doi.org/10.1002/nano.202200222.

Kiani, A. K., Dhuli, K., Donato, K., Aquilanti, B., Velluti, V., Matera, G., Iaconelli, A., Connelly, S. T., Bellinato, F., Gisondi, P., & Bertelli, M. (2022). Main nutritional deficiencies. Journal of Preventive Medicine and Hygiene, 63(2 Suppl 3), E https://doi.org/10.15167/2421-4248/jpmh2022.63.2S3.2752.

Makkar H.P.S & Goodchild, A.V. (1996). Quantification of Tannins: a laboratory manual. ICARDA; Aleppo, Syria.

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.

Rahayu, W. D., Syafitri, U. D., & Soleh, A. M. (2019). D-optimal Design for Ordinal Responses in Mixture Experiments. Journal of Physics, 1-6. https://doi.org/10/10.1088/1742-6596/1317/1/012017

Salim, R., Nehvi, I. B., Mir, R. A., Tyagi, A., Ali, S., & Bhat, O. M. (2023). A review on anti-nutritional factors: Unraveling the natural gateways to human health. Frontiers in Nutrition. https://doi.org/10.3389/fnut.2023.1215873

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. Food Production, Processing and Nutrition, 2(6). https://doi.org/10.1186/s43014-020-0020-5

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.

Weyh, C., Krüger, K., Peeling, P., & Castell, L. (2022). The Role of Minerals in the Optimal Functioning of the Immune System. Nutrients, 14(3). https://doi.org/10.3390/nu14030644

World Health Organization (2012). Guideline: Sodium intake for adults and children.

Yamada, S., & Inaba, M. (2021). Potassium Metabolism and Management in Patients with CKD. *Nutrients*, 13(6). https://doi.org/10.3390/nu13061751.