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Optimizing maize-pigeon pea flour blends for *Ukpo-Oka,* a traditional maize-based diet, using response surface methodology

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Abstract Results showed that protein content ranged from 18.65% to 26.53%. Ash content ranged from 1.45%- 1.97%. Iron and zinc content ranged from 4.01-5.29 mg/100g and 3.13-3.69 mg/100g respectively.Based on the description of the response models for the maize-pigeon pea blends, all models except zinc had a significant p-value of < 0.05 and were reliable in predicting the responses and therefore were used for the optimisation. All response models had regression coefficients (R^2) ranging from 0.67-0.947 for the maize-pigeon pea flour blend. The predictive models subjected to a numerical technique gave an optimal maize-pigeon pea blend of 54.3% maize and 45.7% pigeon pea with desirability factor of 0.605 and corresponding protein content, ash content, zinc and iron were 24.31%, 1.45%, 5.03mg/100g and 3.47mg/100g respectively. This study is provided optimal maize-pigeonpea blend capable of enhancing the nutritional quality of *ukpo-oka* thereby serving as a tool for mitigating against protein energy malnutrition (PEM) and micronutrient deficiencies in the areas where this diet is consumed as a staple diet. This innovation is found to be the potential to contribute significantly to dietary diversification and nutritional security in the region.

Keywords: Design expert, Mineral composition, Nutritional quality, Proximate composition

Introduction

Ukpo Oka is a traditional maize-based steamed pudding that is widely consumed in various parts of Nigeria. It is known with this name in Delta state, but also as *abari* in Ekiti State and *ekpanakpapa* in Efik-speaking part of Nigeria (Adejuyitan *et al.,* 2022). *Ukpo- oka* is a popular meal option for breakfast or lunch widely consumed across various age groups. This diet is prepared from maize with the addition of ingredients such as peppers, salt, onions, and palm oil (Anosike *et al.,* 2020). Typically lacking in protein content, *Ukpo-oka* is often consumed without any additional protein sources (Anosike *et al.,* 2020).

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Protein-energy malnutrition (PEM) continues to pose significant health challenges in developing nations, primarily due to the consumption of nutritionally inadequate diets. A key contributing factor to the prevalence of nutritional deficiencies is the excessive reliance on single food items, leading to monotonous diets lacking in essential micronutrients and macronutrients. One promising approach to effectively mitigate the issue of PEM is blending cereals and legumes to create more nutritious, balanced food options and this has been suggested as a cost-effective approach (Koyum *et al.,* 2023).

Maize (*Zea mays*) a cereal grain is one of the most commonly produced grain crops in Nigeria, and is a major staple food containing about 43% of the total calories (Adiaha, 2018). However, research studies on the potential synergies of integrating underutilized legumes like pigeon peas to enhance the quality of traditional cereal-based dishes like *ukpo-oka* appear very limited.

Pigeon pea (*Cajanus cajan* L.,) also reffered to as red gram and Arhar, boasts a historical significance as one of the earliest cultivated crops and is recognized as the sixth most vital legume for human consumption worldwide. In Nigeria, it is recognized by various names across different regions such as *"fio-fio"* in Igbo, "*otile"* in Yoruba, and "*wake masa"* by the Hausas (Ekpo *et al.,* 2022).

Although pigeon pea is relatively insignificant as a pulse in West Africa, it holds considerable importance for low-income farmers in Nigeria, Benin and Ghana (Ikhajiagbe *et al.,* 2022). The pigeon pea is regarded as a versatile plant with multiple applications. It is primarily grown for its palatable and nutrient-dense seeds (Sarkar *et al.,* 2020). Pigeon pea serves as an ideal protein supplement for traditional cereal-based diets to prevent protein deficiency as it has one of the highest protein contents (average 25 g/100 g) among legumes (Kuraz Abebe, 2022). It is also rich in calcium, iron, phosphorus, potassium, various vitamins, and essential amino acids (particularly lysine), contributing to its high nutritional value (Haji *et al.,* 2024).

Despite pigeon pea being high in nutrients, its utilization is still limited in certain regions of Nigeria, and it has not yet found industrial applications. This initiative therefore presents opportunities for broader utilization of pigeon peas and also enhanced support for farmers engaged in pigeon pea cultivation.

Optimisation is a statistical technique employed in achieving tailor-made objectives in food systems (Roy *et al.,* 2020). Optimization techniques, such as Response Surface Methodology (RSM), enable researchers to identify optimal blending ratios that harmoniously balance sensory attributes such as taste and texture with enhanced nutritional profiles (Babu 2024). The utilization of an optimum blend of maize-pigeon pea flour for *ukpo-oka* production presents an opportunity to develop a more nutritionally balanced food product.

The main objective was to optimize the blend ratio of maize and pigeon pea flours utilizing Response Surface Methodology for the production of enriched *upko-oka*, with protein, ash, iron, and zinc as indicators of nutritional quality in the optimization process.

Materials and methods

Materials

Freshly harvested maize and pigeon peas were bought from Arena market Oshodi and Oyingbo market respectively in Lagos state. All reagents utilized in this study were of analytical grade. The analytical and processing equipment employed were accessed from the Food Laboratory and the Central Teaching and Research Laboratories at Bells University of Technology, Ota, Nigeria

Methods

Processing of maize flour

Maize flour was obtained using a modified version of the method described by Anosike *et al.* (2020). Freshly harvested maize was dehusked and shelled. The shelled maize was then properly dried using a cabinet air dryer at 70 °C to reduce the moisture content. The dried maize was further milled with a disc attrition milling machine to obtain maize flour which was stored in an airtight container at ambient temperature (25 °C) to reduce moisture absorption, prevent contamination, and maintain quality.

Processing of pigeon pea flour

Pigeon pea flour was obtained using the method described by Atuna *et al.* (2021). Pigeon pea seeds were sorted manually rinsed thoroughly and soaked for 12 hrs after which the seeds were drained and allowed to undergo sprouting for 72 hrs at room temperature by spreading in a single layer on a malting bed. During this period, the grains were intermittently sprayed with water at 12hrs interval until the last day of sprouting with turning at 8hrs interval. After sprouting, the germinated seeds were dried at 70 °C for 3 hrs. The dried seeds were dehulled and rootlets removed then finally milled using a disc attrition mill into flour. The flour was packaged into polyethylene bag at room temperature (25 °C).

Experimental design

The experimental design and statistical analysis were conducted using the i-optimal mixture design approach, which was integrated within the framework of response surface methodology (RSM). The design specified maize flour ratios ranging from 50% to 80% and pigeonpea flour ratios ranging from 20% to 50%, resulting in a total of 13 blends of maize and pigeonpea formulations for analysis.

Determination of protein and ash content

The protein content and ash content of the blends of maize and pigeon pea flours were determined using AOAC (2012) methods.

Determination of iron and zinc content

The mineral elements were determined by the method described by AOAC (2012). Zinc and iron concentrations were determined by using Atomic Absorption Spectroscopy (Model AA670 Shimadzu, Kyoto, Japan). The analysis involved aspirating the solution into the oxygen-acetylene flame. Before the the analysis of the sample, the instrument was calibrated by using standard solutions of various metal salts to generate a calibration curve for accurate quantification of the desired elements (Nanda *et al.,* 2003).

Optimization of blends using Response surface methodology (RSM)

Maize and pigeon pea flours were optimized using response surface methodology. An i-optimal mixture design with two independent variables namely; maize (X1) and pigeon pea (X2) were examined in relation to dependent variables namely; protein content, ash content, iron content, and zinc content. The total maize and pigeonpea proportion was 100%. Consequently, the maize proportion in the blend was between 50-80%, while the pigeon pea proportion was 20-50%. These proportions were computed into DE software. Experimental maize-pigeon pea blends were formulated for each independent variable and coded as -1 and 1 using DE software. These blends were then utilized to examine the impact of the independent variables on the dependent variables as shown in Table 1 respectively. Each dependent variable data for the maize-pigeon pea blends was fitted into a Scheffe model shown in Equation 1:

$$Y = \beta_1 M + \beta_2 P \ \beta_{10} MP \quad (1)$$

Where Y represents the response function.β_1 and β_2 *are* the linear coefficients, β_{10} *is* the interactive coefficient, M is maize and P is pigeon pea.

Analysis of variance (ANOVA) at a significance level of $P<0.05$ was conducted to evaluate the suitability of each response model to be a predictive

model. A numerical technique was employed to determine the optimized factor conditions, keeping the independent variables within a specified range. The goal was to maximize all dependent variables, and this optimization was achieved using a desirability function. (Eq. 2) (Myers *et al.,* 2016).

$$D(x)=(d_1 \times d_2 \times \ldots\ldots d_n)1/n \quad (2)$$

Where $D(x)$ is desirability function, d_1, d_2 to d_n are the desirability indices for each response variable and n is the number of considered dependent variables. The desirability function generated a combination of optimal maize-pigeon pea blend and assigned to them score which ranges from 0 (undesirable) to 1 (most desirable).

Results

Experimental design

An I-optimal mixture design within the framework of Response Surface Methodology (RSM) was utilized for the experimental design, resulting in 13 runs of maize and pigeon pea flour blends. The independent variables were maize and pigeon pea, while the responses included protein content, ash content, and concentrations of iron and zinc as illustrated in Table 1.

Protein and ash contents determination

The protein and ash contents of the maize flour and pigeon pea flour blends are presented in Table 1.

The addition of pigeon pea significantly enhanced the protein content of the flour blend. The protein content in this study ranged from 18.65% to 26.53%. The 50:50 flour blend exhibited the highest protein content, while the 72.5:27.5 blend had the lowest. The ash content of the blends ranged from 1.45% to 1.97%, with an increase observed as the proportion of pigeon pea flour increased.

Iron and zinc concentration of maize-pigeon pea flour blend

Table 2 presents the iron and zinc contents of the different formulations of maize-pigeon pea flour blend ratios generated by the design expert tool. The result showed that pigeon pea is characterized with its high iron content. The iron content of the blends ranged from 4.01 to 5.29 mg/100 g, increasing with a higher proportion of pigeon pea. The 50:50 blend had the highest iron content

at 5.29 mg/100 g. Zinc content ranged from 3.13 to 3.69 mg/100 g across the flour blends. The highest zinc content was found in the blend 50: 50 and the lowest was found in the blend 80:20.

Statistical analysis and optimization

The impact of blending maize flour and pigeon pea flour on protein content is shown in Figure 1. As the amount of both flours increased so also was a significant increase observed in the protein response. The influence of maize flour and pigeon pea flour blend on the ash content is shown in Figure 2. As the amount of both flours increased so also was an increase observed in the ash response. The influence of maize flour and pigeon pea flour blend on the iron content is shown in Figure 3. As the amount of both flours increased so also was an increase observed in the iron response. The influence of maize flour and pigeon pea flour blend on the zinc content is shown in Figure 4. As the amount of both flours increased no significant difference was observed in the zinc response.

All response models had significant p-values ($p<0.05$), except zinc response model which had a significant p-value of >0.1. This indicates that the zinc content values showed no significant difference. All response models had regression coefficients (R^2) ranging from 0.67-0.947 for the maize-pigeon pea flour blend.

Optimum blend

Based on the description of the response models for the maize-pigeon pea blends, all models reliable in predicting the responses were used for the optimisation. The predictive models subjected to a numerical technique gave an optimal maize-pigeon pea blend of 54.3% maize and 45.7% pigeon pea with desirability factor of 0.605 as shown in Figure 5 and corresponding protein content, ash content, zinc and iron were 24.31%, 1.45%, 5.03mg/100g and 3.47mg/100g respectively.

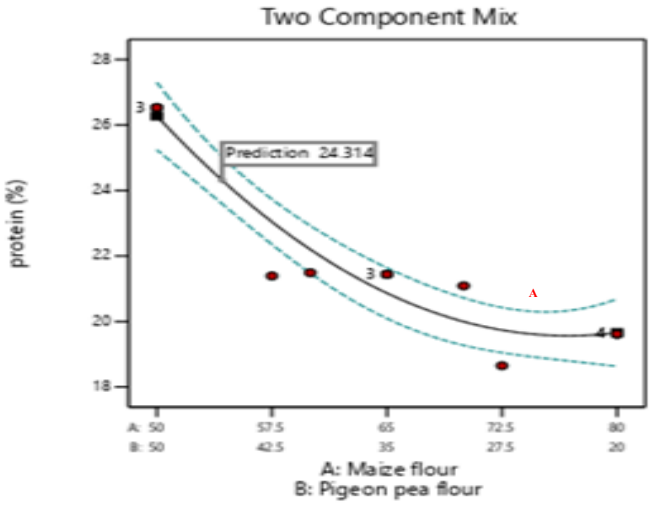


Figure 1. The influence of maize flour and pigeon pea flour blends on protein

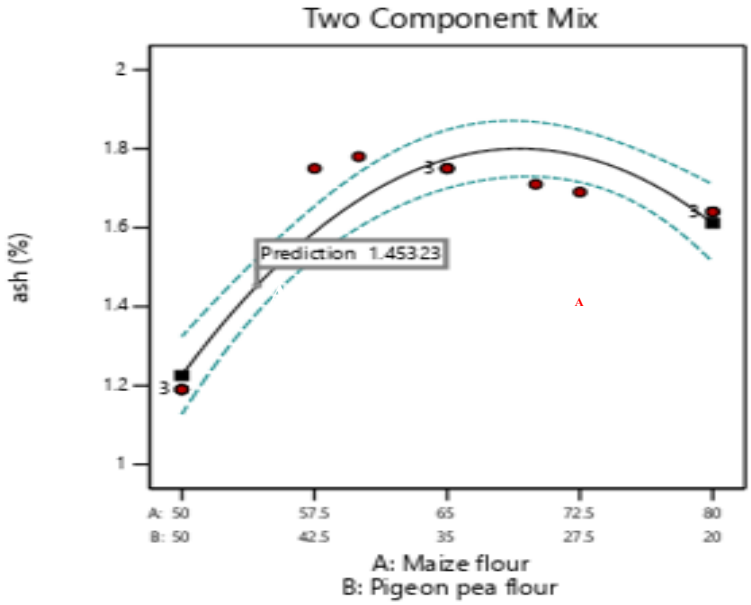


Figure 2. The influence of maize flour and pigeon pea flour blends on ash

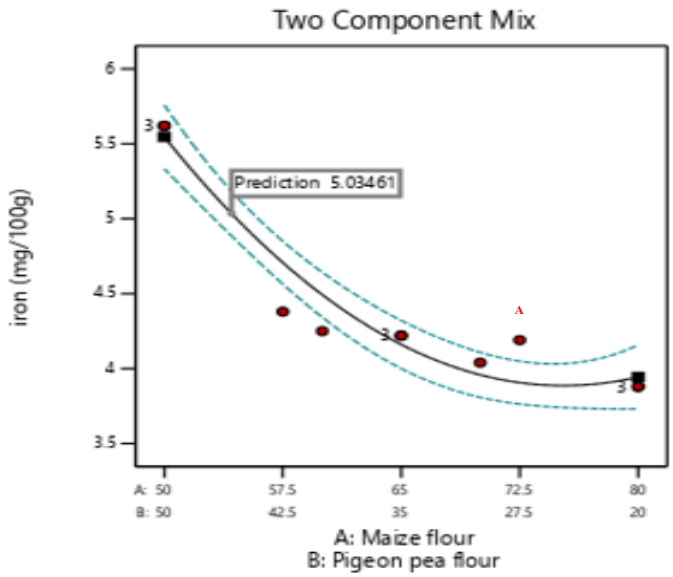


Figure 3. The influence of maize flour and pigeon pea flour blends on iron

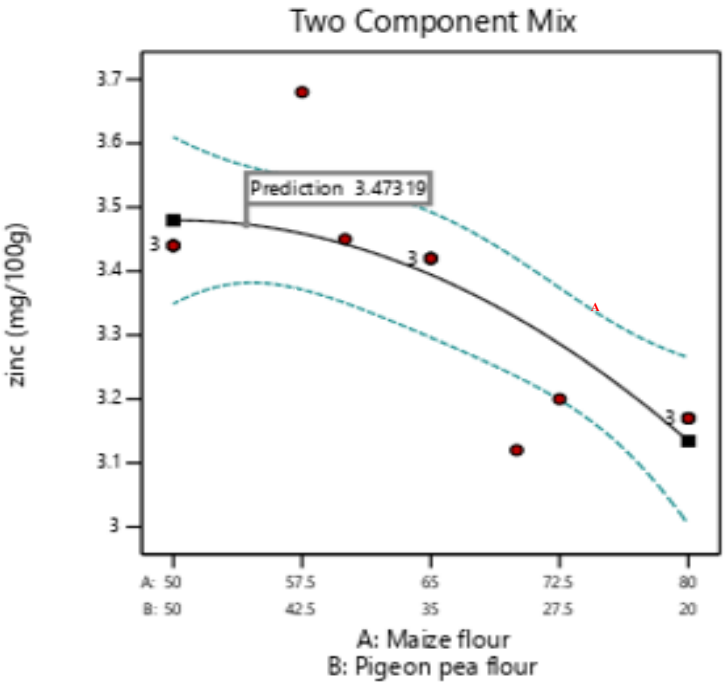


Figure 4. The influence of maize flour and pigeon pea flour blends on zinc

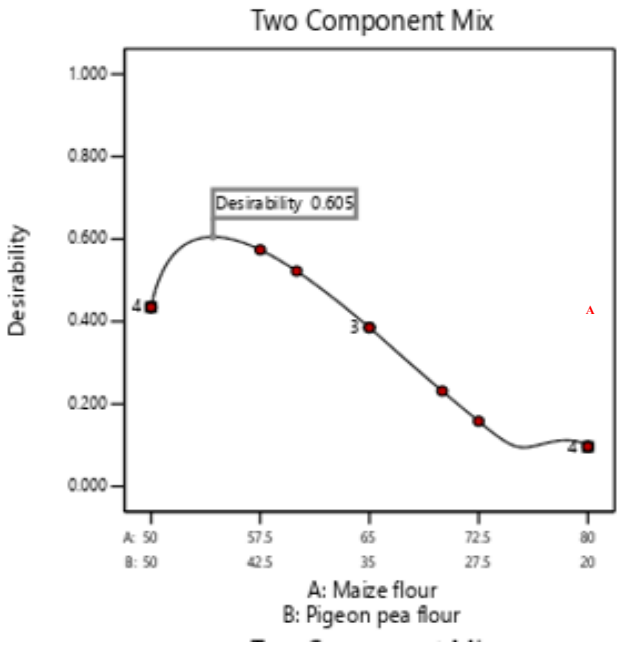


Figure 5. Graph showing the optimal blend ratio and the desirability factor

Table 1. Crude protein and ash contents (%) of experimental runs

Runs	Maizeflour	Pigeon pea flour	Crude protein (%)	Ash (%)
1	60	40	21.50±0.05^{b}	1.72±0.03^{d}
2	80	20	19.62±0.10^{d}	1.45±0.04^{f}
3	50	50	26.53±0.05^{a}	1.91±0.01^{b}
4	65	35	21.44±0.29^{b}	1.83±0.01^{c}
5	80	20	19.62±0.10^{d}	1.45±0.04^{f}
6	57.5	42.5	21.39±0.30^{b}	1.97±0.01^{a}
7	65	35	21.44±0.29^{b}	1.83±0.01^{c}
8	80	20	19.62±0.10^{d}	1.45±0.04^{f}
9	50	50	26.53±0.05^{a}	1.91±0.01^{b}
10	70	30	21.10±0.05^{c}	1.85±0.04^{c}
11	65	35	21.44±0.29^{b}	1.83±0.01^{c}
12	72.5	27.5	18.65±0.05^{e}	1.57±0.03^{e}
13	50	50	26.53±0.05^{a}	1.91±0.01^{b}

The values are expressed as means ± standard deviations based on three replicate samples.
At the 5% level, there is no significant difference between the means of any column containing a similar letter.

Table 2. Iron and Zinc content of experimental runs

Runs	Maize flour (g/100g)	Pigeon pea flour (g/100g)	Iron (mg/100g)	Zinc (mg/100g)
1	60	40	4.19±0.05^{c}	3.36±0.03^{bc}
2	80	20	3.56±0.21^{de}	3.13±0.04^{d}
3	50	50	5.44±0.17^{a}	3.69±0.02^{a}
4	65	35	4.28±0.09^{c}	3.33±0.02^{c}
5	80	20	3.56±0.21^{de}	3.13±0.04^{d}
6	57.5	42.5	4.60±0.12^{b}	3.44±0.12^{b}
7	65	35	4.28±0.09^{c}	3.33±0.02^{c}
8	80	20	3.56±0.21^{de}	3.13±0.04^{d}
9	50	50	5.44±0.17^{a}	3.69±0.02^{a}
10	70	30	3.83±0.10^{d}	3.39±0.04^{bc}
11	65	35	4.28±0.09^{c}	3.33±0.02^{c}
12	72.5	27.5	3.53±0.27^{e}	3.18±0.04^{d}
13	50	50	5.44±0.17^{a}	3.69±0.02^{a}

The values are expressed as means ± standard deviations based on three replicate samples.
At the 5% level, there is no significant difference between the means of any column containing a similar letter.

Discussion

Protein is vital for body growth, development, and tissue repair. There was a significant increase ($p< 0.05$) in the protein content of the flour blend with the addition of pigeon peas. Incorporating pigeon pea flour improves protein content by providing a complementary amino acid profile. (Adeoye *et al.,* 2024). The highest protein value was observed in the flour blend 50:50 while the 72.5:27.5 flour blend recorded the least protein content. Higher values of protein were recorded in this work compared to the values reported by Iombor *et al.* (2023) (13.95-17.95%) for maize-groundnut-fluted pumpkin composite in "*Aba*" preparation. Feyera (2020) reported a notable improvement in protein content of cereal-based products when fortified with legume flours such as soybean, chickpea, cowpea, and African yam bean flour. The protein content obtained in this study was similar to that recorded by Babarinde *et al.* 2020 in a study for Fonio-pigeon pea flour (16.51% to 24.85%). The ash content of a sample is a quantitative indicator of its mineral composition, serving as a valuable tool for evaluating nutritional value (Nath *et al.,* 2022). It was observed that the ash content of the blends increased with the inclusion of pigeon pea flour ($p<0.05$). This finding is consistent with previous work which has also reported an increase in ash content with the incorporation of pigeon pea. For instance, an increase in ash content due to the inclusion of pigeon peas

was also reported in maize-carrot-pigeon pea flour blends by Bello *et al*. (2020).

Iron is an essential component of hemoglobin which is an oxygen-carrying pigment in the blood. Regular consumption of iron-rich foods can help prevent anemia. (Singh *et al.,* 2024).The iron content of the blend ratios ranged from 4.01- 5.29mg/100g. The iron content increased significantly ($p<0.05$) with increased ratio of pigeon pea. The highest iron content (5.29mg/100g) was found in 50:50. Pigeon pea is recognized as a valuable source of iron, and iron deficiencies can result in severe malnutrition, cognitive impairments, anemia, and immune system abnormalities (Kuraz Abebe, 2020). The value was similar to iron content of 5.4mg/100g for fortified and extruded Maize-Bambara groundnut reported by Happiness *et al.* (2023).

Zinc is a crucial cofactor for over 300 enzymes, playing a vital role in cell division, protein synthesis, and growth (Hubne and Haase, 2024). Zinc deficiency can lead to growth failure, anemia, enlargement of the liver and spleen, and impaired skeletal development (Islam *et al.,* 2023). The zinc content of the flour blends ranged from 3.13 - 3.69mg/100g. The result of this study was higher than the values of zinc for maize, pea, and anchote flours (2.77 to 3.2mg/100g) reported by Gemede (2020) and reported by Tadesse and Gutema (2023) for a complementary diet from sorghum and pigeon pea flour blends. However, the values obtained in this study are consistent with those reported by Ademulegun *et al.* (2021) for complementary foods formulated from fermented, sprouted, and toasted maize soybean blends. Also, the findings align with the results of Adigwe *et al*. (2023), who reported a comparable value of 3.17 mg/100g for Maize-Cowpea and Orange-Fleshed Sweet potato-based complementary foods. In mixture design within response surface methodology (RSM), response models are mathematical equations used to predict the relationship between the proportions of components in a mixture and the response variable of interest (Cornell, 2002). All response models had significant p-values ($p<0.05$), except the zinc response model, which had a significant p-value of >0.1, indicating no significant difference in the zinc content values. The protein content model had a p-value of 0.0019 and R^2 of 0.93. This result indicated that there was a strong relationship between the predictor and the response variable. This implied that the maize and pigeon pea flour ratio determined the protein content. Also, the regression coefficient implied that the model could explain 93% of the overall variations in the protein content values and thus the protein model was fit for prediction.

Ash content model had a p-value of < 0.0001 and R^2 of 0.90. This model indicated that 90% of the overall variations in the ash content values could be

explained by the model and also a strong relationship existed between the predictor and the response variable (ash). This model was also fit for prediction.

Iron content model had a p-value of 0.0002 and R^2 of 0.93. It showed that the maize flour and pigeon pea flour proportion determined the iron content. The Regression coefficient value implied that the model could explain 93% of the overall variations in the iron content values. The iron content response model was thus reliable for prediction.

Zinc content model had a p-value of 0.2 and R^2 of 0.67. This model having p-value >0.05 and regression coefficient <0.7 is an indicator that the response model was not reliable for prediction. This result suggested that the inherent zinc content in maize and pigeon pea flours may not differ sufficiently to show significant effects when combined in varying proportions and as such changes in the proportion of maize and pigeon pea flour in the blend did not have a consistent effect on zinc concentration within the tested range.

The resulting optimal maize-pigeon pea blend of 54.3% maize and 45.7% pigeon pea with desirability factor of 0.605 which selected after subjecting the predictive models to numerical optimisation had corresponding protein content, ash content, zinc and iron concentrations of 24.31%, 1.45%, 5.03mg/100g and 3.47mg/100g respectively.

This study is provided an optimal blend of maize-pigeon pea flour with a superior nutritional profile for the production of *ukpo-oka*. By incorporating pigeon pea flour into the maize-based formulation, the nutritional profile of the flour blend was significantly enhanced. The use of RSM enabled a systematic evaluation of the interactions between maize and pigeon pea flour proportions, providing a predictive model for optimal blending ratio. The results demonstrated that including pigeon pea flour at an optimized level could serve as a tool for mitigating PEM and micronutrient deficiencies in the areas where this diet is consumed as a staple diet. However, these findings are preliminarily based solely on some proximate parameters and essential minerals. Further studies should incorporate functional, sensory, and shelf-life evaluations to ensure the optimized blend meets consumer acceptability and storage stability requirements.

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Conflicts of interest

The authors declare no conflict of interest.

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