



Optimization of Protein and Ash contents of Pearl Millet-Based Composite Flour using a

D-Optimal Design

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ABSTRACT

The increasing demand for nutrient-dense, plant-based food products has driven research into the development of composite flours, particularly in regions where cereal-based diets dominate and protein-energy malnutrition is prevalent. Optimization of the production of composite flour from Pearl millet, bambara nut and sesame seed flour using D optimal mixture design of Design Expert software with the levels for pearl millet (40-60%), Bambara nut (10-30%), and defatted sesame seed flour (20-40 %), was carried out. The responses were protein and ash content. Run 3 recorded the highest crude protein (21.4%) while run 14 and 16 recorded the highest total ash content (5.20 %). The model terms were significant ($p < 0.05$) for the two responses of the blends with R^2 values for protein and ash content are 0.9977 and 0.9747 respectively. In conclusion, composite flours formulated from pearl millet, bambara nut, and defatted sesame seed exhibited acceptable protein and ash contents, indicating their suitability for the development of nutrient-dense food products capable of addressing malnutrition.

Keywords: Pearl millet, Bambara nut, Sesame seed, D-optimal, protein, optimization

INTRODUCTION

The increasing demand for nutrient-dense, plant-based food products has driven research into the development of composite flours, particularly in regions where cereal-based diets dominate and protein-energy malnutrition is prevalent.

Cereals such as pearl millet (*Pennisetum glaucum*) are widely cultivated in arid and semi-arid regions due to their drought tolerance and adaptability [1]. Pearl millet provides a substantial source of carbohydrates and energy; however, its protein content is limited and deficient in

essential amino acids like lysine, which restricts its nutritional quality when used as a sole ingredient in human diets [2,3].

Bambara groundnut is known by different names in sub-Saharan Africa (SSA). For instance, it is referred to as earth pea, jugo bean, nyimo beans, or ditloo in Southern Africa. Bambara nut is believed to have originated from the Timbuktu areas in central Mali, West Africa [5]. Legumes, including Bambara nut (*Vigna subterranea*), are recognized for their high-quality protein and essential amino acids, particularly lysine, which complements the amino acid profile of cereals [4]. The grain of Bambara groundnut is regarded as a source of a complete and balanced diet. It contains carbohydrates (51–71%), crude protein (18–24%), oil (4–12%), fiber (3–12%), and ash (3–12%) [6]. Furthermore, it has essential and non-essential amino acids at 32.72% and 67.28%, respectively, per 100 g of grain [7]. Lysine is the major essential amino acid (10.3%) present in the grain. Due to higher protein and amino acid contents, Bambara groundnut is an ideal food to complement most cereal-based diets [8]. Additionally, Bambara nut flour contributes bioactive compounds and functional properties such as water absorption and emulsification, which are valuable in food formulation [9].

Sesame (*Sesamum indicum*), an oilseed, is rich in protein, lipids, and minerals, particularly calcium, magnesium, and phosphorus, making it a valuable component for enhancing both the protein and ash content of composite flours [10]. The incorporation of sesame flour into cereal–legume blends can therefore improve the nutritional profile while potentially influencing functional and sensory properties.

Despite the nutritional advantages of blending cereals, legumes, and oilseeds, the formulation of composite flours poses significant challenges due to the complex interactions among multiple ingredients. Linear, quadratic, and higher-order interactions can affect protein availability, mineral retention, and functional characteristics of the resulting flour. Traditional optimization approaches, such as the one-factor-at-a-time method, are inefficient and often fail to capture these interactions, leading to suboptimal formulations. To address these challenges, Response Surface Methodology (RSM) has emerged as a robust statistical tool for modeling and optimizing multi-component systems. Within RSM, mixture designs are particularly suited to food systems, where the response depends on the relative proportions of components rather than

their absolute amounts [11]. Among mixture designs, the D-optimal design is advantageous because it allows efficient exploration of constrained formulation spaces, reduces the number of experimental runs required, and provides high precision in estimating model parameters [12]. It is especially effective for systems with non-linear responses and multiple interactions, such as the protein and mineral composition of cereal–legume–oilseed blends. By employing D-optimal mixture design, researchers can identify the combination of ingredients that maximizes targeted nutritional responses while minimizing experimental resources [12].

The present study focuses on the optimization of protein and ash content in composite flours formulated from pearl millet, bambara nut, and sesame seed. Using a D-optimal mixture design, the study aims to develop predictive models for nutritional responses and identify the optimal blend ratios that enhance protein quality and mineral content. Such optimized composite flours have potential applications in the formulation of functional foods, weaning foods, and dietary supplements, contributing to improved nutrition in populations reliant on cereal-based diets.

MATERIALS AND METHODS

Materials: The materials used for this study were: Pearl millet (*Pennisetum glaucum*) Bambara nuts (*Vigna subterranean*) flour, sesame seed flour (*sesamum indicum*), high density polythene bags, plastic containers, crucibles, and food-grade reagents.

Source of raw materials and preparation of experimental samples

Pearl millet (*Pennisetum glaucum*) Bambara nuts (*Vigna subterranean*) flour and sesame seed (*Sesamum indicum*) were purchased from a local market in Ilishan-remo, Ogun State, Nigeria. The pearl millet, Bambara seeds, and sesame seeds were sorted to remove unwanted materials and milled into flour using a grinding machine [13]. The flour samples were packaged in plastic containers and stored at room temperature until further use.

Preparation of defatted Sesame seed flour

Defatted sesame seed flour was prepared using a modified method [14]. The finely ground sesame flour sample was defatted with n-hexane in a 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.

Experimental design

A three-component constrained D-optimal mixture process experimental design, with 16 randomized experimental runs, was employed. The formulation design constraints were process design. The raw materials and their ranges were: Pear millet (40-60 %), Bambara nut (10-30 %), and sesame seed flour (20-40 %). The formulation of the composite blend was based on the D-Optimal mixture. The responses were the crude protein and total ash. The outline of experimental design is shown in Table 1.

Preparation of composite flour

Composite flours consisting of pearl millet, bambara nut and sesame seed 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.

Sample analysis

The samples were analyzed for crude protein and total ash according to the method described by AOAC [15].

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 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 (version 13.0) statistical software, 3-D response surfaces plots were generated based on the fitted models for the responses.

Table 1: Design matrix for blends of Pearl millet, Bambara flour and sesame flour blends

Component	Unit	Low	High
Pearl millet flour	%	40	60
Bambara groundnut flour	%	10	30
Sesame flour	%	20	40

Table 1 presents the formulation space used to develop composite flour blends consisting of pearl millet, Bambara groundnut, and sesame flours. The selected ranges for each component reflect the nutritional considerations within the blend. Pearl millet flour constitutes the major cereal base in the blends, with proportions varying from 40-60%. Sesame flour, included at 20–40%, introduces both lipid and mineral richness, particularly calcium and essential fatty acids.

RESULTS AND DISCUSSION

The experimental design results are presented in Table 2, whereas Table 3 provides a statistical design summary.

Table 2: Experimental design for the Pearl millet, Bambara flour and sesame flour blends

Run	Pearl millet seed flour	Bambara nut flour	Defatted Sesame seed flour
1	55	25	20
2	40	25	35
3	55	10	35

4	50	20	30
5	60	10	30
6	50	30	20
7	50	20	30
8	60	15	25
9	40	30	30
10	60	20	20
11	45	30	25
12	55	10	35
13	50	30	20
14	40	20	40
15	40	30	30
16	40	20	40

Table 3: Design summary for the protein and ash content of Pearl millet, Bambara flour and Sesame flour blends

Response	Name	Unit	Transformation	Minimum	Maximum	Model
Y ₁	Protein	%	None	15.99	21.40	Cubic
Y ₂	Total Ash	%	None	2.93	5.20	Special Quartic

Table 3 provides a statistical design summary for protein content (Y₁) and total ash content (Y₂) derived from experimental blends of pearl millet, Bambara nut flour, and sesame flour. The protein content (Y₁) ranged from 15.99 - 21.40% across the sixteen experimental runs, indicating substantial variation attributable to differences in the proportions of (Bambara) and sesame flour. The use of a cubic model for protein prediction suggests the presence of significant interaction effects among the three components. This model choice indicates that synergistic and possibly antagonistic effects occur when the ingredients were combined, highlighting the complex nature of protein distribution in composite flours. For total ash content (Y₂), the response ranged from 2.93 - 5.20%. The use of a special quartic model reflects the highly nonlinear behavior of ash

content within the blend. This advanced model form typically captures curvature and higher order interactions beyond those represented in simpler mixture models. The ‘none’ in the two responses (crude protein and total ash) indicated that no mathematical transformation was performed on the data.

Table 4 presents the analysis of variance (ANOVA) results for the cubic mixture model developed to predict the protein composition of composite blends formulated from pearl millet, Bambara flour, and sesame flour.

Table 4: Analysis of Variance (ANOVA) for Response Surface Mixture Cubic model for protein composition of Pearl millet, Bambara flour and Sesame flour blends

Source	Sum of square	Df	Mean square	F-value	P-value	
Model	34.65	9	3.85	284.78	< 0.0001	Significant
Linear						
Mixture	5.19	2	2.60	192.09	< 0.0001	
AB	0.0006	1	0.0006	0.0458	0.8377	
AC	0.9255	1	0.9255	68.46	0.0002	
BC	5.88	1	5.88	435.00	< 0.0001	
ABC	1.32	1	1.32	97.37	< 0.0001	
AB(A-B)	5.58	1	5.58	412.41	< 0.0001	
AC(A-C)	0.3061	1	0.3061	22.64	0.0031	
BC(B-C)	0.0938	1	0.0938	6.93	0.0389	
Residual	0.0811	6	0.0135			
Lack of Fit	0.0611	1	0.0611	15.28	0.0113	Significant
Pure Error	0.0200	5	0.0040			
Cor Total	34.73	15				

$R^2 = 0.9977$; Adjusted $R^2 = 0.9942$, Adequate precision= 56.3689, Std. Dev = 0.1163, Mean= 19.43, C.V. % = 0.5986

The ANOVA evaluates the significance, adequacy, and predictive capability of the fitted model, thereby forming the basis for interpreting ingredient interactions and optimizing protein content in the mixture design. The model summary indicates that the cubic mixture model is highly significant ($F = 284.78$, $p < 0.0001$), demonstrating its strong explanatory power regarding protein variation across the blend formulations. The model accounts for nearly all of the variability observed in the response, as evidenced by the extremely high coefficient of determination ($R^2 = 0.9977$) and adjusted R^2 (0.9942). The adjusted R^2 and predicted R^2 were well-aligned, indicating strong model performance [16, 17]. These values confirmed that over 99% of the variation in protein content is successfully explained by the fitted model. The model with $R^2 > 0.75$ was considered acceptable according to [18]. The closer the R^2 value to unity, the better and more significant an empirical model fits the actual data. The model's adequate precision of 56.37 was well above the threshold of 4 which further confirmed a strong signal-to-noise ratio, and thereby validated its suitability for navigating the design space.

The breakdown of variance components reveals that the linear mixture terms are highly significant ($p < 0.0001$), illustrating that the individual proportions of pearl millet, bambara nut, and sesame flour exert strong effects on protein content. Among the binary interactions, AC and BC showed very high significance ($p < 0.001$), highlighting important interactive effects between millet–sesame and bambara–sesame blends. The BC interaction, in particular, exhibits a remarkably high F-value (435.00), indicating synergistic effect between bambara nut flour and sesame flour which are both protein-rich. Conversely, the AB interaction is non-significant ($p = 0.8377$), suggesting limited interaction between millet and bambara at the levels tested.

Higher-order interaction terms, including the ternary interaction (ABC) and the mixture-dependent terms AB(A–B), AC(A–C), and BC(B–C), were all significant, with the AB(A–B) and BC(B–C) components showing especially large F-values (412.41 and 6.93, respectively).

The residual error is low (sum of squares = 0.0811; mean square = 0.0135), which indicated excellent model fit with minimal unexplained variation. However, the significant lack of fit ($F = 15.28$, $p = 0.0113$) suggested that while the model predicted well, minor deviations existed between the model and actual data points possibly due to nonlinearities not fully captured or to experimental variability inherent in the blends. The low standard deviation (0.1163) and

coefficient of variation (0.5986%) indicate high precision and reliability in the measured protein values. The ANOVA results confirmed that the cubic mixture model provided a robust and highly predictive framework for describing protein variation in millet-bambara-sesame composite flour blends.

Table 5 presents the Analysis of Variance (ANOVA) results for the special quartic mixture model used to predict the total ash content of composite flour blends.

Table 5: Analysis of Variance (ANOVA) for Response Surface Mixture Special Quartic Model for Total Ash composition of Pearl millet, Bambara flour and Sesame flour Blends

Source	Sum of square	DF	Mean square	F-value	P-value	
Model	7.90	8	0.9873	33.75	< 0.0001	significant
Linear	2.36	2	1.18	40.39	0.0001	
Mixture						
AB	0.3529	1	0.3529	12.06	0.0104	
AC	1.35	1	1.35	46.17	0.0003	
BC	0.6709	1	0.6709	22.93	0.0020	
A ² BC	0.1833	1	0.1833	6.26	0.0408	
AB ² C	0.1915	1	0.1915	6.54	0.0377	
ABC ²	0.2487	1	0.2487	8.50	0.0225	
Residual	0.2048	7	0.0293			
Lack of Fit	0.2035	2	0.1018	407.08	< 0.0001	significant
Pure Error	0.0013	5	0.0003			
Cor Total	8.10	15				

$R^2 = 0.9747$; Adjusted $R^2 = 0.9458$, Adequate precision= 17.8088, std. Dev = 0.1710, Mean= 3.51, C.V. % = 4.87

The overall model is highly significant ($F = 33.75$, $p < 0.0001$), demonstrating strong predictive capability for ash content variation across the experimental runs. The high coefficient of determination R^2 (0.9747) and adjusted R^2 (0.9458) indicated that over 94% of the variability in total ash was explained by the model. The adequate precision of 17.81, well above the recommended threshold of 4, confirmed a strong signal-to-noise ratio, validating the model for exploring the mixture design space [19]. A low coefficient of variation (4.87%) and high adequate precision (118.25) further reinforced the model's precision and predictive power [19]. The linear mixture terms were significant ($F = 40.39$, $p = 0.0001$), reflecting the primary contributions of individual component to ash content. Among the binary interactions, AC (millet–sesame) exhibited the largest effect ($F = 46.17$), followed by BC (bambara-sesame, $F = 22.93$) and AB (millet-bambara, $F = 12.06$). These results suggested that sesame flour strongly modulated ash content, both independently and in combination with millet or Bambara flour, while millet and Bambara interactions were comparatively moderate.

The higher-order special quartic terms (A^2BC , AB^2C , ABC^2) were all significant ($p < 0.05$), highlighting the importance of non-linear and composition-dependent effects. These terms capture complex synergistic or antagonistic interactions among the three ingredients that could not be explained by linear or simple binary effects alone.

The crude protein equation (Equation 1) shows how pearl millet (A), bambara nut flour (B), and sesame flour (C) individually and jointly influenced protein levels in the composite blend. The linear terms indicated that bambara and sesame strongly increased protein content, whereas millet contributes very little. The interaction terms (AB, AC, BC) revealed how component combinations modified protein levels, with the large negative BC coefficient showing a strong antagonistic effect when bambara and sesame were blended together. The three-way interaction (ABC) showed how all ingredients jointly influenced protein beyond simple pairwise effects. The higher-order nonlinear terms further described complex blending behavior, showing that shifts in the relative proportions of ingredients could enhance or suppress protein content in ways not explained by linear effects alone.

The ash-content model (Equation 2) showed how pearl millet (A), Bambara flour (B), and sesame flour (C) influence the ash contents of the blend. The positive linear terms indicated that all three ingredients increase ash, with sesame contributing the most. The negative AB, AC, and BC terms showed that combining ingredients could reduce ash due to antagonistic interactions. The higher-order terms (A^2BC , AB^2C , ABC^2) captured nonlinear effects, showing that ash content increased sharply when sesame was present in high proportions and interacted with balanced levels of millet.

$$\text{Crude protein} = -7.82A + 30.43C - 1.05AB + 39.84AC - 78.46BC + 68.94ABC + 114.87AB(A-B) + 31.05(AC(A-C) - 18.87BC(B-C)) \quad (1)$$

$$\text{Total Ash} = +8.19A + 5.53B + 12.38C - 14.61AB - 28.19AC - 21.81BC - 58.18A^2BC + 53.97AB^2C + 148.21ABC^2 \quad (2)$$

Figures I and II are the 3D plots showing the effect of pearl millet, bambara nut and sesame seed flour blends on protein content and total ash, respectively, whereas Figures III and IV show the plots of actual value vs residual value for crude protein and Ash content, respectively.

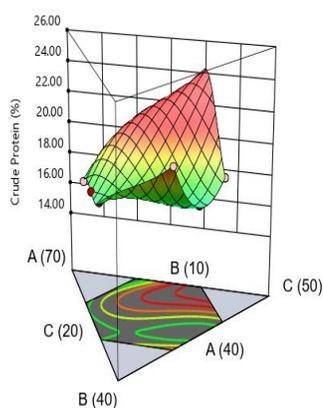


Figure I

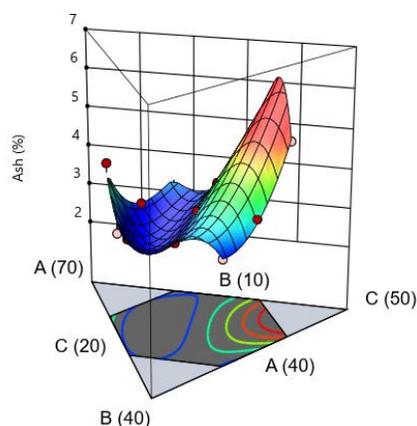


Figure II

Figures I and II: 3D plot showing the effect of pearl millet, bambara nut and sesame seed flour blends on protein content and total ash, respectively

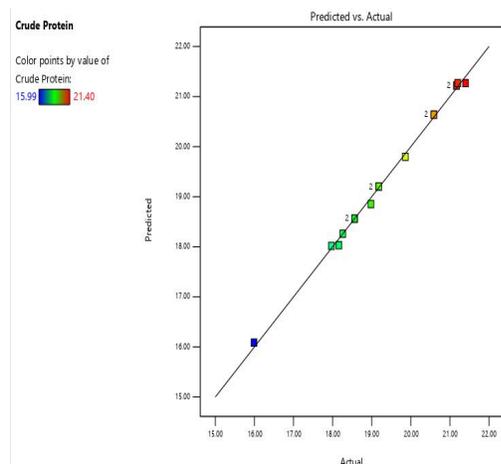


Fig III

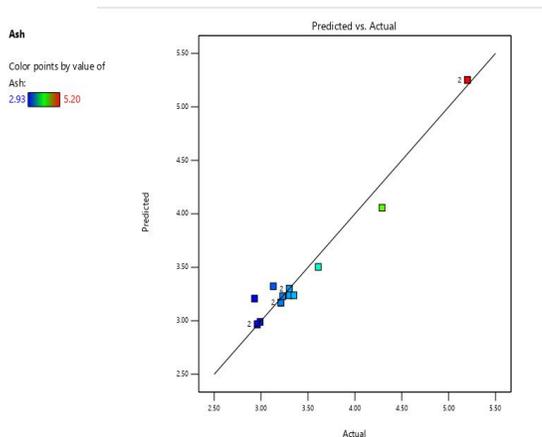


Fig IV

Fig III and IV: Plot of actual value vs residual value for crude protein and Ash content, respectively.

Table 6 presents the results of crude protein and total ash contents of the composite flour blends across the experimental runs.

Table 6: Protein and ash content of pearl millet, bambara flour and sesame flour blends

Run	Pearl millet seed flour	Bambara nut flour	Defatted Sesame seed flour	Crude Protein	Total ash
1	55	25	20	18.26±0.02d	2.93±0.02
2	40	25	35	18.16 ±0.04c	3.61±0.04c
3	55	10	35	21.40 ±0.02k	3.30±0.02b
4	50	20	30	20.59±0.02i	3.30±0.03ab
5	60	10	30	15.99±0.00a	3.13±0.06ab
6	50	30	20	18.56±0.03e	3.21±0.01b
7	50	20	30	20.59±0.02i	3.30±0.03ab
8	60	15	25	18.98±0.03j	2.99±0.05a

9	40	30	30	21.17±0.05b	2.96±0.03a
10	60	20	20	17.97±0.03b	4.29±0.07b
11	45	30	25	19.86±0.02h	3.23±0.07b
12	55	10	35	21.40 ±0.02k	3.30±0.02b
13	50	30	20	18.56±0.03e	3.21±0.01b
14	40	20	40	19.18±0.02h	5.20±0.01c
15	40	30	30	21.17±0.03j	2.96±0.03a
16	40	20	40	19.18±0.03g	5.20±0.01c

Values are means of triplicate determination ± SD; Means followed by the different alphabet within the same column are significantly ($P \leq 0.05$)

Protein content ranged from 15.99 - 21.40%, The highest crude protein content (21.40%) was observed in Run 3 (55% millet, 10% bambara, 35% sesame) followed by Run 4 and Run 7 (50:20:30), with protein content of 20.59%. This could be attributed to the combination of sesame and millet at relatively high levels, which are both rich in protein, coupled with a lower proportion of bambara [20]. The high sesame content, in particular, appears to exert a strong protein-enhancing effect, consistent with the modeled interactions showing positive contributions of sesame (C) and synergistic AC interactions. However, the protein content obtained in this study was lower than values (33.72 - 40 82 g/100 g) reported for amaranth–Bambara-based composite flours [21]. Conversely, run 5 (60:10:30) exhibited the lowest protein (15.99%), likely due to the high millet and low bambara contents

The total ash content ranged from 2.93-5.20%. Runs 14 and 16 had the highest value (5.20%). The highest ash content reported in these runs implied that sesame flour had the major contribution to ash content. The relatively lower millet content in these blends may also enhance the ash concentration by reducing dilution from starch-rich millet. The higher ash content recorded in blends with increased sesame flour is consistent with earlier reports showing that sesame is a major contributor to ash content in composite flour [21]. The observed high ash content is consistent with the special quartic model, which identified significant nonlinear and synergistic effects of sesame flour on total ash composition.

CONCLUSION

Based on the study, the blends exerted a significant effect on the crude protein and total ash composition among the blends, emphasizing the importance of strategic formulation. There were high R^2 values and low coefficients of variation for crude protein and total ash content of the blends indicating the reliability of the experimental design in optimizing nutritional properties of the blends. Run 3 (55% millet, 10% Bambara, 35% sesame) had the highest crude protein content (21.40%) while run 14 and 16 had the same highest ash content. These findings justified the need for a cubic model, as they highlight the presence of complex nonlinear blending effects that cannot be captured by linear or quadratic mixture models. Composite blends of pearl millet, Bambara nut and sesame seed flour through advanced experimental design methodologies hold potential for the production of nutrient dense food products capable of addressing issues of protein-energy malnutrition.

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