TIMBOU-AFRICA PUBLICATION INTERNATIONAL JOURNAL MAY, 2024 EDITIONS.

INTERNATIONAL JOURNAL OF SCIENCE RESEARCH AND TECHNOLOGY

VOL. 4 NO. 2 E-ISSN 3026-8796 P-ISSN 3026-8095

STATISTICAL EVALUATION OF PYRIDOXINE IN RED GUINEA CORN-MILLET COMPOSITE

ODEWORITSE EYIDE[1]; EJIROGHENE THELMA AKHIHIERO[2]; SUNDAY IWERIOLOR[3]; BLESSING JOSEPHINE OSSAI[4]; ONORIODE ERHINYODAVWE[5]; AYODEJI ARNOLD OLASEINDE[6]; OHIRI AUGUSTINE CHIMEZIE[7]

[1]Department of Chemical Engineering, Faculty of Engineering, University of Delta, Agbor, Delta State, Nigeria. [2]Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria. [3]Department of Mechanical Engineering, Faculty of Engineering, University of Delta, Agbor, Delta State, Nigeria. [4]Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria. [5]Department of Mechanical Engineering, Faculty of Engineering, University of Delta, Agbor, Delta State, Nigeria. [6]Department of Materials Science and Engineering, College of Engineering, Clemson University, Clemson, South Carolina, U.S.A. [7]Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria.

odeworitse.eyide@unidel.edu.ng

ABSTRACT

This research work focused on the statistical optimization of pyridoxine in red guinea corn and millet composite. The concentration of pyridoxine (vitamin B_6) was investigated under the following conditions: blending time (1.5-5hours), amount of red guinea corn (10-50g) and amount of agro residue (50-100 g) using Box-Behnken design. Statistically significant model (< 0.0001) was developed to represent the relationship between the response (concentration of pyridoxine) and the independent variables. The model showed a significant

INTRODUCTION

Red guinea corn (*Sorghum bicolor*) and millet (*Pennisetum glaucum*) are two staple grains widely consumed by human or animals and they are widely cultivated in the northern part of Nigeria (Ojattah and Oguche, 2023). Both grains are valued for their nutritional content, including proteins, carbohydrates, minerals, and vitamins (Kutyauripo and Mutombo, 2020). Integrating these grains into composite food products can offer enhanced nutritional benefits, potentially enriching the diet with essential micronutrients such as pyridoxine. According to Food and Agricultural Organization FAO, (2007) both grains are regarded as valuable sources of healthy diet.

fit with experimental data with R^2 values of 0.99. Analysis of variance (ANOVA) results showed that the concentration of pyridoxine was influenced by the blending time, amount of red guinea corn and amount of millet used. Response surface methodology (RSM) was used to optimize the concentration of pyridoxine and the optimization results showed that the maximum concentration of 61.36 µg/100g for pyridoxine was obtained at the optimum production conditions of blending time of 5hours, 47.45g of red guinea corn and 100g of millet. Also the recommended amounts of red guinea corn and millet composite for all groups as shown in table 11 were formulated in conformity with World Health Organization (WHO)/Food and Agricultural Organization (FAO) specification for recommended safe intake for all age groups, pregnant and nursing mothers with deficiencies in pyridoxine.

Keywords: Composite, Millet, Statistical, Red Guinea Corn, Pyridoxine.

Composite food products, formulated by blending different ingredients, have gained popularity as a suitable and nutritious substitutes to traditional foods (Akhihiero et al., 2022 , Nwokem et al. 2019, Ikokoh et al., 2019). The combination of red guinea corn and millet as a composite offers a promising avenue for enhancing dietary diversity and nutritional quality, particularly in regions where these grains are staple foods.

One key area of interest in food engineering is the design and formulation of right nutrition in other to have the right diet quality which serve as a great significant in addressing the issues of hunger, food insecurity and malnutrition. Statistically, in 2019, two billion people, or 25.9 percent of the global population, experienced hunger or did not have regular access to nutritious and sufficient food. This is linked to the issue of harnessing the right diet quality in other to address the challenges of food insecurity which have negatively contributed progressively to the risk of child malnutrition (FAO,IFAD,UNICEF, WFP and WHO. 2020). According to WHO, malnutrition as well as non-communicable diseases (NCDs) such as diabetes, heart disease, stroke and cancer can be prevented when the right diet quality is consumed (Poore et al., 2018).

Diet quality is the overall nutritional value and composition of an individual's diet, based on the types and quantities of foods consumed. It contains balanced, diverse, and variety of nutrients obtained from food in line with individual's nutritional needs and health goals (Springmann et al., 2018). Diet quality (healthy diet) ensures that a person's needs for macronutrients (proteins, fats and carbohydrates including dietary fibers) and essential micronutrients (vitamins and minerals) are met, based on their gender, age, physical activity level and physiological state (Springmann et al., 2018).

Vitamins are either fat-soluble or water-soluble, and a lack of either can result in vitamin deficiencies, leading to health issues (Ottaway, 2008). These vitamins are frequently reduced or washed out of the body due to malnutrition and processes of preparation (Keservani et al., 2014). Because the human body cannot store water-soluble vitamins such as vitamin C and the B-complex, these vitamins must be supplemented daily from different variety of foods via multiple methods by blending different food together with the right diet quality. However, it is not easy to specify the intake ranges for a particular food, which should be provided in each combination to meet nutritional requirements. But utilizing the right nutrition design and formulation from Plant source with high nutritional content and functional qualities such as cereals, legumes, and vegetables (Keservani et al., 2014, Kunyanga et al., 2013), will help in addressing the problem of vitamin deficiencies.

One of the micronutrient of interest in this study is vitamin B6 (pyridoxine) which is an essential nutrient needed in small amounts by humans for the normal functioning of coenzyme in metabolic activities, neurotransmitter synthesis, hemoglobin formation, glycogen metabolism, immune system, cardiovascular system and nervous System (Iqbal Ahmad et al., 2013). They occur in variety of related form known as vitamers which includes pyridoxine (pyridoxol), pyridoxal, and pyridoxamine and their phosphorylated derivatives. However, pyridoxine is the predominantly used active vitamer of vitamin B6 in clinical treatment of diseases (Iqbal Ahmad et al., 2013). Its importance in human nutrition emphasizes the significance of assessing its presence and concentration in dietary sources.

Table 1: Recommended nutrient intakes for pyridoxine for all age groups, pregnant and nursing mothers

Group	Recommended nutrient intake μg/day
Infants and children	
0-6 months	100
7-12 months	300
1-3 years	500
4-6 years	600
7-9 years	1000
Adolescents, 10-18 years	
Females	1200
Males	1300
Adults	
Females, 19-50 years	1300
Males, 19-50 years	1300
Females, >50 years	1500
Males, >50 years	1700
Pregnancy	1900
Lactation	2000

Source: FAO/WHO. 1988.

However, despite the nutritional importance of pyridoxine and the potential benefits of red guinea corn-millet composites, limited research has investigated the pyridoxine content of such composite formulations. Understanding the pyridoxine levels in these composites is crucial for assessing their nutritional value and informing dietary recommendations. To the best of our

E-ISSN 3026-8796
P-ISSN 3026-8095

knowledge, none of these researches have attempted to statistically evaluate the concentration of pyridoxine in several typical Nigerian cereals (Red Guinea corn and millet).
Hence, this study aims to statistically evaluate the pyridoxine content of a red guinea corn-millet composite. By quantifying pyridoxine levels in the composite, we seek to contribute to the existing knowledge on the nutritional composition of composite foods and explore the potential of this composite as a source of dietary pyridoxine.

MATERIALS AND METHODS

Collection and Pretreatment of Raw Materials

The millet (*Pennisetum glaucum*) and red guinea corn (*Sorghum bicolor*) grains used in this study were purchased from the midwifery market, a local market, in Oshimili North Local Government Area, Delta State, Nigeria. The millet and red guinea corn grains were sorted to remove sand, dust, dirt and other unwanted materials, and then washed in clean water and sun-dried for 7 to 14 days.

Experimental Design

A three-factor Box-Behnken design for response surface methodology was used to study the composite effect of the mass of red guinea corn, the mass of millet and blending time on the concentration of pyridoxine. The range and levels of the independent variables are shown in Table 2.

Table 2: Coded and actual levels of the factors for three factors of Box-Behnken design for the statistical optimization of pyridoxine.

Independent Variables	Symbols	Coded and Actual Levels		
		-1	0	+1
Mass of red guinea corn (g)	X_1	10.00	30.00	50
Mass of millet (g)	X_2	50.00	75.50	100.00
blending time(hours)	X_3	1.50	3.25	5.00

According to Amenaghawon et al., (2013), the Box-Behnken design has been established to be appropriate for the investigation of quadratic response surfaces and this design generates a second-degree polynomial model which can be used for optimization purposes.
The number of experimental runs for this design was obtained from Equation (1).

$$N = k^2 + k + c_p \quad (1)$$

Where k is the number of factors and c_p is the number of replications at the center point. The design for the evaluation of the concentration of pyridoxine in millet and guinea corn was developed using Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA) and 17 experimental runs were obtained. The coded and actual values of the independent variables were calculated using Equation (2).

E-ISSN 3026-8796
P-ISSN 3026-8095

$$x_i = \frac{X_i - X_o}{\Delta X_i} \qquad (2)$$

Where x_i and X_i are the coded and actual values of the independent variable respectively. X_o is the actual value of the independent variable at the center point and ΔX_i is the step change of X_i. Below is the generalized second-degree polynomial equation used to estimate the response of the dependent variable (Amenaghawon et al., 2013).

$$Y_i = b_o + \sum b_i X_j + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \qquad (3)$$

Where Y_i is the dependent variable or predicted response, X_i and X_j are the independent variables, b_o is the offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the error term. The Design Expert software was used for regression and graphical analysis of the experimental data. The goodness of fit of the models for the concentration of pyridoxine was evaluated by the coefficient of determination (R^2) and analysis of variance (ANOVA).

Table 3: Box Behnken Experimental Design

Std	Run	Block	Factor 1 A: mass of red guinea corn (g)	Factor2 B: mass of millet (g)	Factor3 C: Blending Time (hours)
13	1	Block1	30	75	3.25
15	2	Block1	30	100	5
10	3	Block1	50	100	3.25
16	4	Block1	30	75	3.25
9	5	Block1	10	75	5
11	6	Block1	10	50	3.25
7	7	Block1	30	75	3.25
1	8	Block1	50	75	1.5
5	9	Block1	30	50	1.5
6	10	Block1	10	75	1.5
8	11	Block1	30	50	5
12	12	Block1	30	75	3.25
2	13	Block1	30	100	1.5
17	14	Block1	50	75	5
14	15	Block1	50	50	3.25
3	16	Block1	10	100	3.25
4	17	Block1	30	75	3.25

Analysis of Samples

The analyses were carried out at the Central Research and Diagnostic Laboratory, Tanke, Ilorin, Kwara State, Nigeria. The proximate analysis was done on the samples as well as the determination of the concentration of pyridoxine for each composite according to the Box Behnken experimental design in Table 3. The concentration of pyridoxine for each composite was determined using Agilent 6890 Gas Chromatography (GC).

Proximate Analysis

The method of the Association of Official Analytical Chemists (AOAC 2005) was used to determine the amount of moisture, ash, fat, protein, crude fiber, and carbohydrate contents of the pure samples. The percentage of protein and caloric value were evaluated by Eq (4) and (5) respectively (Eyide et al., 2023).

Protein (%) = 100 - Carbohydrate (%) + Moisture (%) + Ash (%) + crude Fibre (%) + Fat (%)). (4)

While the Energy or Caloric Value was determined by Eq (5)

(KJ/100g) = (Protein X 16.7) + (Lipids X 37.7) + (Carbohydrate X 16.7) (5)

Sample Preparation

Based on the experimental design in Table2, prior to analysis, extraction process for each homogenized sample was carried out using acid hydrolysis of 0.1N HCl at 121°C at the respective blending time. It was then cool and pH adjusted to 4.5, follow by treating each homogenized sample with hexane to remove fat. Thereafter, autoclaving of the each homogenized sample with 0.1N HCl at 121°C at the respective blending time follow by corresponding increase in the pH value to 6.0, thereafter diluted with water then filter. In the next stage, enzymatic hydrolysis was carried out; acid phosphatase was added and incubates at 45°C overnight. Proceeded was proteins precipitation using Trichloroacetic Acid (TCA) 50% w/v for 5min and adjustment pH to 4.5 at temperature of 100°C with simultaneous reaction of glyoxylic acid in presence of Fe^{+2} catalyst, to convert pyridoxamine in each of the homogenized sample into pyridoxal, which is subsequently reduced to pyridoxine by the action of sodium borohydride in the alkaline medium. Finally each prepared homogenized sample was analyzed using Agilent 6890 gas chromatography (GC) with a suitable stationary phase and detector.

RESULTS AND DISCUSSION

Proximate Analysis Results

E-ISSN 3026-8796
P-ISSN 3026-8095

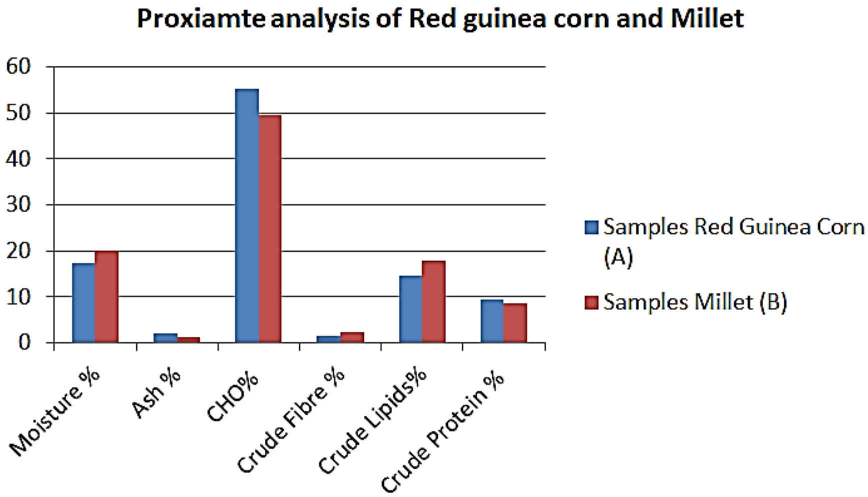

Figure 1: graphic representation of proximate analysis for pure samples of Red guinea corn and Millet

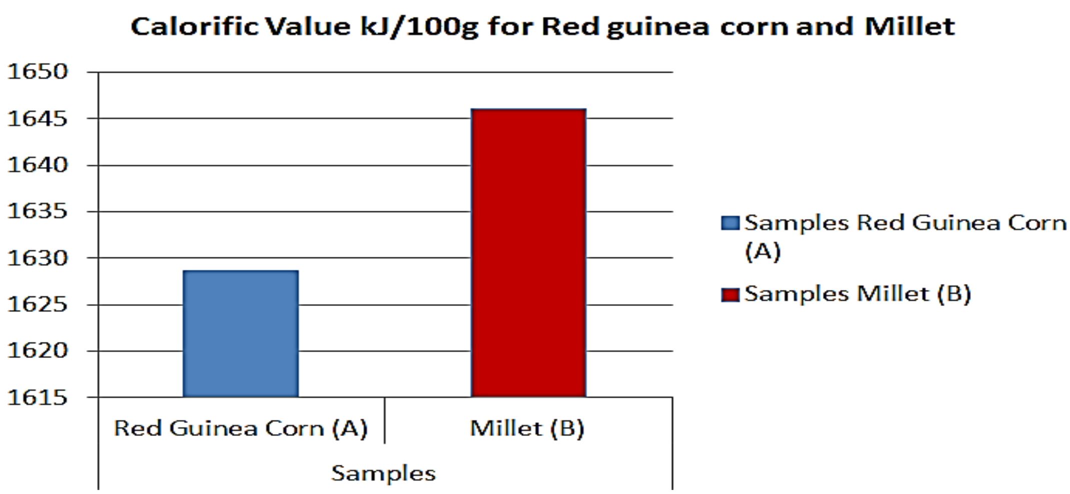

Figure 2: graphic representation of caloric value kJ/100g for pure samples of Red guinea corn and Millet

From figure 1, millet has a moisture content of 20.04% which is slightly higher than that of red guinea corn at 17.28%. The ash content of 2.07% observed with of red guinea corn was higher as compared to 1.38% observed in millet. Red guinea corn has 55.23% of carbohydrates while millet has 49.54%. The crude fiber was higher in millet (2.44%) as compared to that of red guinea corn (1.48%). Crude lipids content was higher in millet (17.84%) as compared to the one observed with red guinea corn (14.59%). Red guinea corn was higher in crude protein contents (9.34%) compared to that millet of 8.76%). While in figure 2, show that calorific value for both red guinea corn and millet. A calorific value of 1646.02kJ/100g was observed in millet which was higher than 1628.62kJ/100g in red guinea corn.

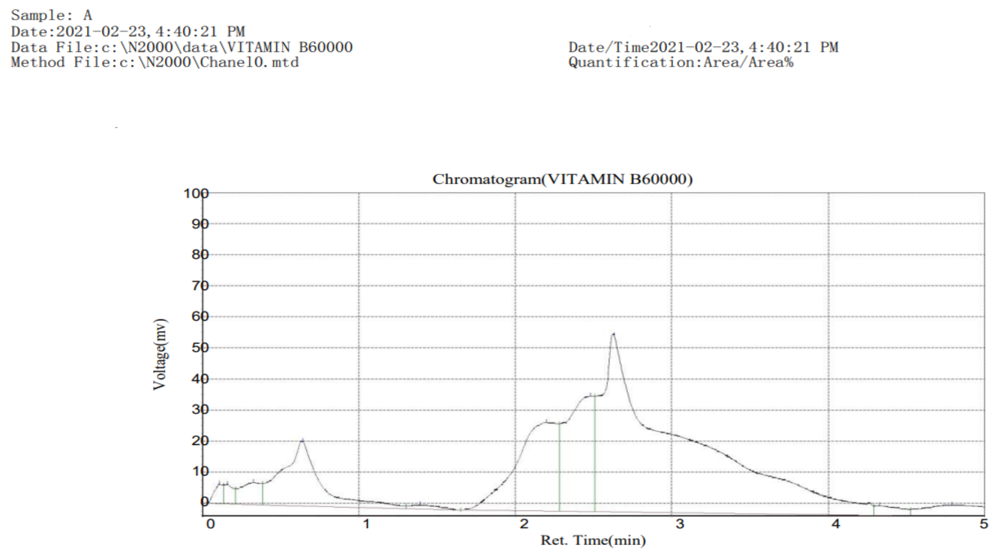

Figure 3: Chromatogram for sample A (red guinea corn)

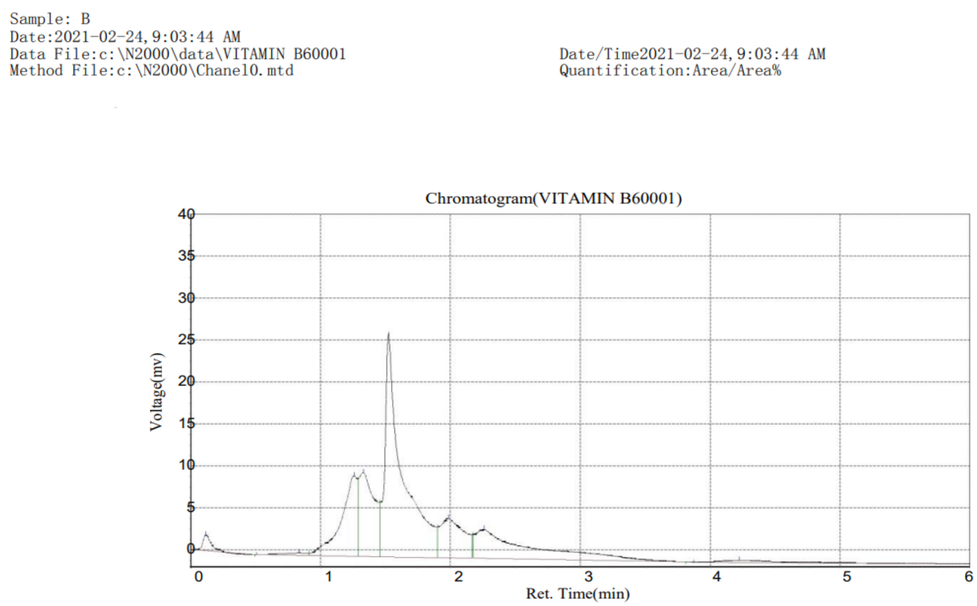

Figure 4: Chromatogram for sample B (millet)

Table 5: Concentration of pyridoxine presents in sample A (red guinea corn)

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100g
1	Unidentified	0.107	6240.436	24532.768	0.6669
2	Unidentified	0.157	6275.098	25574.207	0.6952
3	Unidentified	0.323	7077.969	66554.898	1.8091
4	Unidentified	0.640	20755.826	355498.844	9.6633
5	Unidentified	1.390	1234.749	16520.977	0.4491
6	Unidentified	2.198	28531.902	534681.063	14.5339
7	Unidentified	2.482	37187.063	449291.844	12.2129
8	Pyridoxine	2.632	57092.855	2029718.500	55.1727
9	Unidentified	4.323	3110.612	35929.457	0.9767
10	Unidentified	4.790	3533.407	140540.688	3.8202

Table 6: Concentration of pyridoxine present in sample B (millet)

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100g
1	Unidentified	0.115	1857.714	7997.000	1.3365
2	Unidentified	0.832	301.697	4149.809	0.6936
3	Unidentified	1.257	9577.454	85653.813	14.3154
4	Unidentified	1.332	9971.000	81918.883	13.6912
5	Pyridoxine	1.523	26394.949	245264.047	40.9911
6	Unidentified	1.982	4686.394	60503.988	10.1121
7	Unidentified	2.257	3462.061	104164.125	17.4090
8	Unidentified	4.223	221.027	8682.650	1.4511

From Tables 5 and 6, it can be depicted that sample A (red guinea corn) has a higher amount of Pyridoxine of 55.1727 (µg/100g) compared to sample B(millet) with 40.991 (µg/100g) of pyridoxine.

Statistical Analysis

The Box-Behnken design resulted in 17 experimental runs as shown in Table 2. Eq.(6) was obtained after applying multiple regression analysis to the experimental data. This second-degree polynomial equation was used to estimate the response (concentration of pyridoxine).

$$Y_2=+40.67 + 6.00X_1 +4.98X_2 - 0.12X_3 +8.84X_1X_2 -0.000X_1X_3+0.25X_2X_3 -3.26X_1^2-5.14 X_2^2+10.32X_3^2 \qquad (6)$$

Where, Y_1 , Y_2 and Y_3= predicted responses for the concentration of pyridoxine (µg/100g), $X_1X_2X_3$ = A,B,C coded values for the mass of red guinea corn , mass of millet and blending time respectively.

The values of the concentration of pyridoxine (µg/100g), as predicted by model Equation (6), were shown in Tables 6, in line with their experimental data. The significance of the fit of the equation representing the concentration of pyridoxine (µg/100g), was evaluated by carrying out analysis of variance (ANOVA). ANOVA result depicted that the model for the concentration of pyridoxine (µg/100g) was statistically significant with p values (< 0.0001), as shown in Table 7. The model did not show a lack of fit as seen from the "lack of fit" p values (0.0705). For the model, the terms representing the mass of red guinea corn and mass of millet were significant for response (the concentration of pyridoxine (µg/100g) while the term representing blending time was significant for the response indicating that it significantly influenced the concentration of pyridoxine (µg/100g).

Table 7: Box Behnken Design Matrix for the optimization variables and response values of concentration of pyridoxine (µg/100g).

Run No	Variables						Response	
	Coded levels			Actual values			Pyridoxine (µg/100g)	
	X1	X2	X3	X1	X2	X3	Actual	Predicted
1	0	0	0	30	75	3.25	40.11	40.67
2	0	1	1	30	100	5.00	50.32	50.96
3	1	1	0	50	100	3.25	52.11	52.09
4	0	0	0	30	75	3.25	40.89	40.67
5	-1	0	1	10	75	5.00	41.23	48.37
6	-1	-1	0	10	50	3.25	30.12	30.13
7	0	0	0	30	75	3.25	40.11	40.67
8	1	0	-1	50	75	1.50	54.23	33.21
9	0	-1	-1	30	50	1.50	41.87	41.00
10	-1	0	-1	10	75	1.50	41.23	41.85
11	0	-1	1	30	50	5.00	40.89	40.50
12	0	0	0	30	75	3.25	41.11	40.67
13	0	1	-1	30	100	1.50	50.32	50.70
14	1	0	1	50	75	5.00	54.23	53.61
15	1	-1	0	50	50	3.25	23.43	24.45
16	-1	1	0	10	100	3.25	23.42	22.41
17	0	0	0	30	75	3.25	41.11	40.67

Table 8: ANOVA results for a model representing the concentration of pyridoxine (µg/100g).

Sources	Sum of Squares	Df	Mean Squares	F value	p-value [Prob>F]
Model	1375.00	9	152.78	202.39	< 0.0001
X_1	288.00	1	288.00	381.53	< 0.0001
X_2	198.60	1	198.60	263.10	< 0.0001
X_3	0.12	1	0.12	0.16	0.7019
X_1X_2	312.94	1	312.94	414.56	< 0.0001
X_1X_3	0.000	1	0.000	0.000	1.0000
X_2X_3	0.24	1	0.24	0.32	0.5904
X_1^2	44.69	1	44.69	59.21	0.0001
X_2^2	111.15	1	111.15	147.25	< 0.0001
X_3^2	448.60	1	448.60	594.29	< 0.0001
Residual	5.28	7	0.75		

Lack of Fit	4.22	3	1.41	5.30	0.0705
Pure Error	1.06	4	0.27		
Cor Total	1380.28	16			

Table 9: Statistical information for ANOVA concentration of pyridoxine (µg/100g), in the composite.

Parameter	Response
	Concentration of Pyridoxine (µg/100g).
R-Squared	0.99
Mean	41.57
Standard Deviation	0.87
C.V%	2.09
Adeq. Precision	47.18
Adjusted R-Square	0.99

From table 9, the statistical information for the ANOVA shows that the model describing the concentration of pyridoxine (µg/100g) had a high coefficient of determination (R^2) of 0.99. This shows that the model was able to adequately represented the relationship between the chosen variables (mass of red guinea corn, mass of millet and blending time) and response (concentration of pyridoxine). R^2 values of 0.99 means that the model was able to account for 99.00% of the variability observed in the values of concentration of pyridoxine. The standard deviation was observed to be relatively small compared to the mean, which reveal the concentration of the experimental values around the mean. The coefficient of variation of 2.09 was obtained for the model. This parameter shows the degree of precision with which the runs were carried out. The values obtained are in line with high reliability as recommended by (Montgomer, 2005). The Adequate precision for the model indicates adequate signal meaning that the model can be used to navigate the design space (Cao, 2009).

Optimization of Concentration of Pyridoxine *(µg/100g)* in the blend

Response surface methodology was used to optimise the process. This was achieved by generating response surface plots showing the three-dimensional relationships among the mass of red guinea corn, mass of millet and blending time on the concentration of pyridoxine Figure 5, shows composite effect of the mass of red guinea corn and millet on the concentration of pyridoxine. The trend observed shows that the concentration of pyridoxine, increased with an increase in the mass of red guinea corn and mass of millet, this is due to the resultant amount of the pyridoxine present in the respective cereals, with red guinea corn having the highest contribution of pyridoxine concentration to the composite as shown in tables 4 and 5. Similar trends were observed in Figure 6 and Figure 7, shows that an increase in the blending time against the amount of the red guinea corn and amount of millet has a significant effect on the concentration of pyridoxine which is due to the interacting effect between the mass of red guinea and millet.

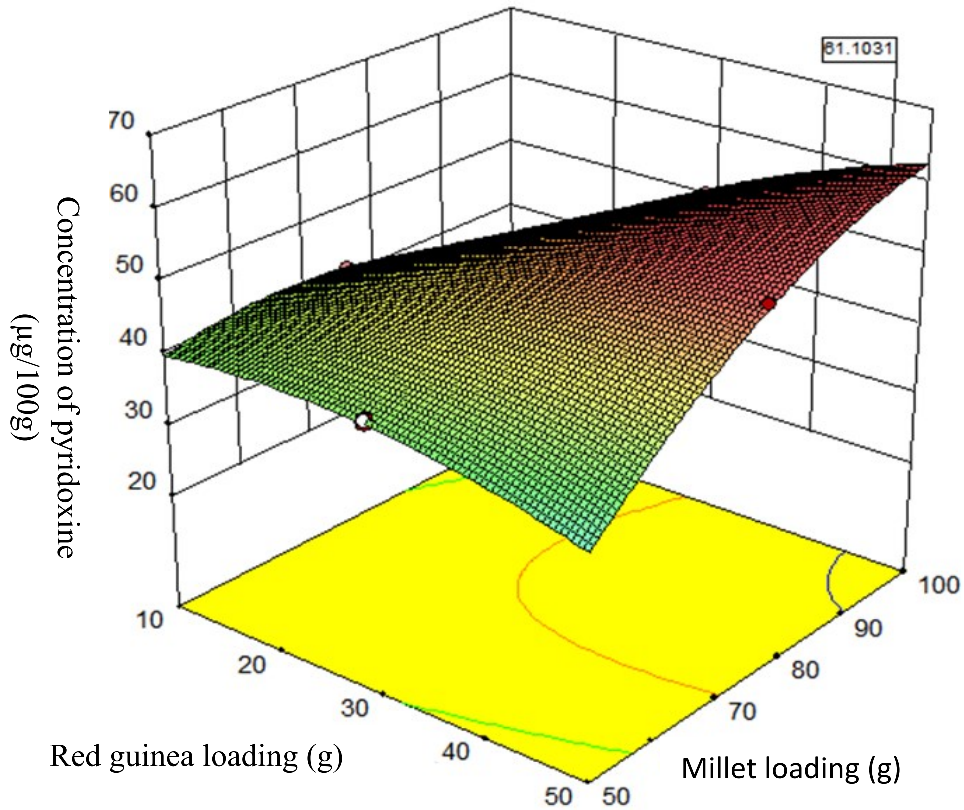

Figure 5. Effect of amount of red guinea corn and millet on concentration of pyridoxine.

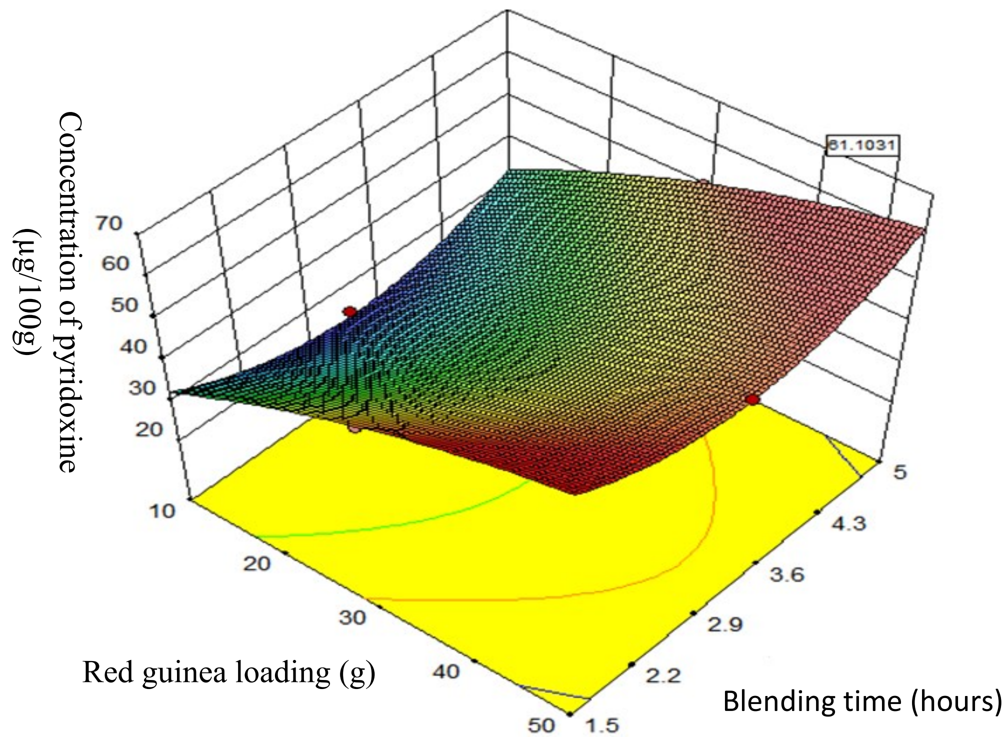

Figure 6. Effect of amount of red guinea corn and blending time on concentration of pyridoxine.

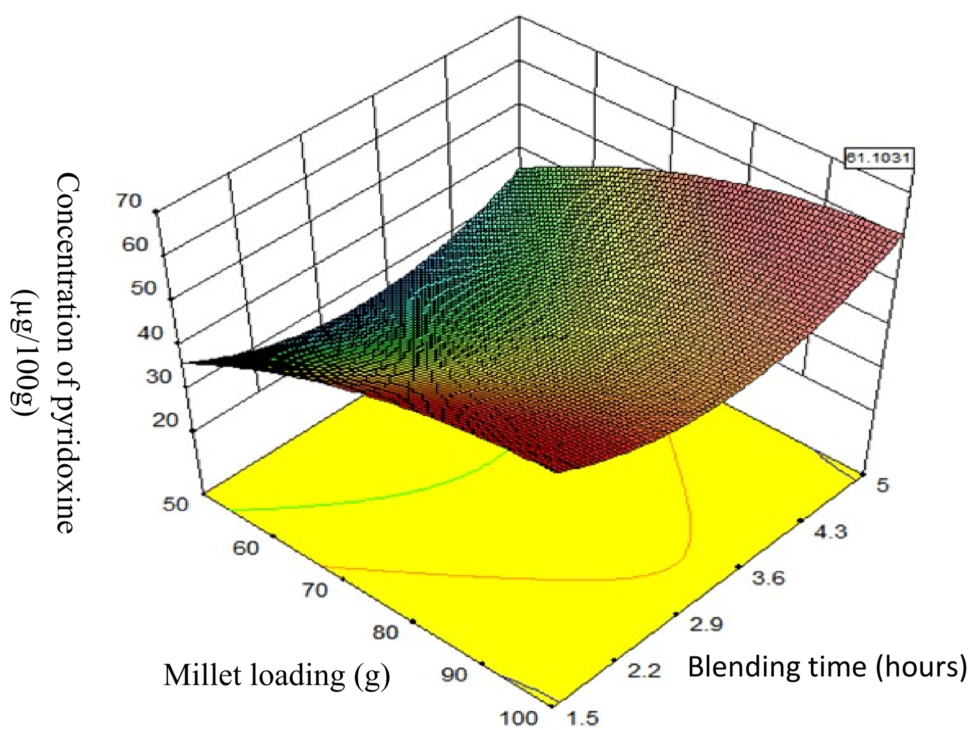

Figure 7. Effect of amount of Millet and blending time on concentration of pyridoxine.

The optimum levels of the independent variables and the response (Concentration of pyridoxine) were determined from numerical optimisation of the statistical model (Equation 6) and the top five results are shown in Table 9, the results show that the optimal value of 61.36 (µg/100g) of pyridoxine, was obtained at a blending time of 5.00 hours, 47.45g of red guinea corn and 100g of millet.

Table 10: Solutions for optimum conditions for concentration of pyridoxine.

Solution Number	Red guinea corn (g)	Millet(g)	Blending Time(hours)	Concentration of pyridoxine (µg/100g)
1	47.45	100.00	5.00	61.36
2	47.31	100.00	5.00	61.19
3	46.78	100.00	4.99	61.12
4	46.75	99.98	4.98	61.10
5	46.50	99.96	4.97	60.98

Recommended amount of red guinea corn and millet composite in grams based on the optimal results.

Table 11: Recommended amount of red guinea corn and millet composite in grams for pyridoxine

Group	Recommended nutrient intake μg/day	Recommended amount of red guinea corn and millet composite g/day for concentration of pyridoxine at optimal value for 61.36 (µg)	Recommended amount of red guinea corn and millet composite g/day for concentration of pyridoxine at optimal value for 61.19 (µg)	Recommended amount of red guinea corn and millet composite g/day for concentration of pyridoxine at optimal value for 61.12 (µg)	Recommended amount of red guinea corn and millet composite g/day for concentration of pyridoxine at optimal value for 61.10 (µg)	Recommended amount of red guinea corn and millet composite g/day for concentration of pyridoxine at optimal value for 60.98 (µg)
Infants and children						
0-6 months	100	162.97	163.43	163.61	163.67	163.99
7-12 months	300	488.92	490.28	490.83	490.99	491.96

1-3 years	500	814.86	817.13	818.06	818.33	819.94
4-6 years	600	977.84	980.55	981.68	981.99	983.93
7-9 years	1000	1629.73	1634.25	1636.12	1636.66	1639.88
Adolescents, 10-18 years						
Females	1200	1955.67	1961.10	1963.35	1963.99	1967.85
Males	1300	2118.64	2124.53	2126.96	2127.66	2131.85
Adults						
Females, 19-50 years	1300	2118.64	2124.53	2126.96	2127.66	2131.85
Males, 19-50 years	1300	2118.64	2124.53	2126.96	2127.66	2131.85
Females, >50 years	1500	2444.59	2451.38	2454.19	2454.99	2459.82
Males, >50 years	1700	2770.53	2778.23	2781.41	2782.32	2787.79
Pregnancy	1900	3096.48	3105.08	3108.64	3109.65	3115.78
Lactation	2000	3259.45	3268.51	3272.25	3273.32	3279.76

$$Y = \frac{X * 100g}{Z}$$

Where Y = Recommended amount of red guinea corn and millet composite (g/day)
X = Recommended nutrient intake μg/day
Z = Concentration of pyridoxine at optimal value (μg)

Validation of Statistical Models

Three validation experimental runs were carried out at the chosen optimum conditions to validate the statistical model representing concentration of pyridoxine (μg/100g). The result shows that the maximum Concentration value of 61.34μg/100g for pyridoxine, obtained was close to the predicted values of 61.36μg/100g. This shows validity of statistical models due to the excellent correlation between the predicted and measured values of these experiments.

CONCLUSION

The concentration of pyridoxine, in the composite was influenced at a blending time of 5 hours, 47.45g of red guinea and 100 g of millet. A quadratic statistical model developed to represent concentration of pyridoxine, showed a good fit with the experimental data with R^2 values of 0.99.

The best composite was produced at the optimized conditions of 61.36 µg/100g.The blend produced at the optimized conditions satisfied the World Health Organization (WHO), Food and Agricultural Organization (FAO) specification for recommended safe intake for all age groups, pregnant and nursing mothers.

ACKNOWLEDGEMENT

We express our sincere gratitude to God Almighty for giving us the strength to succeed in the research work. Also acknowledged are our supervisor, families and friends for supporting us during the course of this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

Akhihiero E. T.; Olaseinde A.A; Eyide O. (2022). Effect of Blending Ratio on the Nutritional Value of Millet and Guinea Corn using Mixture Design. Int'l J. Innovative Science and Research Technology, Page: 757-765 http://www.ijisrt.com/

Amenaghawon, NA; Nwaru, KI; Aisien, FA; Ogbeide, SE; Okieimen, CO (2013). Application of Box-Behnken Design for the Optimization of Citric Acid Production from Corn Starch Using Aspergillusniger Br. Biotechnol. J. (3): 236.

Cao, G., Ren, N., Wang, A., Lee, D.-J., Guo, W., Liu, B., … Zhao, Q. (2009). Acid hydrolysis of corn stover for biohydrogen production using Thermoanaerobacterium thermosaccharolyticum W16. International Journal of Hydrogen Energy, 34(17), 7182–7188. https://doi.org/10.1016/j.ijhydene.2009.07.009

Eyide O.; Iweriolor S.; Ossai B.J; Akhihiero E.T; Olaseinde A.A (2023). Optimization of Vitamin A in Red Guinea Corn-Millet Mix. AZOJETE, 19(4):759-770. ISSN 1596-2490; e-ISSN 2545-5818, www.azojete.com.ng

FAO, IFAD, UNICEF, WFP and WHO (2020).The State of Food Security and Nutrition in the World Transforming food systems for affordable healthy diets. Rome, FAO. https://doi.org/10.4060/ca9692en

FAO/WHO. 1988. Requirements of vitamin A, iron, folate and vitamin B12. Report of a Joint FAO/WHO Expert Consultation. Rome, Food and Agriculture Organization

Ikokoh, PP., and Owa, OJ. 2019. Nutritional Evaluation of Some Grains (Millet, Guinea-Corn, Wheat and Sorghum) in Bwari Area Council FCT Abuja. EAS Journal of Nutrition and Food Science; 1(1): 20-23.

Iqbal Ahmad1 ,Tania Mirza, Kiran Qadeer , Urooj Nazim and Faiyaz HM Vaid (2013). Vitamin B6: Deficiency diseases and methods of analysis. Pak. J. Pharm. Sci., Vol.26, No.5, , pp.1057-1069

Keservani, R. K., Sharma, A. K., Ahmad, F., &Baig, M. E. (2014). Nutraceutical and functional food regulations in India. In *Nutraceutical and functional food regulations in the United States and around the world* (pp. 327-342). Academic Press.

Kunyanga, C.N.; Imungi, J.K.; Vellingiri, V (2013). Nutritional evaluation of indigenous foods with potential food-based solution to alleviate hunger and malnutrition in Kenya J. of Appl. Biosci.https://dx.doi.org/10.4314/jab.v67i0.95049

Kutyauripo I; Mutombo P (2020), "Determinants of Production of Sorghum (Sorghum bicolor) and Millet (Eleusine coracana) in Zvimba District of Zimbabwe", International Journal of Scientific Research in Multidisciplinary Studies, Vol.6, Issue.9, pp.37-42, 2020

Montgomery, DC (2005). Design and Analysis of Experiments. 6. ed. New York: John Wiley & Sons, Inc.

Nwokem C.O, Nwokem N.C, Adewuyi J; Rabia M.A. (2019). Quality assessment of baby food produced from cereals enriched with date palm science world journal Vol 14(No 1) ISSN 1597-6343

Ojattah G.U, Oguche S.O (2023). Assessment of Five Macro Elements in Some Cereals Obtained from Ajaka Market in Igalamela/Odolu Local Government Area, Kogi State, Nigeria International Journal of Scientific Research in Multidisciplinary Studies Vol.9, Issue.1, pp.19-24.

Ottaway, P. B. (2008). Principles of food fortification and supplementation. *Food fortification and supplementation: Technological, safety and regulatory aspects*, 1-10.

Poore, J. & Nemecek, T. 2018. Reducing food's environmental impacts through producers and consumers. Science, 360(6392): 987–992.

Springmann, M., Clark, M., Mason-D'Croz, D., Wiebe, K., Bodirsky, B.L., Lassaletta, L., de Vries, W., Vermeulen, S.J., Herrero, M., Carlson, K.M., Jonell, M., Troell, M., DeClerck, F., Gordon, L.J., Zurayk, R., Scarborough, P., Rayner, M., Loken, B., Fanzo, J., Godfray, H.C.J., Tilman, D., Rockström, J. & Willett, W. 2018. Options for keeping the food system within environmental limits. Nature, 562(7728): 519–525.

WHO/FAO, "World Health Organization/Food and Agricultural organization)" The state of Food Insecurity in the World (Report), FAO/UN, Rome, 2007.