



OPTIMIZATION OF VITAMIN A IN RED GUINEA CORN-MILLET MIX

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ABSTRACT

This study focused on the optimization of vitamin A in guinea corn and millet mix. The concentration of vitamin A was investigated under the following conditions: blending time (1.5 - 5 hours), amount of red guinea corn (10 - 50g) and amount of agro residue (50-100 g) using Box-Behnken design. Statistically significant model ($p < 0.05$) was developed to represent the relationship between the response (concentration of vitamin A) and the independent variables. The model showed a significant fit with experimental data with R^2 values of 0.94. Analysis of variance (ANOVA) results showed that the concentration of vitamin A was influenced by the blending time, amount of red guinea corn and amount of millet used. Additionally, response surface methodology (RSM) was used to optimize the concentration of vitamin A. The results showed that maximum concentration of 98.76 $\mu\text{g}/100\text{g}$ for vitamin A was obtained at the optimum production conditions of blending time of 5 hours, 49.79g of red guinea corn and 100g of millet. 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

1.0 Introduction

Nutrition is an integral part of human life, that deals with the study of nutrients which is the relationship between food and health. Nutrients are chemical compounds found in foods used by the body for growth, maintenance and repair. Examples of nutrients are carbohydrates, proteins, fats, vitamins, minerals and water. The right balance diets are vital for human existence, physical growth, mental development, performance and productivity throughout the entire lifetime (Akhiehiero et al., 2022).

One of the 17 goals of Sustainable Development Goals (SDGs) adopted by the United Nations in 2015 is to address global challenges such as hunger, food insecurity, improved nutrition and sustainable agriculture. Goal 2.1 of the SDGs targeted access to affordable, reliable, sustainable and right nutrition for all while target 2.2 has to do with eliminating all forms of malnutrition. In line with this Goal #2, the issue of hunger and malnutrition is of great concern to humans, which is linked to poor feeding habits, the high rate of junk food consumption, the high cost of nutritional foods and negligence of pregnant and nursing mothers which can be addressed via developing value-added products from millets and guinea corn.

The issue of malnutrition may arise from lack of adequate or excess intake of one or more nutrients (Kunyanga et al., 2013), In 2019, two billion (25.9 percent) of the global population,

experienced hunger or did not have daily access to nutritious and enough food. While in Nigeria, malnutrition is a significant public health concern. According to World Food Program in Nigeria, an estimated 22 million people are affected by acute food insecurity and malnutrition, including 2.5 million children under the age of five. Also, malnutrition is estimated to contribute to more than one-third of all child deaths (Younis et al., 2022).

It is noteworthy that food insecurity affects diet quality, such as human diet and people's health. Therefore, child malnutrition remains a global challenge. In 2019, it was reported that 21.3 percent (144.0 million) of children fewer than 5 years of age were estimated to be stunted, 6.9 percent (47.0 million) wasted and 5.6 percent (38.3 million) were overweight, while at least 340 million children suffered from micronutrient deficiencies.

One of the micronutrients of interest in this study is vitamin A (retinol) which is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system, growth, bone development, reproduction, protective effect on the skin, immune function, contributes to the development of normal teeth and hair ((Sabina et al., 2019, D'Ambrosio et al., 2011, Gutierrez-Mazariegos et al., 2011) Vitamin A is a fat soluble micronutrient that is vital for pregnant women and their fetus, they are needed for the maintenance of maternal night vision and fetal ocular health, also for the development of organs and the fetal skeleton and maintenance of the fetal immune system (Sabina et al., 2019; El-Khashab et al., 2013; Sommer et al., 2012,). During the period of pregnancy, there is a high demand for vitamin A, particularly in the third quarter because of the fast development of the fetus (WHO, 2013). According to the WHO report of 2009, in some developing countries, Vitamin A deficiency (VAD) is still addressed as a public health issue affecting approximately 19 million pregnant women. According to Younis et al. (2015), the issue of VAD among age groups in developing countries is linked to poor feeding habits and almost 130 million of all preschool children are suffering from a deficiency of vitamin A (UNSCN., 2005).

The issue of malnutrition can be addressed via a composite blend of cereals such as millet and guinea corn processed into valuable food products (Akhiero et al., 2022). According to Nwokem et al. (2019) report that composite blends from locally available food commodities such as cereals have great potential in providing nutritious foods that can address the issues of malnutrition among age groups, pregnant mothers and nursing mothers in Nigeria in particular, and developing countries in general. In a recent study carried out by Akhiero et al. (2022), revealed that guinea corn and millet can be processed into valuable food products with an additional quantity of the vitamins and minerals needed to boost our immune system. Thus, value-added products produced from cereals are good sources of micronutrients that are needed for body building, they can be used to formulate nutritious and more cost-effective complementary food and drink products that will improve child nutrition, reduce morbidity and mortality rates (Ikokoh et al., 2019).

To the best of our knowledge, none of these studies have attempted to statistically optimize the concentration of vitamin A, in Red guinea corn and Millet. This study, thus, aims to investigate the optimization of vitamin A in red guinea corn-millet mix using the Response Surface Methodology.

2.0 Materials and Methods

2.1 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 Asaba, 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.

2.2 Experimental Design

A three-factor Box-Behnken design for response surface methodology was used to study the combined effect of the blending ratio of the mass of red guinea corn, the mass of millet and blending time on the concentration of Vitamin A. The range and levels of the independent variables are shown in Table I (Eyide et al., 2023).

Table I: Coded and actual levels of the factors for three factors of Box-Behnken design for optimization of vitamin A.

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

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 (Amenaghawon et al., 2013).

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 vitamin A 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).

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

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 . The following generalized second-degree polynomial equation was used to estimate the response of the dependent variable (Amenaghawon et al., 2013).

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

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 vitamin A was evaluated by the coefficient of determination (R^2) and analysis of variance (ANOVA). The grains were weighed separately, then mixed together and finally homogenized to get each blend according to the design of experiment in Table 2.

Table 2: Box Behnken Experimental Design

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

2.3 Analysis of millet, red guinea corn and their blends

The analyses were carried out at the Central Research and Diagnostic Laboratory, Tanke, Ilorin, Kwara State, Nigeria. The proximate analysis was done on the grains of millet and red guinea corn respectively as well as the determination of the concentration of vitamin A for each blend according to the Box Behnken experimental design in Table 2. The concentration of vitamin A was determined using Agilent 6890 Gas Chromatography (GC).

2.4 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 grains of millet and red guinea corn. The percentage of carbohydrates was determined by Eq (4)

$$\text{Carbohydrate (\%)} = 100 - (\text{protein (\%)} + \text{Moisture (\%)} + \text{Ash (\%)} + \text{crude Fibre (\%)} + \text{Fat (\%)}). \quad (4)$$

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

$$(\text{KJ}/100\text{g}) = (\text{Protein} \times 16.7) + (\text{Lipids} \times 37.7) + (\text{Carbohydrate} \times 16.7) \quad (5)$$

2.4 Preparation and analysis of each blend using 6890 GC

Based on the experimental design in Table 2, each blend was converted into a more volatile derivative for analysis. Firstly, mix grains were milled into homogenized sizes. Thereafter the milled grains were placed inside flat-bottomed flasks and were suspended in 20 to 40 ml of water according to the mixture times for the respective experimental runs. Care was taken to ensure that no lumps were formed; the blend must be fully wetted with water. While spinning the flask, 100 ml ethanol, 1g sodium ascorbate or L-ascorbic acid and 25 ml potassium hydroxide solution (60%) were added to the suspended sample. The flat-bottomed flasks were sealed with a glass stopper and shaken vigorously. The reaction mixture is now heated to boiling point under reflux in a water bath that has been preheated to 80 - 90°C and maintained at boiling point for 30 minutes under a slow flow of nitrogen to hydrolyze the ester bonds in the vitamin A. The saponified solutions were transferred quantitatively to a separating funnel through multiple rinses with water. To avoid the formation of emulsions, sufficient water was

added to the saponified sample solution to ensure that the alcohol/water ratio in the resulting solution was about 1:1; thereafter, 150 ml petroleum ether was added and shaken mechanically for about 10 minutes to extract the vitamin A. The extraction solution is now washed neutrally with purified water through multiple shaking and the washing water is allowed to drain away after the phases have separated. The extraction solution was filtered through a phase separation filter into a 50 ml volumetric flask to remove any potential suspended water drops. Finally, the extracted vitamin A was derivatized at 70 - 80°C for 2 hours to form a more volatile derivative for GC analysis. After derivatization, the volatile derivative of vitamin A, was analyzed using Agilent 6890 gas chromatography (GC) with a suitable stationary phase and detector.

3. Results and Discussion

3.1 Proximate Analysis Results

Table 3 shows that millet has a moisture content of 20.04% which is slightly higher than that of red guinea corn, 17.28%. Similarly, the ash content of 2.07% observed with red guinea corn was higher when compared to 1.38% observed in millet. Red guinea corn has 55.23% of carbohydrates while millet has 49.54%.

Table 3: Proximate Composition of Red Guinea corn and Millet grain samples

Proximate Parameter	Samples	
	Red Guinea Corn (A)	Millet (B)
Moisture %	17.28	20.04
Ash %	2.07	1.38
CHO%	55.23	49.54
Crude Fibre %	1.48	2.44
Crude Lipids%	14.59	17.84
Crude Protein %	9.34	8.76
Calorific Value kJ/100g	1628.62	1646.02

A calorific value of 1646.02kJ/100g was observed in millet which was higher than 1628.62kJ/100g in red guinea corn. 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%.

Figure 1 is a chromatogram showing the different peak identification for the vitamers present in red guinea corn. The major vitamers present are retinol and dihydroretinol with highest peaks identified for retinol and dihydroretinol at retention time of 1.798mins and 2.573mins with a corresponding voltage values of 13mv and 2mv respectively. The other vitamers were unidentified by the GC analyzer because they are present in minute amount.

Sample: A
 Date:2021-02-23, 3:38:10 PM
 Data File:c:\N2000\data\VITAMIN A0003
 Method File:c:\N2000\Chanel10.mtd

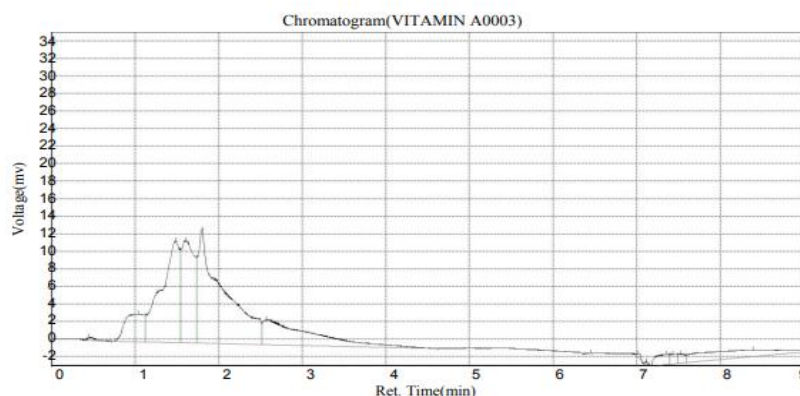


Figure 1: Chromatogram for sample A (red guinea corn)

Figure 2 is a chromatogram showing the different peak identification for the vitamins present in millet. The major vitamins present are retinol and dihydroretinol, with highest peaks identified for retinol and dihydroretinol at retention time of 1.648mins and 2.032mins with a corresponding voltage value of 12mv and 7mv respectively. The other vitamins were unidentified by the GC analyzer because they are present in minute amount.

Sample: B
 Date:2021-02-23, 3:55:28 PM
 Data File:c:\N2000\data\VITAMIN A0004
 Method File:c:\N2000\Chanel10.mtd

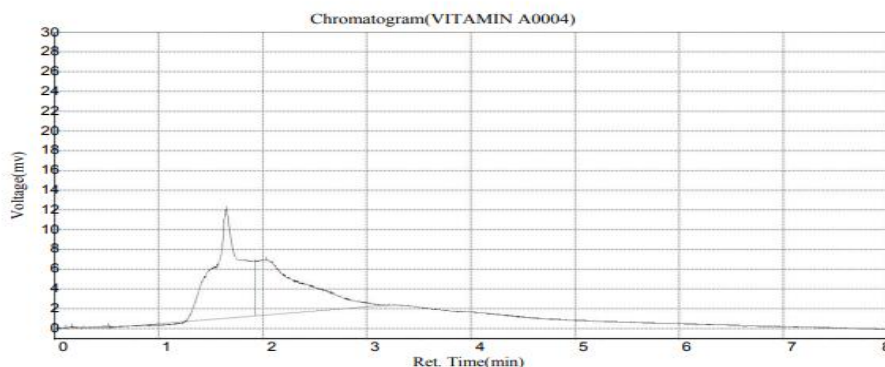


Figure 2: Chromatogram for sample B (millet)

Table 4: Concentration of vitamin A vitamins present in sample A (red guinea corn)

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100g
1	Unidentified	0.440	373.054	2378.150	0.2838
2	Unidentified	1.040	3125.500	50568.363	6.0355
3	Unidentified	1.482	11540.948	180443.406	21.5366
4	Unidentified	1.607	11634.680	124055.344	14.8065
5	Retinol	1.798	12931.667	277205.438	33.0855
6	Dihydroretinol	2.573	2820.397	115160.055	13.7448
7	Unidentified	6.448	30.727	286.050	0.0341
8	Unidentified	7.015	379.923	1361.050	0.1624
9	Unidentified	7.115	331.500	841.100	0.1004
10	Unidentified	7.357	1170.514	13425.724	1.6024
11	Unidentified	7.432	1137.589	6626.280	0.7909
12	Unidentified	7.532	1066.687	6043.773	0.7213
13	Unidentified	8.390	729.868	59449.668	7.0955

Table 5: Concentration of vitamin A vitamers present in sample B (millet)

Peak No.	Peak ID	Ret Time	Height	Area	Conc µg/100g
1	Unidentified	0.098	52.923	200.490	0.0523
2	Unidentified	0.165	106.846	509.710	0.1331
3	Unidentified	0.515	122.387	1044.000	0.2725
4	Retinol	1.648	10966.510	202220.953	52.7872
5	Dihydroretinol	2.032	5538.132	179112.234	46.7549

From Tables 4 and 5, it can be depicted that sample B (millet) has a higher amount of retinol and dihydroretinol of 52.7872 (µg/100g) and 46.7549 (µg/100g) compared to red guinea corn with 33.0855 (µg/100g) of retinol and 13.7448 (µg/100g) of dihydroretinol respectively. Due to high Concentration of A vitamers present in millet than red guinea corn, we decided to used 1:2 of red guinea corn and millet in our design of experiment as shown in Table 2, in other to optimized the concentration of vitamin A in the cereals blends.

3.2 Statistical model equation for the concentration of vitamin A

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. These second-degree polynomial equations were used to estimate the response (concentration of Vitamin A).

$$Y_1 = +82.34 + 9.56X_1 + 9.47 X_2 + 0.75 X_3 + 5.45X_1X_2 - 0.75 X_1X_3 + 0.75 X_2X_3 - 11.20 X_1^2 - 1.22 X_2^2 + 3.56X_3^2 \quad (6)$$

Where, Y_1 = predicted responses for the concentration of Vitamin A (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.

Results from the laboratory for the 17 experimental runs where inputted and tally according to the runs in the Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA) software, thereafter the data were analyzed to generate the statistical model equation and anova for the concentration of vitamin A. The inputted values are referred to as the actual values while the values gotten from the model equation are referred to as predicted values.

Table 6: Box Behnken Design Matrix for the optimization variables and response values of concentration of vitamin A (µg/100g).

The values of the concentration of vitamin A (µg/100g), as predicted by model Equation (6), were shown in Tables 6, alongside with their experimental. It was observed from the data in the table 6, that there is correlation between the actual and predicted values.

Run No	Variables						Response	
	Coded levels			Actual values			Vitamin A (µg/100g)	
	X1	X2	X3	X1	X2	X3	Actual	Predicted
1	0	0	0	30	75	3.25	84.34	82.34
2	0	1	1	30	100	5.00	92.32	94.9
3	1	1	0	50	100	3.25	96.21	94.4
4	0	0	0	30	75	3.25	84.34	82.34
5	-1	0	1	10	75	5.00	65.12	66.64
6	-1	-1	0	10	50	3.25	54.53	56.34
7	0	0	0	30	75	3.25	79.34	82.34
8	1	0	-1	50	75	1.50	85.78	85.76

9	0	-1	-1	30	50	1.50	78.53	75.21
10	-1	0	-1	10	75	1.50	62.12	64.39
11	0	-1	1	30	50	5.00	78.53	75.21
12	0	0	0	30	75	3.25	84.33	82.34
13	0	1	-1	30	100	1.50	89.32	85.53
14	1	0	1	50	75	5.00	85.78	84.26
15	1	-1	0	50	50	3.25	59.71	64.56
16	-1	1	0	10	100	3.25	69.23	64.38
17	0	0	0	30	75	3.25	79.36	82.34

Table 7: ANOVA for a model representing the concentration of vitamin A ($\mu\text{g}/100\text{g}$). ANOVA result depicted that the model for the concentration of vitamin A ($\mu\text{g}/100\text{g}$) was statistically significant with p values of 0.0016, as shown in Table 7. The model did not show a lack of fit as seen from the “lack of fit” p values (0.0822). For the model, the terms representing the mass of red guinea corn, mass of millet and blending time were significant for response (the concentration of vitamin A ($\mu\text{g}/100\text{g}$)).

Sources	Sum of Squares	Df	Mean Squares	F value	p-value [Prob>F]
Model	2153.40	9	239.27	12.24	0.0016
X_1	731.15	1	731.15	37.40	0.0005
X_2	717.83	1	717.83	36.71	0.0005
X_3	4.50	1	4.50	0.23	0.6460
X_1X_2	118.81	1	118.81	6.08	0.0431
X_1X_3	2.25	1	2.25	0.12	0.7444
X_2X_3	2.25	1	2.25	0.12	0.7444
X_1^2	527.93	1	527.93	27.00	0.0013
X_2^2	6.29	1	6.29	0.32	0.5882
X_3^2	53.29	1	53.29	2.73	0.1427
Residual	136.86	7	19.55		
Lack of Fit	107.06	3	35.69	4.79	0.0822
Pure Error	29.80	4	7.45		
Cor Total	2290.27	16			

Table 8: Statistical information for ANOVA concentration of Vitamin A ($\mu\text{g}/100\text{g}$), in the mix. Statistical information for ANOVA shows that the model describing the concentration of vitamin A ($\mu\text{g}/100\text{g}$) had a high coefficient of determination (R^2) of 0.94 as shown in Table 8. This shows that the model was able to adequately represented the relationship between the chosen factors (mass of red guinea corn, mass of millet and blending time) and response (concentration of vitamin A). R^2 values of 0.94 means that the model was able to account for 94.02% of the variability observed in the values of concentration of vitamin A. The standard deviation was observed to be relatively small compared to the mean. The coefficient of variation of 5.65 was obtained for the model. This parameter shows the degree of precision with which the runs were carried out. The values obtained show high reliability as recommended by (Montgomer, 2005). The Adequate precision for the model signifies adequate signal meaning that the model can be used to navigate the design space (Cao, 2009).

Parameter	Response Concentration of Vitamin A ($\mu\text{g}/100\text{g}$).
R-Squared	0.94
Mean	78.17
Standard Deviation	4.42
C.V%	5.66
Adeq. Precision	11.59
Adjusted R-Square	0.86

3.3 Optimization of concentration of Vitamin A ($\mu\text{g}/100\text{g}$) 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 vitamin A. Figure 3, shows the effect of blending ratios of the mass of red guinea corn and millet on the concentration of vitamin A. The trend observed shows that the concentration of vitamin A, increased with an increase in the mass of red guinea corn and mass of millet, this is due to the resultant amount of the vitamers (retinol and dihydroretinol) present in the respective cereals, with millet having the highest contribution of vitamers concentration to the blend as shown in tables 4 and 5. Similar trends were observed in Figure 4 and Figure 5, 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 Vitamin A which is due to the interacting effect during homogenization between the mass of red guinea and millet.

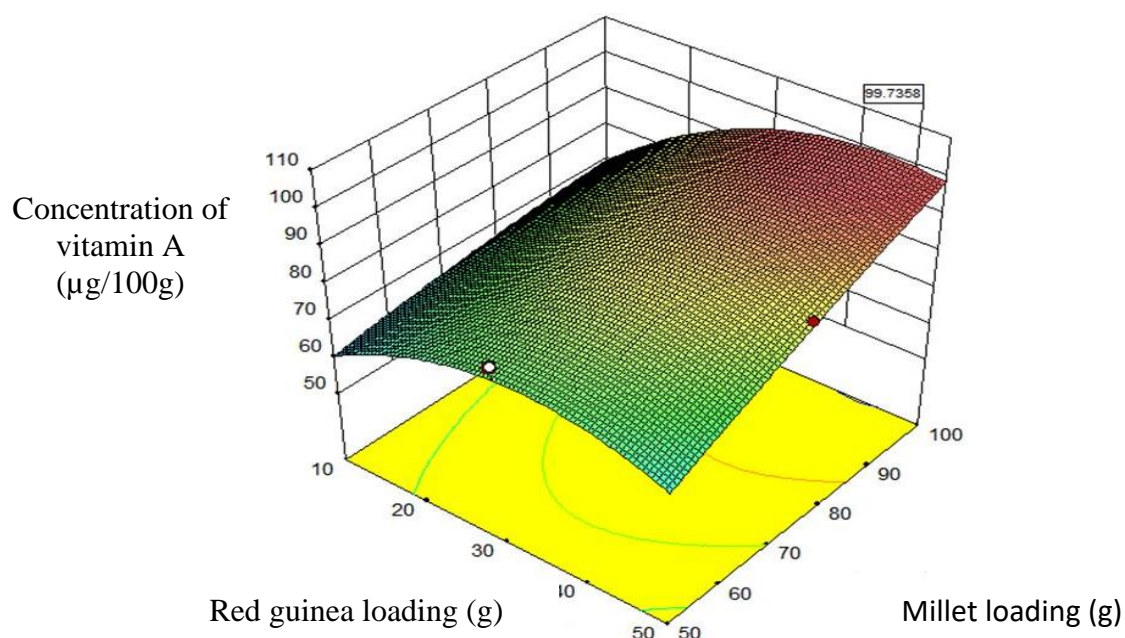
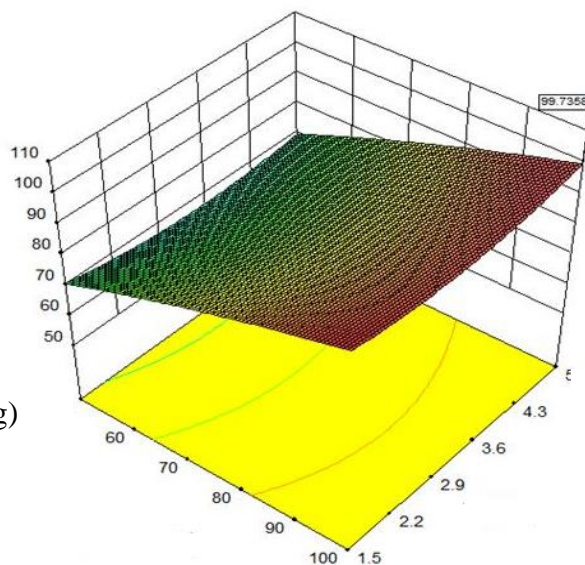


Figure 3. Effect of amount of red guinea corn and millet on concentration of vitamin A.

Concentration of vitamin A ($\mu\text{g}/100\text{g}$)

Red guinea loading (g)



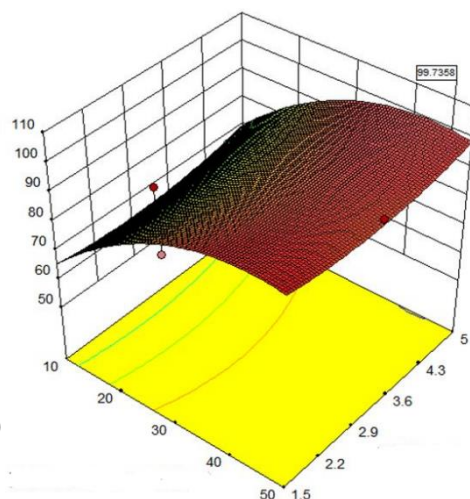
Blending time (hours)

Figure 4. Effect of guinea corn and on concentration of

amount of red blending time vitamin A.

Concentration of vitamin A ($\mu\text{g}/100\text{g}$)

Millet loading (g)



Blending time (hours)

Figure 5. Effect of amount of millet and blending time on concentration of vitamin A.

The optimum levels of the independent factors and the response (Concentration of Vitamin A) were determined from numerical optimisation of the statistical model (Eq 6) and the top five results were obtained as shown in Table 9. The best optimal result of 98.79 ($\mu\text{g}/100\text{g}$) of Vitamin A, was obtained at a blending time of 5.00 hours, 49.79g of red guinea corn and 100g of millet.

Table 9: Solutions for optimum conditions for concentration of vitamin A

Solution Number	Red guinea corn (g)	Millet(g)	Blending Time(hours)	Concentration of Vitamin A ($\mu\text{g}/100\text{g}$)
1	49.79	100.00	5.00	98.79
2	50.00	100.00	5.00	98.71
3	50.00	100.00	4.99	98.64
4	50.00	99.77	5.00	98.59
5	50.00	100.00	4.96	98.51

3.4 Validation of Statistical Models

Three validation experimental runs were performed at the chosen optimum conditions to validate the statistical model representing concentration of Vitamin A ($\mu\text{g}/100\text{g}$). The result shows that the maximum Concentration value of $98.76 \mu\text{g}/100\text{g}$ for vitamin A, obtained was close to the predicted values of $98.79 \mu\text{g}/100\text{g}$. The excellent correlation between the predicted and measured values of these experiments shows the validity of statistical models.

4. Conclusions

The concentration of vitamin A, in the blends were influenced at a blending time of 5 hours, 49.79g of red guinea and 100 g of millet. A quadratic statistical model developed to represent concentration of Vitamin A, showed a good fit with the experimental data with R^2 values of 0.94. The best blend was produced at the optimized value of $98.79 \mu\text{g}/100\text{g}$ which conforms with World Health Organization (WHO)/Food and Agricultural Organization (FAO), specification for recommended safe intake for all age groups, pregnant and nursing mothers.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise

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