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Application of Response Surface Methodology (RSM) and Central Composite Design (CCD) to Optimize Minerals Composition of Rice-Cowpea Composite Blends during Extrusion Cooking

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Abstract Significant loss of nutrients has been reported during extrusion cooking, and processing food with this technology therefore requires careful optimization of the process parameters. In this experiment, the effect of process variables on the mineral content of rice-cowpea extrudates was investigated using response surface methodology and central composite design. Barrel temperature (X_1), feed moisture composition (X_2) and blend composition (X_3) were the independent variables considered, while mineral composition was the response variables. Results showed that X_1, X_2 and X_3 all had a significant effect on the mineral composition ($p< 0.001$). The interactions between the threefactors were also found to be significant at 0.001 level of probability. At X_1 between 86 - 140°C, X_2 between 15 - 25% and X_3 between 8 - 24% the mineral contents in terms of Mn, Fe, Cu, Zn and Ca contents of rice-cowpea extrudates increased. The optimization of the analyzed responses demonstrated that the best peak conditions for extrusion under the different variables were 12.06mg/100g, 5.59mg/100g, 10.98mg/100g, 2.36mg/100g, 4.24mg/100g, and 25.99mg/100g for Mg, Mn, Fe, Cu, Zn and Ca respectively. At moisture content slightly above 22% and blend composition of 20%, Mg content start to decrease. Calcium and Cu contents decrease with increasing moisture and feed blend contents and gradually increases when moisture content raise above 20% and feed blend composition greater than 10%. The correlation coefficients of 0.992, 0.987, 0.969, 0.866, 0.974 and 0.980 observed between the predicted and actual values for the response variables are evidence that the regression model can represent the experimental data well. It can be concluded therefore that minerals present in the extrudates may be maximized when process conditions are carefully adjusted within the reported values.

Keywords Rice, Cowpea, Mineral content, ANOVA, Response Surface Methodology

1. Introduction

In Nigeria and indeed most countries of sub-Saharan Africa, frequent conflicts, poverty, poor agricultural practices, productivity and climate changes has caused food shortage and most vulnerable populations survive majorly on sole starchy staples such as rice, maize, sorghum, millet, cassava, potatoes and cowpea with little or no animal products to supply protein required for normal growth and development. These problems are further aggravated by the menace of HIV/AIDS epidemic that increased the number of vulnerable populations. Cereals processed using technology that minimize nutrient loss and combination of cereals with local legumes has been reported as one of the vehicle for delivering protein and minerals to at-risk populations because of their wide spread in consumption, stability during storage and versatility in the production of arrays of food products [20]. The production of cereals-legume based products to supply additional protein and minerals to the daily diet of the vulnerable groups of the population has increased significantly over the years. Such products include nutritionally enhanced biscuits, breads, cakes, porridges and extruded snacks. Extrusion cooking technology has played a central role in modern cereal-based industries especially for the production of snacks from wheat, corn, oats and rice [34]. Nevertheless, fewer rice-based extruded products are available in the market compared to those from corn and wheat. But, rice flour has become an attractive ingredient in the production of extruded products due to its bland taste, attractive white colour, hypoallergenicity, ease of digestion

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and the ability to expand well and make excellent extrudate [21, 23, 34]. The bland flavour of rice therefore makes it favourable for preserving more expensive flavour attributes. Though, reliable statistics are not available for Nigeria and other developing countries, data from United State of America indicated that there is increasing interest in gluten-free food products as while as the number of people having celiac disease grows and society are becoming more informed about health implications of gluten-free products [26].

Food minerals are solid, crystalline chemical elements that cannot be decomposed or synthesized by ordinary chemical reactions. They are classified as macro- and microminerals. The macro minerals include calcium, phosphorus, sodium, potassium and chloride, and of these, calcium and phosphorus are needed in large quantity. The microminerals include magnesium, manganese, zinc, iron, copper, molybdenum, selenium, iodine, cobalt and chromium which are required by the body in minute quantity for normal metabolic activities. Although minerals represent a small portion of food composition, they play major roles in food chemistry and nutrition [22]. Minerals such as iron and calcium are added to foods for improvement of its nutritional value [19]. Elements such as iron (Fe), copper (Cu), magnesium (Mg) and calcium (Ca) act as catalyst for enzymes during normal metabolic processes, while Fe is essential for the prevention of anaemia, Ca is required for bone health [19]. But despite the huge importance of minerals in human health, and wide application of extrusion cooking in food processing, relatively few studies has examine mineral stability during extrusion cooking, probably because they are stable in other food processing techniques [19] [22]. Minerals are heat stable and are unlikely to become lost in steam distillate at the die end [22]. But Alonso *et al*., [32] reported that iron content of flour increased after extrusion, while Singh *et al*., [22] reported that when wheat bran is incorporated into a broken rice flour in extrusion (300 rpm screw speed, 27kg/h feed rate, 5/32 inches die size, 93-97 ℃ outlet temperature) there was increase in the calcium, phosphorus, iron and copper contents, which they attributed to type of feed composition and the water used during the extrusion exercise. They recommend further research in this area, particularly if extrudate foods are produced as vehicle for mineral fortification.

During extrusion, variation in feed composition such as moisture content, type of carbohydrate, and protein contents and extruder conditions directly influence qualities of finished products [24, 27]. In order to obtain good quality extruded product, the multivariate inputs must be set at the correct levels to allow the dependent physical and chemical changes within the barrel of the machine to continue at a steady state. Once the relationships between the independent and dependent variables are established for a particular product, they must be maintained close to optimum levels to ensure that the extrudate variables are also kept at required level [24].

Response surface methodology (RSM) has been used widely for modelling extrusion cooking processes [1, 4, 19]. It is a collection of mathematical and statistical techniques useful for analysing and optimizing the response of multivariate systems [10]. Perez *et al* [35] used RSM to study the effects of extrusion variables on extrudate quality, adjusting grit moisture content (14-18%) and extrusion temperature between 155^{o}C and 185^{o}C. Pansawate *et al*., [28] studied the effect of extrusion variables on secondary extrusion variables of fish, rice-based snacks considering primary extrusion variables such as barrel temperature of 125 to 145^{o}C, screw speed of 150-300rpm and feed moisture content of 19-23%. Giri and Bandyopadhyay [29] reported on the effect of extrusion factors on extrudate qualities of fish-rice flour blend in a single screw extruder. In the extrusion of blends of flour made from starch and protein rich materials. While [31, 32] earlier reported the optimization of extrusion variables and the application of RSM in the manufacture of *Fura* a traditional cereal-based porridge of northern Nigeria, blending millet with cowpea, millet and soybean and millet mixed with Bambara groundnut [31, 32].

It is in line with these assertions and the need to improve the competiveness of low grade broken rice fractions that the current study was designed to assess the effect of twin-screw extruder extrusion parameters (barrel temperature -100 to140^{o}C, feed moisture content – 15 to 25% and feed blend composition – 8 to 24%) on the mineral compositions of rice-cowpea based extruded instant porridge were examined using RSM and central composite design (CCD) to optimize the process for maximum mineral retention as vehicle for fortification and value addition to rice based extrudates.

2. Materials and Methods

2.1. Raw Materials Collection and Preparation

The raw materials included rice (*Oryzasativa* L.) and Cowpea (*Vignaunguiculata*). About 100kg of rice (FARO 52) was obtained from the Breeding Program of National Cereals Research Institute (NCRI) Badeggi, Nigeria, and 40kg of Cowpea (*Vignaunguiculata*) was purchased from Central Market, Bida, Niger State, Nigeria and were manually cleaned and kept in a dry condition (30±2^{o}C) until required. For the preparation of rice flour, samples were cleaned in a pneumatic cleaner (locally fabricated) and milled using rubber roll mill (Satake, Japan) and graded, the broken rice fraction was then used for this study. The broken fractions were milled to flour in a small scale disc mill (locally fabricated) and sieved using 150µm screen size sieve. Cowpea seeds were soaked in tap water at ambient temperature (30±2^{o}C) for 30 min to loosen seed coat in stainless steel buckets and decorticated using pestle and mortar before washing several times in clean tap water and drying under the sun for 4hrs (30±2^{o}C) to approximately 14% moisture content. The decorticated dried seeds were mill in a small scale disc mill (locally fabricated) and sieved using 150μm screen size sieve. Both rice and cowpea flours were packaged in a polyethylene bags and stored in a cardboard at ambient conditions (32 ± 2°C) until required for analysis.

2.2. Composite Flour Formulation

Rice and cowpea flours were mixed at defined ratios (2.55%, 8%, 16%, 24% and 29.45%, wet-wet bases). The moisture content of flour of different ratios was measured by hot air oven method. After getting the initial moisture content of the blends (M_1), the blended samples were conditioned to appropriate moisture content by spraying with a calculated amount of water and mixing continuously at medium speed in a blender. The samples were put in closed buckets and stored overnight. The amount of water to be added was calculated using the equation proposed by Ascheri [18]

$$W_W = W_d \text{ x } \frac{(M_2-M_1)}{(1-M_1)(1-M_2)} \quad (1)$$

Where W_w is the amount of water to be added, W_d is dry weight of the raw flour, M_1 is initial moisture content and M_2 the desired moisture content in grams.

2.3. Preliminary Extrusion Exercise

In order to define the experimental range, preliminary experiments were first. As the design ranges were established, they were coded to lie at ±1α for the factorial points, 0 for the centre points and ±1α for axial points. The codes were calculated as a function of the range of interest of each factor as shown in Table 1. In this study, a small scale laboratory twin-screw extruder with three zones (feeding, cooking and die zones) equipped with a screw feeder and a 3mm die was used to extrude the different formulations. Based on preliminary runs, the feeding, cooking and die zones were set at 100, 120 and 140°C respectively. Other extruder parameters were screw speed 260rpm, and feeder screw speed (150rpm). When the twin screw extruder output are at steady state extrusion conditions, samples were collected and dried overnight in oven at 80°C. These samples were removed from oven and stored in a desiccator for further analysis.

2.4. Extrusion Experimental Design

Considering the extruder limitations, a central composite design was used for this study, based on five-levels of the three variables (Table 2). The independent extrusion variables considered were barrel temperature (100 – 140°C), feed moisture content (15 to 25%) and blend composition (8 to 24%). All other parameters were kept constant. The operating ranges and five standardized levels were established after several preliminary runs as described above. Based on CCD, the experimental runs comprises of 15 trials (8 factorial points, 6 axial points and 1 central point).All treatments were performed in a randomized order. RSM and second-order CCD for three-variables (Barrel temperature X_1, Feed moisture content X_2, and Feed composition X_3), five level combinations coded -1.68, -1, 0, +1, and +1.68 (Table 2) as modelled by Snedecor and Cochran [15] was adopted to determine the effects of the independent variables on response variables. Using the coded levels, the natural levels were calculated and outlined as in Table 3, comprising of 15 experimental runs and different formulation composition.

Table 1. Relationship between the coded and un-coded values of the independent variables

Code	Actual value of independent variable
-α	X_{min}
-1	$\frac{(\alpha-1)Xmax + (\alpha+1)Xmin}{2}$
0	$\frac{Xmax + Xmin}{2}$
+1	$\frac{(\alpha-1)Xmin + (\alpha+1)Xmax}{2}$
+α	X_{max}

X_{max} and X_{min} are maximum and minimum values of the independent variables

Table 2. Independent Variables and natural levels used for Central Composite Rotatable Design

Independent variables	Levels of coded variables				
	-α	Low	Medium	High	+α
	-1.68	-1	0	1	+1.68
Barrel Temperature (X_1)	86.36	100	120	140	153.64
Feed Moisture content (X_2)	11.59	15	20	25	28.41
Feed Composition (X_3)	2.55	8	16	24	29.45

Level of each variable was established based on a preliminary extrusion. The distance of the axial points from the centre point was ± 1.68, and calculated from Equation $\alpha = (2^n)^{1/4}$ where n is the number of variables.

Table 3. Outline of experimental design with coded and un-coded values

Design point	Independent variables in coded form			Independent variables in their natural form		
	X_1	X_2	X_3	X_1	X_2	X_3
1	-1	-1	-1	100	15	8
2	1	-1	-1	140	15	8
3	-1	1	-1	100	25	8
4	1	1	-1	140	25	8
5	-1	-1	1	100	15	24
6	1	-1	1	140	15	24
7	-1	1	1	100	25	24
8	1	1	1	140	25	24
9	-1.68	0	0	86.36	20	16
10	1.68	0	0	153.64	20	16
11	0	-1.68	0	120	11.59	16
12	0	1.68	0	120	28.41	16
13	0	0	-1.68	120	20	2.55
14	0	0	1.68	120	20	29.45
15	0	0	0	120	20	16

X_1 = Barrel temperature, X_2 = Feed moisture content, X_3 = Feed composition. Duplicate runs were carried out all design point and average recorded. The experimental runs were randomized.

2.5. Chemical Analysis

The moisture content was determined by taking 2g of each sample and placed in petri dishes and heated in an oven at 105°C for 2h, after which it was removed and cooled in a glass jar containing silica gel desiccants for about 15min. Weight of petri dish containing the cooled samples was measured using a digital weight balance (ED 2201-CW, Sartorius, Berlin, Germany). The samples were further dried for 30min and reweighed until a constant weight was observed. Moisture content was calculated as:

$$\%\text{Moisture content (dry basis)} = \frac{\text{Initial weight} - \text{Final weight (g)}}{\text{Initial weight (g)}}\ x\ 100 \qquad (2)$$

Mineral contents of exrudate were determined by Atomic Absorption Spectrophotometric [Iron (Fe), Zinc (Zn), Calcium (Ca), Manganese (Mn) and Magnesium (Mg)] according to AOAC [17].

2.6. Statistical and Mathematical Analysis

To determine if there exist a relationship between the independent variables and the dependent variables, the data collected were subjected to regression analysis using response surface regression procedure of MINITAB 14.13. Regression analysis is used to model a response factor (Y_i) as a mathematical function of a few continuous factors. Each response (Y_i) was represented by a mathematical equation that correlates the response surfaces. The response was then expressed as second-order polynomial equation according to equation 3.

$$Y_i = f(y) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{i=1}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (3)$$

Where Y_i is the predicted response used to relate to the independent variable, k is the number of independent variables (factors) X_i (i = 1, 2, 3); while β is a constant coefficient and β_i, β_{ij} and β_{ii} the coefficient of linear, interaction and square terms respectively and ε is the random error term. Multivariate regression analysis with model equation (3) was carried out on the data using MINITAB 14.13 statistical software (Manitab Inc. USA) to yield equation (4) which was used to optimize the product responses.

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X^2{}_1 + \beta_{22} X^2{}_2 + \beta_{33} X^2{}_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \qquad (4)$$

The model developed for each determination was then examined for significance and lack-of-fit, while response surface plot was designed after removal of the non-significant terms with the same software.

2.7. Test for Significance of the Regression Model

This test was performed as an analysis of variance (ANOVA) by calculating the F-ratio, which is the ratio between the regression mean square and the mean square error. The F-ratio, also called the variance ratio, is the ratio of variance due to the effect of a factor (in this case the model) and variance due to the error term. This ratio is used to measure the significance of the model under investigation with respect to the variance of all the terms included in the error term at the desired significance level, α. A significant model is desired [11].

2.8. Test for Significance on Individual Model Coefficients

Additionally, checks were carried out in order to determine whether the model actually describes the experimental data [16]. The checks performed here include determining the various coefficient of determination, R^2. These R^2 coefficients have values between 0 and 1. In addition to the above, the adequacy of the model was also investigated by the examination of residuals [10]. The residuals are the difference between the respective, observed responses and the predicted responses examined using the normal probability plots of the residuals and the plots of the residuals versus the predicted response. If the model is adequate, the points on the normal probability plots of the residuals should form a straight line. On the other hand the plots of the residuals versus the predicted response should not depict a pattern, that is, the residual graph should contain no obvious patterns.

2.9. Test of Lack-of-fit

Since triplicate measurements were made during analysis of the dependent variables, a lack-of-fit test examines the significance of replicate error in comparison to the model dependent error. This test split the residual or error sum of squares into two parts, one due to pure error as a result of duplicate measurement and the second due to lack-of-fit which is the ratio between the lack-of-fit mean square and pure error mean square. The *F*-test can then me used to measure wither the lack-of-fit is statistically significant or not at the described level of probability.

3. Results and Discussions

3.1. Central Composite Design and Model Building

In regression analysis, model building is the process of developing a probabilistic model that best describes the relationship between the dependent and independent variables. When multivariate biological process therefore satisfies the assumptions that they are measurable, continuous, and controllable by designed experiments and with no statistically significant errors, the RSM using CCD could be applied to develop the model and also optimize the process by first performing series of experimental runs to adequately and reliably measure the response variables before developing mathematical model of the second-order response surface with best-of-fit, and finally determine the optimal set of experimental parameters producing the optimal response value [30]. In this study, effects of barrel temperature (X_1), feed moisture content (X_2) and feed blend composition (X_3) and their interactions each at three levels on the mineral contents of rice-cowpea extrudates were investigated (Table 2). The three independent variables and their different coded and actual values used in the design matrix are presented in Table 2. Fifteen experiments based on CCD were therefore carried out with different combinations of the variables (Table 3). The number of experiment required (N) is given by the expression 2^k (23 = 8, star points) + 2 x k (2 x 3 = 6, axial points) + 6 centre points(6 replications) [7]. Observed response data (in triplicates) from the experimental runs (Table 5) were used to develop models (Table 4) using least square technique as described by [30]. The six (6) response variables (Mg, Mn, Fe, Cu, Zn and Ca) were correlated with the independent variables using the second-order polynomial as represented by equation (4). HereX_1, X_2 and X_3 represent the barrel temperature (BRT), feed moisture composition (FMC) and feed blend composition (FBC) respectively. The coefficients with one factor (X_1,X_2, and X_3) represent the sole effects of that particular factor, while the coefficients with two factors (X_1X_2, X_1X_3, and X_2X_3) and those with second-order terms (X_{11}, X_{22} and X_{33}) represent the interaction between the three factors and the squared effects respectively. A positive value of the regression terms indicates a synergistic effect, while negative sign indicates an antagonistic effect [30]. The regression equation (Y_i) demonstrated that mineral retention during extrusion of rice-cowpea composite flour was an empirical function of test variables in coded units, as shown in Equations in Table 4. The mean observed and predicted values of the response variables are presented in Table 5. The significant changes in Fe, Cu, Zn and Ca in relation to changes in the independent variables ($X_1X_2X_3$) are represented in Table 4. The coefficients in the regression equation can be used to examine the significance of each term relative to each other when used with coded values.

Statistical analysis showed that barrel temperature, feed moisture and feed composition all had a significant effect on the mineral values ($p< 0.001$). The interactions between the three factors were also found significant at 0.001 level of probability. The Fe content of extrudate as indicated in these equations (Table 4) and Table 5 are in agreement with earlier research results reported by Alonso *et al.* [32] who reported that significant change in Fe content of pea and kidney bean seed extrudates and attributed this variation to wearing of metallic pieces, mainly screws of the extruder. Maintaining barrel temperature between 86 and 140°C and feed composition between 15 and 25% moisture content and 8 to 24% cowpea increases Mn, Fe, Cu, Zn and Ca contents of rice-cowpea extrudatesin this study (Table 5). Singh *et al.* [22] reported similar trend. This might be attributed to the addition of these minerals through water used for extrusion and also extruder barrel. Processing variables like feed moisture content and blend ratio has also been earlier reported as playing important role on the quality of extrudates.

Table 4. Second-order quadratic models developed for the response variables

Response variables	Second-order quadratic models	Regression coefficients (%)	
		R^2	$R^2_{adj.}$
Mg	$83.44+ 1.13X_1 + 3.62X_2 – 0.15X_3 – 0.01X_1^2 – 0.07X^2_2 – 0.01X^3_3 - 0.01X_1X_2 + 0.004X_1X_3 + 0.004X_2X_3$	99.0	97.1
Mn	$9.31– 0.16X_1 + 0.27X_2 + 0.39X_3 + 0.001X_1^2 -0.007X^2_2 – 0.003X^3_3 + 0.001X_1X_2 + 0.004X_1X_3 + 0.007X_2X_3$	99.1	98.8
Fe	$11.08+ 0.04X_1 – 0.33X_2 + 0.39X_3 – 0.001X_1^2 – 0.004X^2_2 – 0.002X^3_3 + 0.005X_1X_2 – 0.002X_1X_3 -0.005X_2X_3$	97.0	95.6
Cu	$4.07+ 0.02X_1 - 0.31X_2 - 0.08X_3 – 0.002X_1^2 + 0.005X^2_2 + 0.003X^3_3 + 0.001X_1X_2 + 0.0001X_1X_3 – 0.002X_2X_3$	85.4	78.9
Zn	$14.99– 0.13X_1 – 0.23X_2 + 0.10X_3 + 0.001X_1^2 + 0.005X^2_2 – 0.002_3X^3_3 -0.002X_1X_2 – 0.003X_1X_3 + 0.014X_2X_3$	97.0	95.6
Ca	$79.15– 0.17X_1 – 1.17X_2 – 0.05X_3 + 0.005X_1^2 + 0.07X^2_2 + 0.33X^3_3 – 0.02X_1X_2 – 0.012X_1X_3 + 0.029X_2X_3$	98.1	97.3

X_1 = Barrel temperature, X_2 = Feed moisture content, X_3 = Feed composition

Table 5. Mean mineral composition of composite blend of rice-cowpea flour extrudates

Design point	Independent variables in coded forms			Independent variables in their natural units			Observed and predicted mean mineral contents											
	X_1	X_2	X_3	X_1	X_2	X_3	Mg		Mn		Fe		Cu		Zn		Ca	
							OBS	PRD	OBS	PRD	OBS	PRD	OBS	PRD	OBS	PRD	OBS	PRD
1	-1	-1	-1	100	15	8	13.78	13.91	4.31	4.30	12.11	12.04	2.33	2.12	5.29	5.36	26.81	26.35
2	1	-1	-1	140	15	8	11.50	11.32	4.89	4.63	10.62	10.77	2.11	2.13	6.14	6.05	31.11	31.20
3	-1	1	-1	100	25	8	16.89	16.43	4.23	4.33	11.27	11.44	2.21	2.15	4.21	4.35	28.92	29.02
4	1	1	-1	140	25	8	11.76	11.87	4.51	4.41	12.13	12.10	2.86	2.69	4.13	4.26	28.11	27.50
5	-1	-1	1	100	15	24	13.22	13.03	4.73	4.80	13.34	13.44	2.14	2.11	5.12	5.05	28.03	28.09
6	1	-1	1	140	15	24	12.46	12.80	2.89	2.73	11.27	11.14	2.33	2.22	4.05	3.96	25.71	25.07
7	-1	1	1	100	25	24	16.09	16.18	6.08	5.94	12.09	11.97	2.09	1.86	6.21	6.36	36.08	35.42
8	1	1	1	140	25	24	14.20	13.98	3.67	3.63	11.47	11.61	2.48	2.49	4.51	4.48	26.11	26.04
9	-1.68	0	0	86.36	20	16	14.56	14.68	6.24	6.16	12.41	12.34	1.39	1.53	6.28	6.07	30.12	30.39
10	1.68	0	0	153.64	20	16	10.76	10.65	4.41	4.48	11.09	10.96	2.08	2.07	5.03	5.07	26.11	26.57
11	0	-1.68	0	120	11.59	16	11.56	11.41	3.56	3.49	12.06	12.01	2.12	2.20	5.13	5.21	26.10	26.40
12	0	1.68	0	120	28.41	16	14.38	14.53	4.23	4.27	12.08	11.91	2.36	2.46	5.01	4.80	29.02	29.46
13	0	0	-1.68	120	20	2.55	15.58	15.69	4.05	3.96	11.68	11.52	2.47	2.58	4.52	4.35	28.01	28.27
14	0	0	1.68	120	20	29.45	16.81	16.72	3.69	3.72	12.34	12.29	2.37	2.41	4.28	4.28	28.02	28.50
15	0	0	0	120	20	16	18.12	18.10	4.38	4.34	12.28	12.27	2.03	1.98	4.63	4.64	23.12	22.98

X_1 = Barrel temperature, X_2 = Feed moisture content, X_3 = Feed composition. Duplicate runs were carried out all design point and average recorded. The experimental runs were randomized.

Table 6. Analysis of variance (ANOVA) for full quadratic model for the response variables

Sources of variation	DF	Sum of square	Mean sum of square	F-Value	p-value
Iron					
Model	9	12.14	1.35	71.73	≤0.0001
Linear	3	6.03	1.22	64.75	≤0.0001
Square	3	0.58	0.19	10.36	≤0.0001
Interaction	3	5.52	1.84	97.84	≤0.0001
Residual Error	20	0.38	0.02	-	-
Lack-of-fit	5	0.35	0.07	35.63	0.058
Pure error	15	0.03	0.002	-	-
Total	29	12.51	-	-	-
Magnesium					
Model	9	140.99	15.67	258.57	≤0.0001
Linear	3	65.32	21.61	356.71	≤0.0001
Square	3	65.79	21.93	361.97	≤0.0001
Interaction	3	9.88	3.29	54.34	≤0.0001
Residual Error	20	1.21	0.06		
Lack-of-fit	5	1.19	0.24	130.89	0.062
Pure error	15	0.03	0.002		
Total	29	142.20			
Manganese					
Model	9	21.12	2.35	109.86	≤0.0001
Linear	3	8.35	1.97	92.37	≤0.0001
Square	3	5.68	1.89	88.62	≤0.0001
Interaction	3	7.09	2.36	110.68	≤0.0001
Residual Error	20	0.43	0.02	-	-
Lack-of-fit	5	0.17	0.03	1.92	0.150
Pure error	15	0.26	0.02	-	-
Total	29	21.54	-	-	-
Copper					
Model	9	2.43	0.27	12.99	≤0.0001
Linear	3	0.92	0.24	11.36	≤0.0001
Square	3	1.15	0.38	18.49	≤0.0001
Interaction	3	0.36	0.12	5.70	≤0.005
Residual Error	20	0.42	0.02	-	-
Lack-of-fit	5	0.39	0.08	45.99	0.0210
Pure error	15	0.03	0.002	-	-
Total	29	2.84	-	-	-
Zinc					
Model	9	15.34	1.70	81.45	≤0.0001
Linear	3	2.83	0.35	16.52	≤0.0001
Square	3	3.36	1.12	53.59	≤0.0001
Interaction	3	9.15	3.05	145.71	≤0.0001
Residual Error	20	0.42	0.02	-	-
Lack-of-fit	5	0.41	0.08	81.26	0.079
Pure error	15	0.02	0.0009	-	-
Total	29	15.76	-	-	-
Calcium					
Model	9	239.18	26.58	115.25	≤0.0001
Linear	3	57.86	5.90	25.60	≤0.0001
Square	3	57.15	19.05	82.62	≤0.0001
Interaction	3	124.16	41.39	179.49	≤0.0001
Residual Error	20	4.61	0.23	-	-
Lack-of-fit	5	4.61	0.92	2764.07	0.091
Pure error	15	0.005	0.0003	-	-
Total	29	243.79	-	-	-

Highest and lowest Mg content was observed in the 15th (120°C X_1, 20% X_2 and 16% X_3) and the 10th (153.64°C X_1, 20% X_2 and 16% X_3) experimental runs respectively. This indicated decrease in Mg content with increasing barrel temperature. The Mn content was highest (6.24mg/100g) at lowest barrel temperature (86.36°C) and lowest (2.89mg/100g) at high temperature (140°C). This variation as noted in this study may be attributed to changing processing variables. The Fe content varied between 10.62 and 13.34mg/100g in runs 2 and 5 respectively. At lower temperature and increased cowpea content of the formulations, Fe content is observed to increase. Alonso *et al.* [32] earlier reported significant change in Fe content of pea and kidney bean seed extrudates and attributed this variation to wearing of metallic pieces, mainly screws of the extruder, this may also be true for this results. Harper [25] reported that extrusion conditions of 120-170rpm screw speed, 105-150°C extruder exit temperature and 18-20% blend moisture content raise the level of Fe in extrudates. The Fe content of 10.62 to 13.34mg/100g recorded in this study is worthy to note as most diets in many developing countries are deficient in Fe. Cupper significantly ($p< 0.001$) decreased from 2.86mg/100g at 140°C barrel temperature, 25% moisture content and 8% cowpea blend to about 1.39mg/100g at a lower temperature of 86.36°C, reduced moisture content of 20% and increased blend ratio of 16% (Table 5). Observed value for Zn and Ca composition of the extrudates was significantly ($p< 0.001$) varied between 4.13 to 6.28mg/100g and 23.12 to 31.11mg/100g respectively. Highest Zn content was recorded at lower extrusion temperature (86.36°C) as compared to 140°C where highest Ca content was observed (Table 5). This indicate that low temperature extrusion favours Zn retention and higher temperature favours Ca retention. It was also clear from the data that Ca content was extensively influenced by extrusion cooking under different treatments. Harper [25] reported 2.95mg/100g and 3.7mg/100g change in Ca contents in rice grits during extrusion.

The general, effects of extrusion variables on the response variables studied here are in harmony with Camire [19] who suggested that increase in the intensity of minerals in extrudates during extrusion and credited the changes to the accumulation of this minerals in water used for the extrusion exercise, heat sensitivity and oxidation tendencies of this class of minerals during heat treatment.

3.2. Statistical Significance of Regression Models

The analysis of variance (ANOVA) for the response variables (Mg, Mn, Fe, Cu, Zn and Ca) are presented in Table 6. To evaluate the significance of any regression model in predicting effects of a set of independent variable on response variables, the F-value test has to be carried out. The f-distribution test is a probability distribution tests used to compare variance by examining their ratios. The f-ratio value in the ANOVA table in this study therefore is the ratio of the model mean square to the appropriate error mean square (Table 6). Krishma *et al*. [9] reported that the larger the ratio, the larger the f-value and more likely is it that variance distributed by the observed models are statistically larger than random error. The f-value reported for each response variables are 71.73, 258.57, 109.86, 12.99, 81.45 and 115.25 respectively for Mg, Mn, Fe, Cu, Zn and Ca. The large f-values reported for the model coefficients indicated that the variation in mineral content of the extrudates can be explained by the regression models. It was also clear that the linear and interaction terms are highly significant ($p< 0.0001$). Based on the ANOVA Table 6, therefore, we can conclude that the selected models adequately represent the data for mineral contents obtained from the extrusion cooking of rice-cowpea composite flour blends. The p-value was used to check for the significance of each of model coefficients. Smaller p-value denotes greater significance of the corresponding coefficient [13].

3.3. Validation of Regression Models

It is also necessary in RSM that the developed regression models (Table 4) provide an adequate approximation for application in real systems, and there are principally two methods used for the validation, these are graphical and numerical methods [30]. The graphical method takes into account the nature of residuals (difference between the observed values and its fitted) of the model while the numerical method uses the coefficient of determination (R^2) and adjusted R^2(R^2_{adj}). R^2 is the measure of how much of the observed variability in the experimental data could be accounted for by the model, while R^2_{adj} on the other hand modifies R^2 by taking into account the number of predictors in the model and calculated as in Eq. 5 and 6.

$$R^2 = \frac{Sum\ of\ square\ residual}{Model\ sum\ of\ square + Sum\ of\ square\ residual} \quad (5)$$

$$R^2_{adj\,.} = 1 - \frac{n-1}{n-p}\,(1 - R^2) \quad (6)$$

Where n is the number of experimental runs, and p the number of predictors (term) in the model, not counting the constant term. Koocheki *et al*, [3] suggested that for a good fitted model, R^2 should not be less than 80%, while Chauhan and Gupta [4] reported R^2 greater than 75% as acceptable for fitting a model. In this study, the models developed indicated R^2 ranging between 85.5% and 99.10% while R^2_{adj} ranges between 78.9 and 98.8% indicating appropriateness of the developed model equation in predicting mineral retention in rice-cowpea composite flour when the three independent variables are mathematically combined. The R^2 and R^2_{adj} values are close to unity. Lee and Wang [13] and Zaibunnisa *et al* [14] reported that when R^2 is closer to unity, the better the empirical model fit the experimental data. It is important to note that adding additional variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, a large R^2 does not always imply adequacy of the model. For this reason therefore, Koocheki *et al*, [3] claim that it is more appropriate to use R^2_{adj} of over 90% to evaluate the model adequacy. Higher R^2_{adj} indicated that non-significant terms have not been included in the model as observed in this study.

Table 7. Regression equation coefficients for response variables[a] in rice-cowpea blends

Coefficient	Response variables (minerals)					
	Mg	Mn	Fe	Cu	Zn	Ca
Linear						
β_o	-83.439	9.308	11.080	4.067	14.987	79.15
β_1	1.133	-0.160	0.039	0.018	-0.128	-0.708
β_2	3.617	0.266	-0.327	-0.309	-0.226	-1.173
β_3	-0.149	0.389	0.394	-0.079	0.099	-0.053
Quadratic						
β_{11}	-0.005	0.001	-0.001	-0.0002	0.001	0.005
β_{22}	-0.073	-0.007	-0.004	0.005	0.005	0.070
β_{33}	-0.011	-0.003	-0.002	0.003	-0.002	0.030
Interaction						
β_{12}	-0.005	0.001	0.005	0.001	-0.002	-0.016
β_{13}	0.004	0.004	-0.002	0.0001	-0.003	-0.012
β_{23}	0.004	0.007	-0.005	-0.002	0.014	0.029
R^2	99.1	98.0	97.0	85.4	97.3	98.1
Adjusted R^2	98.8	97.1	95.6	78.8	96.1	97.3
Lack-of-fit	*	NS	*	*	*	*
Model	*	*	*	NS	*	*

Model on which the coefficients were calculated is $Y = \beta_o + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X^2_2 + \beta_{33}X^3_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$; x_1 = Barrel Temperature, X_2 = Feed moisture content, X_3 = Feed composition, significance at $p\leq0.05$; NS = Non significance.

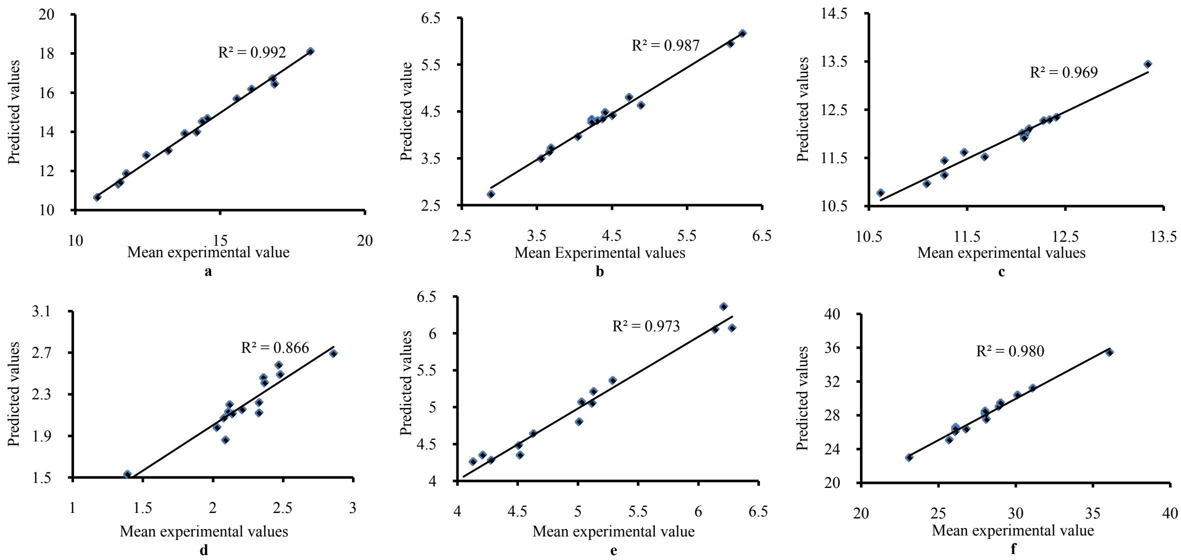

Figure 1. a-f: Parity plots showing the distribution of experimental versus predicted data for response variables (a) Mg (b) Mg (c) Fe (d) Cu (e) Zn and (f) Ca

Figures 1a-f is the plots showing the distribution of experimental versus predicted data for the response variables. In this plots, each of the observed values were compared to the predicted values calculated from the model. The correlation coefficients of 0.992, 0.987, 0.969, 0.866, 0.974 and 0.980 observed between the predicted and actual values for the response variables are evidence that the regression model can represent the experimental data well. It could also be observed that the points on the graph were reasonably distributed near a straight line showing that the underlying assumption of normality in this study was appropriate and therefore validate the models developed. Table 7 presented regression equation coefficients and their estimated values. The coefficients of the response variables Mg, Mn, Fe, Cu, Zn and Ca are -83.439, 9.308, 11.080, 4.067, 14.987 and 79.15 respectively. The negative value for Mg is an indication that when the independent variables are increased, the mg content decreases, while the positive coefficients indicated that the independent variables have significant effects on the dependent variables according to which increase in its value led to an increase in the corresponding mineral in extruded rice-cowpea composite flour [8].

3.4. Analysis of Residual to Check Regression Assumptions

The normal probability plots of residuals are presented in Figures 2a-f, while Figures 3a-f is plot of residual versus the fitted values. In the normal probability plots, the data points forms a straight line indicating that neither response transformation is required nor there was any apparent problem with normality assumption of the regression model equation. This is in line with earlier report by Damirel and Kayan [12]. The plot of residual versus fitted values is presented in Fig. 3a-f. This plot is additionally used to check the underlying assumptions in the regression analysis. If the plot does not show a random scatter of data along the y-axis as presented in this study, then patterns within the plots will indicate problems with the assumptions. In this study, the residual versus fitted value plots shows randomly scattered points spread around 0 without an obvious shape being made by this points indicating that the assumption of the error having zero mean and equal variance have not been violated [5]. This was an indication of better fit for both the models with the experimental data as reported by Chowdhury *et al.* [6].

3.5. Graphical Optimization of Mineral Content of Rice-cowpea Extrudates

The interactive relationship between the independent and dependent variables are illustrated by plotting 3-D graphs representation of the response surfaces generated by the models. The 3-D response surface plots (Figures 4a-f) were plotted based on the coefficients presented in Table 7. The plots were generated by keeping one variable at its zero level and carefully varying the other two variables within the experimental range. As shown in Fig. 4a as the feed moisture content and blend compositions increase, the Mg content in the extrudate increases until it reaches an optimal region. However, increase in both variables beyond the optimum level resulted in decrease in the amount of Mg. At moisture content slightly above 22% and blend composition 20%, Mg content start to decrease.

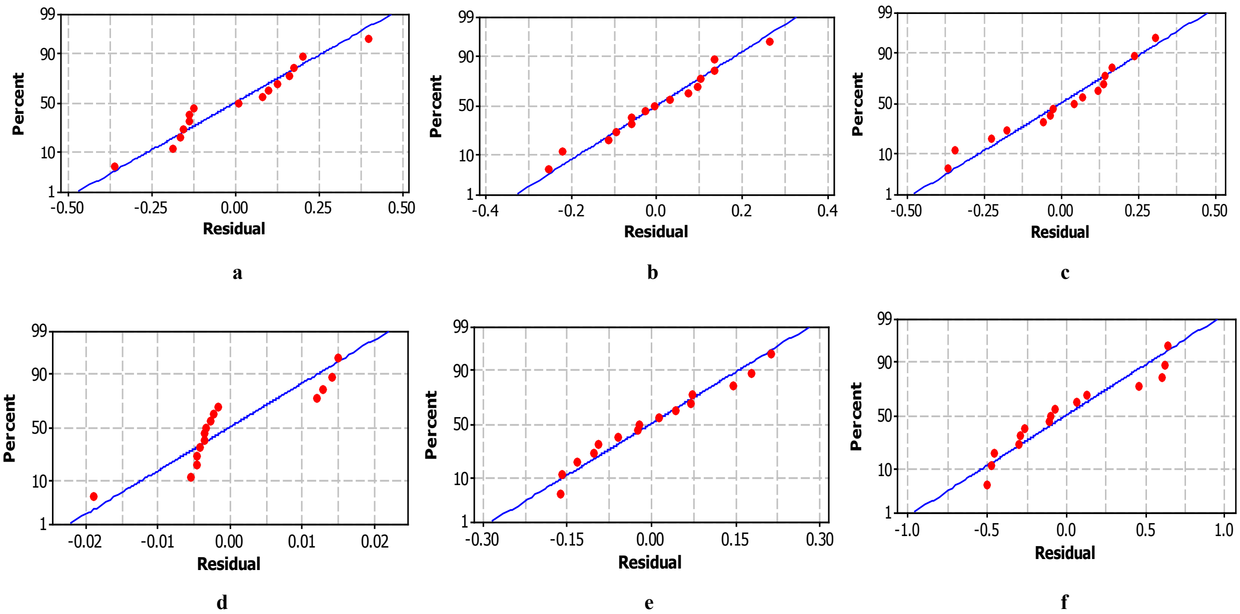

Figure 2. a-f: Normal probability plots of residual for (a) Mg (b) Mg (c) Fe (d) Cu (e) Zn and (f) Ca

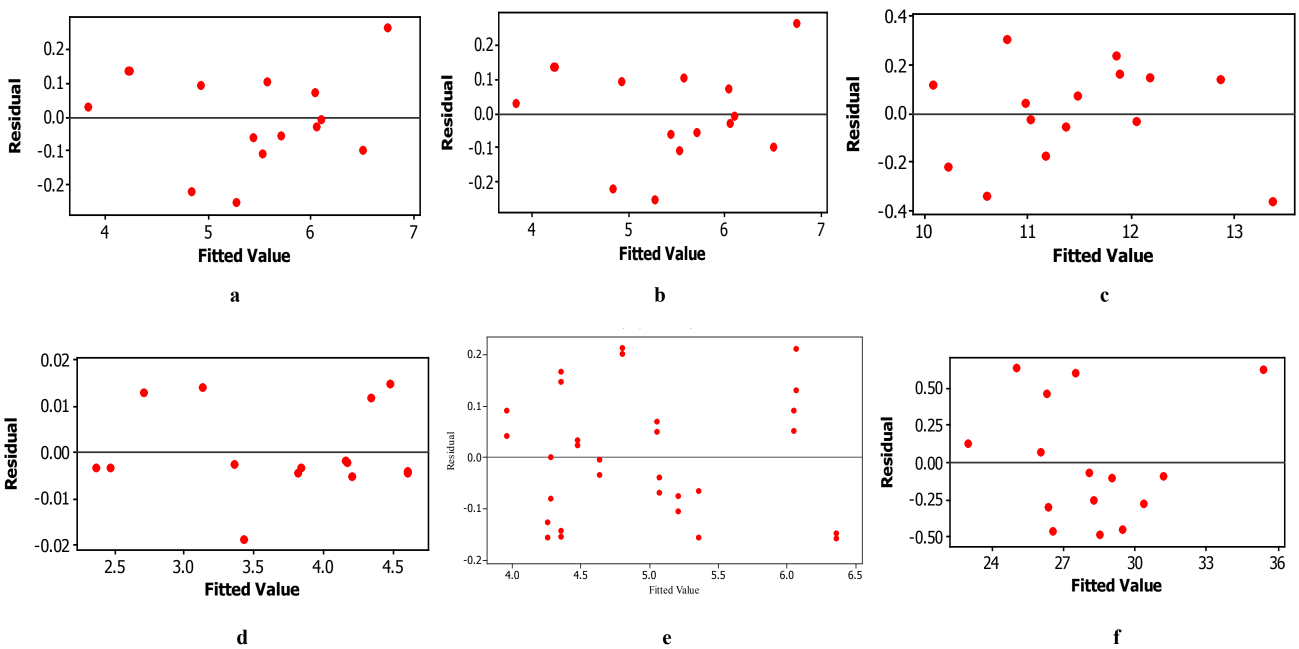

Figure 3. a-f: Plot of residuals versus fitted values for (a) Mg (b) Mg (c) Fe (d) Cu (e) Zn and (f) Ca

Fe an essential nutrient and component of haemoglobin molecule found in red blood cells that carries oxygen in the body on the other hand increase gradually with increasing moisture content and tend to decrease with increasing cowpea content when holding barrel temperature constant (Fig. 4b). Alonso *et al.* [32] earlier reported significant change in Fe content of pea and kidney bean seed extrudates and attributed the variation to wearing of metallic pieces, mainly screws of the extruder and water quality used in the extrusion process and this may also be true for this results. Calcium and Cu contents decrease with increasing moisture and feed blend contents and gradually increases moisture content above 20% and feed blend composition greater than 10% (Fig. 4c and 4f).

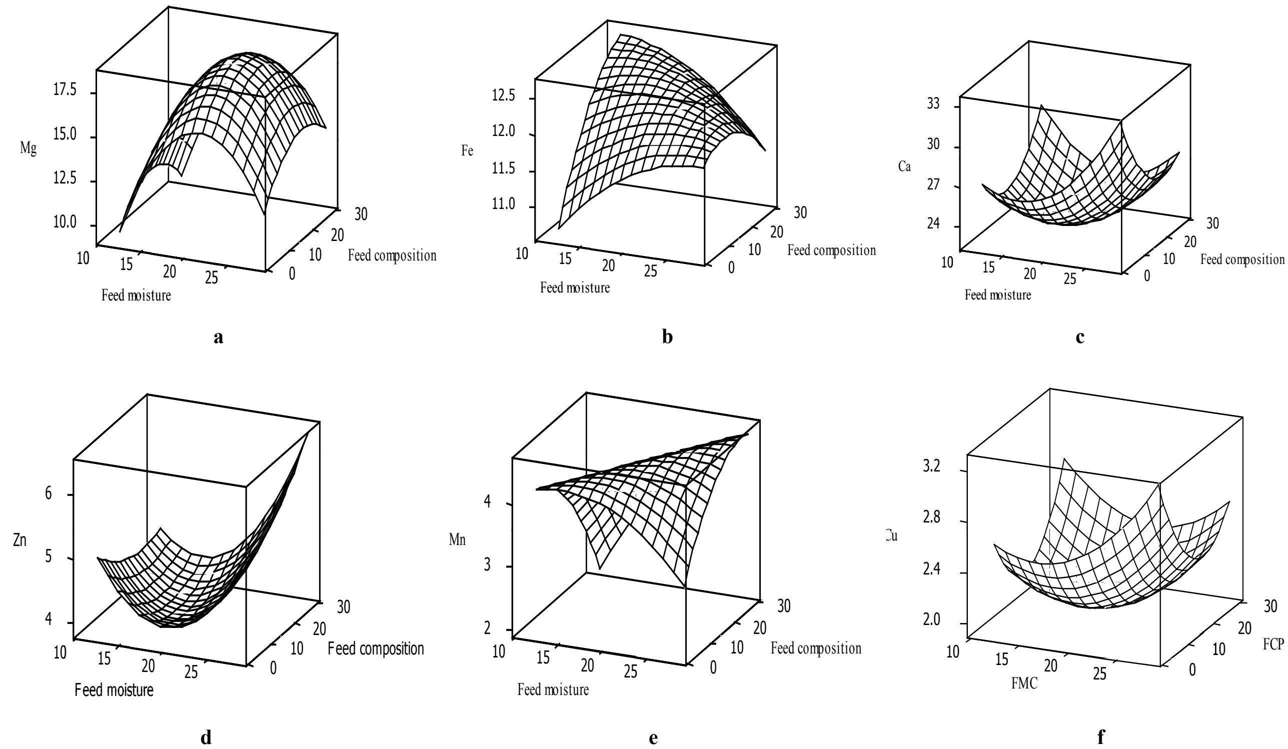

Figure 4. a-f: 3-D Surface plots showing the relationship between independent (feed moisture content (FMC) and feed blend composition (FCP) and dependent[(a) Mg (b) Fe (c) Ca (d) Zn (e) Mn and (f) Cu)] variables holding barrel temperature constant at 120^{o}C

The variation seen in Fig. 4a-f are in harmony with Camire [19] who suggested that increase in intensity of minerals in extrudates that may be accredited to the accumulation of these minerals throughout water used during extrusion.

3.6. Numerical Optimization of Mineral Content of Rice-cowpea Extrudates

A numerical optimization was also performed for the multiple optimization of the response variable (Yi) resulting in the desirable mineral concentration that could achieved in the extrudates. The multiple optimization results indicated that the minimum overall conditions for the minimum reduction in mineral contents of rice-cowpea extrudates could be achieved when the barrel temperature is set at 100^{o}C, feed moisture content of 15% and blend composition of 8%. The peak areas for the response variables were 12.06mg/100g, 5.59mg/100g, 10.98mg/100g, 2.36mg/100g, 4.24mg/100g, and 25.99mg/100g for Mg, Mn, Fe, Cu, Zn and Ca respectively.

4. Conclusions

It clear from this study, that the central composite design and response surface methodology enabled the determination of optimal operating conditions for obtaining stable mineral content in rice-cowpea composite flour during extrusion. The validity of the model was proven by fitting the values of the variables to the model equation and by carrying out experiments using these values. The optimization of the analysed responses demonstrated that the best peak conditions for extrusion under the different variables were 12.06mg/100g, 5.59mg/100g, 10.98mg/100g, 2.36mg/100g, 4.24mg/100g, and 25.99mg/100g for Mg, Mn, Fe, Cu, Zn and Ca respectively. Minerals present in the extrudates may be therefore maximized when process conditions are strictly manipulated.

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